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High CXCR2 expression predicts poor prognosis in adult patients with acute myeloid leukemia

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Abstract

Aims: This study aimed to assess the associations between clinical parameters, long-term outcomes, and expression of chemokine receptor *CXCR2* in patients with acute myeloid leukemia (AML).

Methods: From May 2013 to May 2017, 83 adult patients newly diagnosed with AML in the Affiliated Hospital of BeiHua University and Jilin Chemical Hospital, were enrolled in this study. The expression of *CXCR2* in bone marrow mononuclear cells was determined by quantitative real-time polymerase chain reaction (qRT-PCR). Clinical information and RNA-sequencing datasets of The Cancer Genome Atlas (TCGA) ($n = 136$) were obtained. The associations between clinical parameters, prognosis, and *CXCR2* expression were analyzed.

Results: From both cohorts, patients with AML with M4 and M5 subtypes showed higher *CXCR2* expression levels than those with other French-American-British (FAB) subtypes. Patients with extramedullary leukemia infiltration had higher *CXCR2* levels than those without. In our cohort, patients with high *CXCR2* levels (⩾2.099) had lower relapse-free survival (RFS) ($p < 0.000001$) and overall survival (OS) ($p = 0.000107$) than those with low levels (<2.099). High *CXCR2* levels (⩾2.082) also indicated a poor OS in the TCGA cohort but only in patients younger than 65 years (5-year OS: 7.7% *versus* 29.9% in those with *CXCR2* levels < 2.082). High *CXCR2* levels independently predicted poor prognosis in AML patients, as determined by Cox proportional hazards models.

Conclusion: Our results suggest that high *CXCR2* expression associates with the monocytic lineage of AML and is an independent risk factor for poor patient prognosis.

Keywords: acute myeloid leukemia, clinical features, correlations, *CXCR2* expression, prognosis

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Introduction

Acute myeloid leukemia (AML) is a heterogeneous disorder characterized by the accumulation of poorly differentiated leukemic cells in the bone marrow (BM) and extramedullary organs. The French-American-British (FAB) classification recognizes eight AML subtypes (M0–M7), based on morphological features. Patients with M3, or acute promyelocytic leukemia (APL), have a very favorable prognosis due to the discovery of arsenic trioxide and all-trans retinoic acid.[1,2] However, clinical presentation, treatment response, and prognosis of patients with non-APL AML are highly heterogeneous. Thus, the National Comprehensive Cancer Network (NCCN) and European LeukemiaNet (ELN) have established guidelines to classify newly diagnosed AML patients.[3,4] The presence of specific chromosomal abnormalities and gene mutations is used to stratify the patients into different risk groups (low, intermediate, or high), which help assess prognosis and post-remission treatment-chemotherapy, or allogeneic hematopoietic stem

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cell transplantation (allo-HSCT). Although the NCCN and ELN guidelines are used widely in clinical practice for managing AML patients, heterogeneity still exists in certain risk groups. For example, in AML with *CEBPA* double mutations, a low-risk group, some patients experience continuous remission, while others relapse after cycles of consolidation chemotherapy or stem cell transplantation.[5,6] Studies from different medical centers have attempted to explore new markers for re-stratifying these patients.[7,8] Therefore, there is an urgent need to uncover potential markers for AML stratification. Many studies have attempted to dissect the AML heterogeneity with different molecular markers,[9–11] but it is more convenient to regulate gene expression than to correct gene mutations.

CXCR2 is a seven-transmembrane-domain G-protein-coupled receptor (GPCR) expressed in multiple cell types such as neutrophils, monocytes, eosinophils, and tumor cells.[12] It plays an important role in immunocyte migration and angiogenesis.[12] Furthermore, its upregulation in tumor cells is a poor prognostic factor in various cancers such as prostate, lung, and breast cancer.[13–15] Schinke *et al.* reported that *CXCR2* expression is high in different leukemia cell lines (e.g., KG-1, MOLM-13, HL-60, U937, and THP-1) and primary AML samples. Moreover, using the Cancer Genome Atlas (TCGA) dataset, the authors found that higher levels of *CXCR2* expression led to worse clinical outcomes.[16] Inhibiting or downregulating CXCR2, conversely, leads to decreased viability and clonogenic capability of AML cells.[16] Another group also used TCGA database but failed to establish an association between high *CXCR2* expression and poor patient prognosis.[17] Since the correlation between *CXCR2* expression levels and clinical parameters of AML patients is also unclear, further studies are needed to associate *CXCR2* expression with AML clinical phenotypes. Here, two independent patient cohorts were analyzed, one from our hospitals and the other from the TCGA database, to study the relationships between patient clinical parameters, long-term outcomes, and *CXCR2* expression.

Materials and methods

Patients and controls

From May 2013 to May 2017, 83 patients newly diagnosed with AML in the Affiliated Hospital of BeiHua University and Jilin Chemical Hospital were enrolled in this study. Patients were diagnosed based on the FAB classification (M0–M7), immunological phenotypes, cytogenetics, and gene mutation analyses. All patients received the standard 3+7 regimen for induction therapy (daunorubicin or idarubicin with cytarabine). Some elderly patients in our patient cohort were also treated with the CAG regimen (aclarubicin, cytarabine, and granulocyte colony-stimulating factor). The first consolidation regimen was always similar to that achieved in remission, and the patients were administered a scheduled 3–4 courses of intermediate- or high-dose cytarabine (1.5–2.0 g/m^2) for subsequent therapies. High-risk or relapsed patients underwent allo-HSCT if an appropriate donor was available. Peripheral blood samples from healthy volunteers were collected as controls. Before enrollment, the volunteers, patients, and their relatives gave written informed consent. The ethics committee of the Affiliated Hospital of BeiHua University (No. 2013-003) and Jilin Chemical Hospital (No. 2014-028) approved the study, and it followed the principles outlined in the Declaration of Helsinki.

Cytogenetic analysis and screening of somatic gene mutations

Standard leukemia cell culturing and chromosome-banding techniques determined the karyotypes of AML patients. The results were defined and described according to the International System for Human Cytogenetic Nomenclature 2013.[18] *NPM1*, *FLT3*-ITD, *c-kit*, and *CEBPA* mutations were detected by polymerase chain reaction (PCR).

Quantitative real-time PCR (qRT-PCR)

Bone marrow mononuclear cells (BMMCs) and peripheral blood mononuclear cells (PBMCs) were isolated from the patients and volunteers by density gradient centrifugation. Total RNA was extracted from the cells using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). To synthesize cDNA, the RNA was reverse-transcribed with the PrimeScript RT kit (TaKaRa, Shiga, Japan) according to the manufacturer's instructions. The *CXCR2* expression was determined by qRT-PCR using the SYBR Premix Ex Taq II kit (TaKaRa). *GAPDH* transcript levels were an endogenous control that normalized the variance between the samples. The comparative $\Delta\Delta$Ct method allowed

Table 1. Primer sequences for *CXCR2* and *GAPDH*..

Gene name	Forward primer	Reverse primer
CXCR2	CCTGTCTTACTTTTCCGAAGGAC	TTGCTGTATTGTTGCCCATGT
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG

the calculation of relative gene expression values. The *CXCR2* and *GAPDH* primer sequences (Sangon Biotech, Shanghai, China) are listed in Table 1.

Data mining of public and well-documented datasets

Normalized *CXCR2* and *CXCL1/2/3/5/6/7/8* expression data and clinical information of 136 AML patients were retrieved from the Genomic Data Commons (GDC) TCGA database and downloaded from the website of the University of California, Santa Cruz (UCSC) at http://xena.ucsc.edu/welcome-to-ucsc-xena/. The expression data and clinical information were combined according to patient sample numbers.

Statistical analysis

Statistical Package for Social Sciences (SPSS) software (Version 20, SPSS Inc., Chicago, IL, USA) and Prism 8.0 (GraphPad Software, San Diego, CA, USA) were used to process the data. For continuous variables with normal distribution and homogeneity of variance, independent sample t-test (for two groups) or one-way analysis of variance (ANOVA) (for three or more groups) were used to compare differences. Alternatively, the Mann–Whitney U-test compared the differences between two groups; the Kruskal–Wallis H test, three or more groups. For further two-group comparisons, the least significant difference or Tamhane test was used. Correlation analysis was performed using Pearson's or Spearman correlation. When *CXCR2* and its ligand expression levels were AML prognosticators, a receiver operating characteristic (ROC) curve evaluated the distribution of specificity and sensitivity. The Kaplan–Meier method was employed for survival analysis, and the log-rank test was used to compare differences between groups. The Cox proportional hazard model was used for multivariate analysis, including the low p-value variables ($p < 0.1$). A p value < 0.05 was considered significant for all tests.

Results

Characteristics of AML patients and controls

A total of 219 adult AML patients participated in this study, 83 from our hospital and 136 from the TCGA dataset; 16 healthy volunteers were included as controls, consisting of 10 males and 6 females, with a median age of 42 years (range, 32–58 years). While patients from the TCGA cohort were of advanced age, the median age was 57 years, patients from our hospital were younger with a median age of 46 years ($t = 5.002$; $p = 0.000001$). Concerning FAB classifications, acute myeloblastic leukemia (M2) was the most common subtype in both patient cohorts. Our patients also carried significantly more *CEBPA* mutations ($\chi^2 = 5.349$; $p = 0.021$) and had lower hemoglobin levels ($u = 5.900$; $p < 0.000001$) than those in the TCGA cohort. Detailed information is summarized in Table 2.

CXCR2 expression in AML patients and its association with clinical factors

Compared with healthy controls, AML patients showed higher levels of *CXCR2* expression (Figure 1). There was no significant association between age, sex, white blood cell (WBC) counts, BM blast percentages, and *CXCR2* levels (Table 3 and supplemental Table S1) in both patient cohorts. In both cohorts, patients with acute myelomonocytic leukemia (M4) and acute monocytic leukemia (M5) showed higher *CXCR2* levels than those with other FAB subtypes (Table 3 and supplemental Table S2). Patients with extramedullary leukemia infiltration (EMLI) (lymph nodes, liver, spleen, skin, testicles, Waldeyer ring, or central nervous system) had higher *CXCR2* levels (1.906 ± 0.795, $n = 32$) than those without (1.567 ± 0.660, $n = 51$) ($t = 2.106$, $p = 0.038$). There was no significant difference among patients in different cytogenetic risk groups in our and TCGA cohorts. The number of patients in the low-risk, intermediate-risk, and high-risk groups were 34, 22, and 23, respectively, in our patient cohort according to the NCCN

Table 2. Basic characteristics of patients with AML.

	Our cohort (*n* = 83)	TCGA cohort (*n* = 136)
Age, median (range)	46.0 (18.0–65.0)	57.0 (21.0–88.0)
Gender		
Male	40 (48.19%)	76 (55.88%)
Female	43 (51.81%)	60 (44.12%)
FAB classification		
M0	0 (0.00%)	15 (11.03%)
M1	5 (6.02%)	35 (25.74%)
M2	33 (39.76%)	38 (27.94%)
M4	27 (32.53%)	29 (21.32%)
M5	16 (19.28%)	15 (11.03%)
M6	2 (2.41%)	2 (1.47%)
M7	0 (0.00%)	1 (0.70%)
Others	0 (0.00%)	1 (0.70%)
Cytogenetics		
Low-risk	11 (16.92%)	17 (12.69%)
Intermediate-risk	49 (75.38%)	81 (60.45%)
High-risk	5 (7.69%)	36 (26.87%)
***NPM1* mutation**		
Yes	20 (24.10%)	38 (28.57%)
No	63 (75.90%)	95 (71.43%)
FLT3* mutation		
Yes	24 (28.92%)	37 (27.82%)
No	59 (71.08%)	96 (72.18%)
***c-kit* mutation**		
Yes	6 (7.23%)	7 (5.26%)
No	77 (92.77%)	126 (94.74%)
***CEBPA* mutations#**		
Yes	18 (21.69%)	13 (9.77%)
No	65 (78.31%)	120 (90.23%)
White blood cells ($\times 10^9$/l)	21.26 (4.33, 61.15)	24.5 (6.00, 56.00)
Hemoglobin (g/l)	75.80 ± 28.02	90.00 (90.00, 100.00)
Platelets($\times 10^9$/l)	43.00 (19.00, 89.00)	50.00 (30.00, 87.75)
BM blasts (%)	67.5 (43.00, 82.50)	65.74 ± 21.77

*Information for *FLT3*-ITD or *FLT3*-TKD mutations is lack in TCGA dataset.
#Information for *CEBPA* single or double mutations is lacking in the TCGA dataset. Successful cytogenetic analysis was completed in 78.31% (65/83) of our patients and 98.53% (134/136) of TCGA cohort. There is a lack of information for molecular mutations in some patients from TCGA dataset, so the number of patients with *NPM1, FLT3, c-kit*, and *CEBPA* mutations is less than the total patient number.
AML, acute myeloid leukemia; BM, bone marrow; FAB, French-American-British; TGCA, the Cancer Genome Atlas.

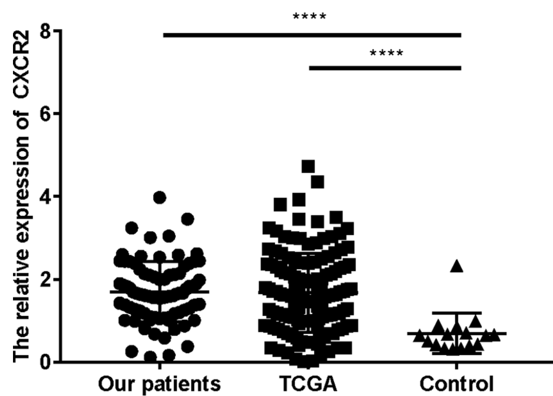

Figure 1. *CXCR2* expression is high in AML patients compared with healthy controls.
****$p < 0.0001$.
AML, acute myeloid leukemia; TCGA, the Cancer Genome Atlas.

guidelines for AML (version 2.0 2020). Patients in the low-risk group (1.450 ± 0.652) had lower *CXCR2* levels compared with those in the intermediate-risk (1.848 ± 0.689) ($p = 0.048$) and high-risk (1.931 ± 0.843) ($p = 0.016$) groups. We also found no significant association between *NPM1, c-kit* mutations, and *CXCR2* levels in either patient cohort. In the TCGA cohort, nonetheless, patients carrying the *FLT3* mutation had higher *CXCR2* levels than those with wild-type *FLT3*, and a tendency of high *CXCR2* expression also could be observed in *FLT3* mutated patients in our cohort. Patients with *CEBPA* mutations (single plus double) showed lower *CXCR2* levels than those without ($p = 0.000394$) in our cohort. Although we observed no significant difference in *CXCR2* expression between patients with and without *CEBPA* mutations in the TCGA cohort, we noticed a tendency of lower *CXCR2* expression in patients with *CEBPA* mutations than in those without (Table 3). Furthermore, patients with *CEBPA* double mutations also showed lower *CXCR2* expression (0.861 ± 0.488) than those without (1.825 ± 0.676) in our cohort ($t = 4.545$; $p = 0.000019$).

The relationship between complete remission and CXCR2 expression

Achieving complete remission (CR) after one course of chemotherapy is an important indicator of chemosensitivity. The *CXCR2* expression levels in our patients who achieved CR after one course of chemotherapy were 1.731 ± 0.719, which was similar to those who did not (1.704 ± 0.835) ($t = 1.123$; $p = 0.902$).

High CXCR2 expression associates with poor prognosis

We used ROC curves to predict relapse-free survival (RFS) and overall survival (OS) of patients with *CXCR2* expression levels. In our patient cohort, we selected an optimal cutoff value of 2.099 for *CXCR2* expression to predict both RFS and OS. The area under the curve (AUC) was 0.837 ($p = 0.001$), and the sensitivity (Se) and specificity (Sp) were 72.7% and 94.1%, respectively, (Figure 2A). For OS, the AUC was 0.740 ($p = 0.035$), and Se and Sp were 62.5% and 86.5%, respectively (Figure 2B). Patients with high *CXCR2* expression levels ($\geqslant 2.099$) showed significantly inferior RFS ($p < 0.000001$) and OS ($p = 0.000107$) than those with low levels (< 2.099) (Figure 3A,B). In the TCGA cohort, however, we could not determine the optimal cutoff value in the whole patient cohort (Figure 2C). Nonetheless, in those younger than 65 years old, a level of 2.082 was found to be an optimal cutoff value (Figure 2D) (AUC = 0.625; Se = 44.9%; Sp = 83.8%; $p = 0.048$). The 5-year OS was 7.7% in patients with high *CXCR2* expression levels ($\geqslant 2.082$), which was significantly lower than that in patients with low levels (< 2.082) (29.9%) ($p = 0.006$) (Figure 3C). The influence of expression levels of CXCR2 ligands on prognosis of patients was also analyzed in this study with TCGA dataset. Among them, both *CXCL1* and *CXCL7* expression levels were associated with outcomes of AML patients (supplemental Figure S1). The 5-year OS in patients with low (< 1.270) and high ($\geqslant 1.270$) *CXCL1* expression levels were 32.4% and 5.1%, respectively ($p = 0.024$) (Figure 4A). Patients with high *CXCL7* expression levels ($\geqslant 3.812$) had an inferior 5-year OS than those with low expression levels (< 3.812) (5.3% *versus* 37.4%) ($p = 0.003$) (Figure 4B).

Next, using univariate analysis, we estimated the prognostic significance of cytogenetic risk groups, molecular markers, and high WBC counts ($\geqslant 100 \times 10^9$/l) (Figures 5, 6 and supplemental Figures S2, S3), and then used these results for multivariate analysis. In our patient cohort, both *CEBPA* double mutations (Figure 5) and *CXCR2* expression associated with RFS and OS. High *CXCR2* expression, furthermore, was the only

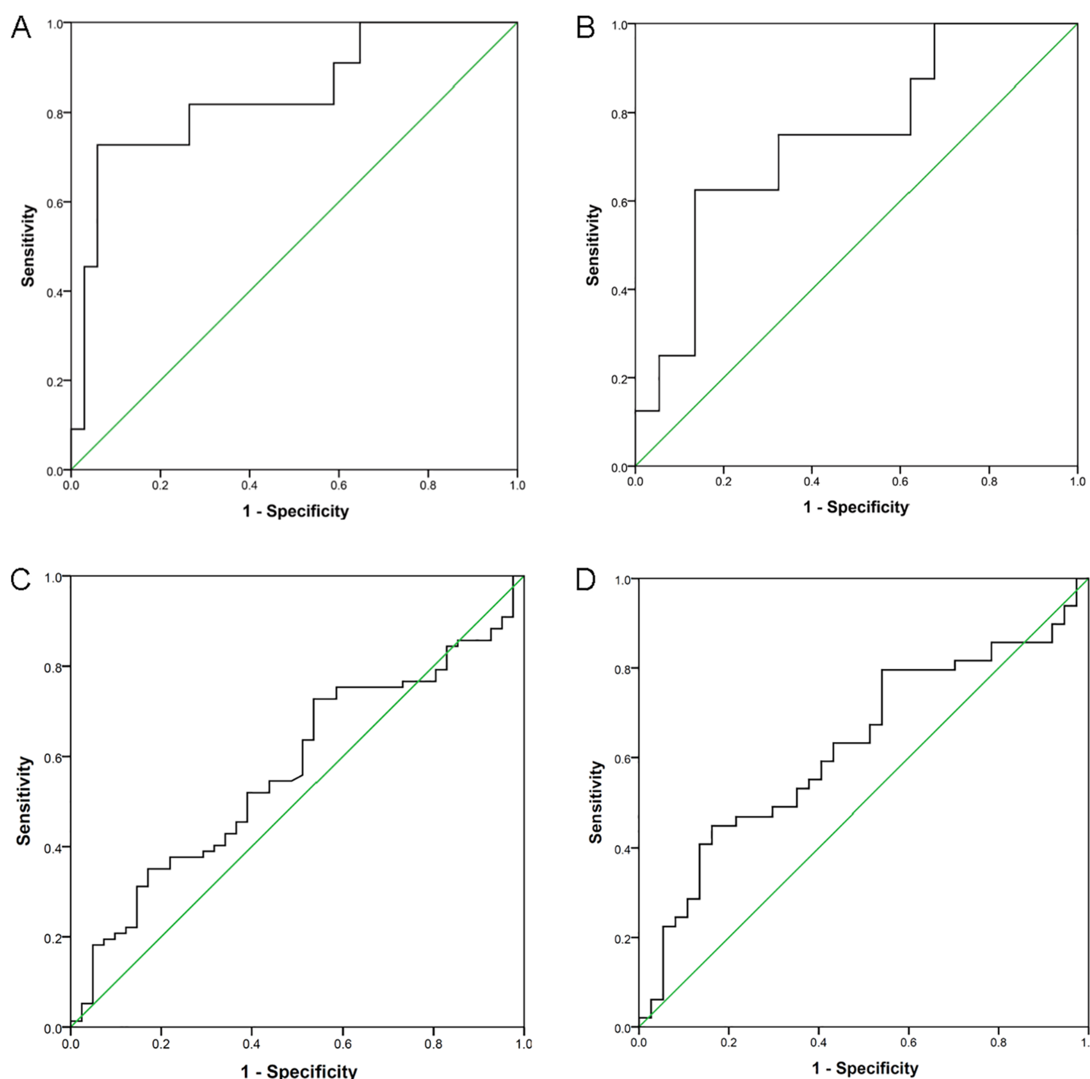

Figure 2. ROC curve was used to predict survival of AML patients with *CXCR2* expression levels. (A) *CXCR2* expression levels predicted RFS in our patient cohort; (B) *CXCR2* expression levels predicted OS in our patient cohort; (C) *CXCR2* expression levels predicted OS in the whole cohort of TCGA patients; (D) *CXCR2* expression levels predicted OS in patients ⩽65 years old in TCGA cohort.
AML, acute myeloid leukemia; OS, overall survival; RFS, relapse-free survival; ROC, receiver operating characteristic; TCGA, the Cancer Genome Atlas.

independent factor for RFS and OS in Cox proportional hazard models (Table 4). In the TCGA cohort, cytogenetic risk groups (Figure 6A), high WBC counts (Figure 6B), and *CXCR2* expression all associated with OS and high *CXCR2* expression was an independent risk factor for unfavorable prognosis (Table 5). Due to the relatively small number of patients receiving allo-HSCT (eight in our cohort) or lack of information for allo-HSCT in the TCGA dataset, we could not analyze the influence of *CXCR2* expression on the survival in the allo-HSCT setting. Taken together, these results suggest that high *CXCR2* expression is an independent unfavorable risk factor for AML patients.

Discussion

Chemokine receptor CXCR2 is expressed in a variety of tumor cells, and plays an important role in their proliferation, metastasis, and chemoresistance.[19–21] The CXCR2 pathway is also

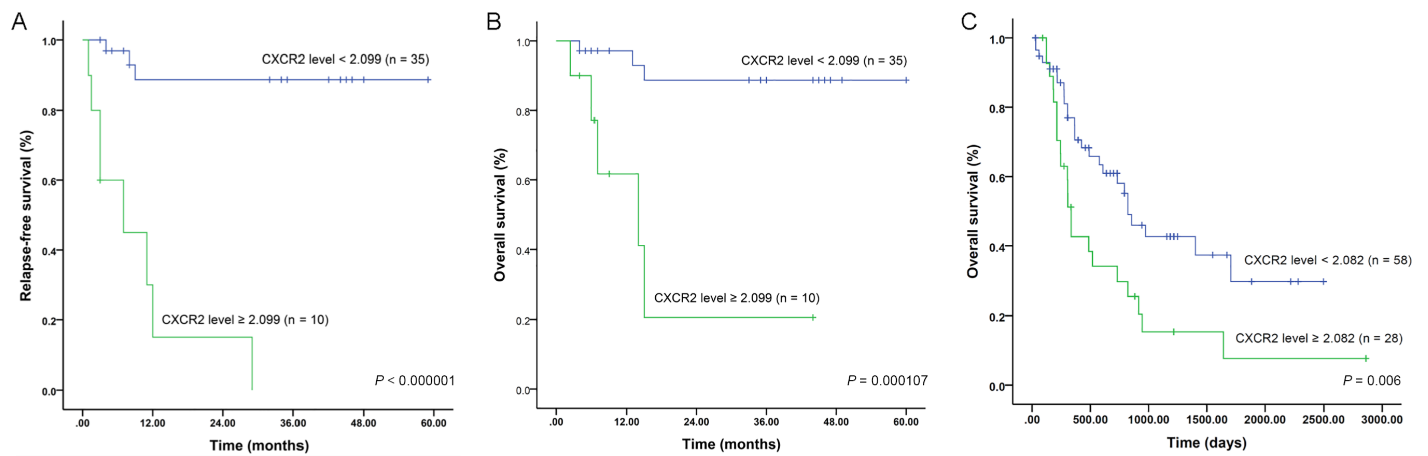

Figure 3. Survival of AML patients based on *CXCR2* expression levels. (A) RFS of our patient cohort; (B) OS of our patient cohort; (C) OS of TCGA patient cohort (⩽65 years old).
AML, acute myeloid leukemia; OS, overall survival; RFS, relapse-free survival; TCGA, the Cancer Genome Atlas.

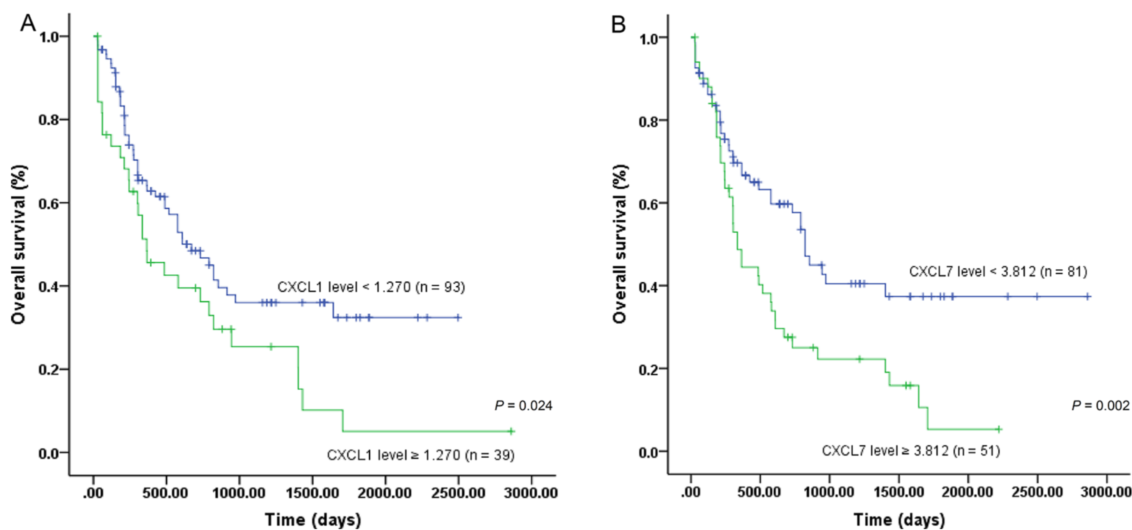

Figure 4. Survival of AML patients based on *CXCL1* and *CXCL7* expression levels. (A) OS of patient with low and high *CXCL1* levels; (B) OS of patient with low and high *CXCL7* levels.
AML, acute myeloid leukemia; OS, overall survival.

involved in angiogenesis and the activation of cancer stem cells (CSCs).[16,22,23] Although high *CXCR2* expression is an indicator of poor prognosis in many solid tumors,[13–15] its role in AML still needs to be elucidated as limited studies are available and controversial results exist. Therefore, in this study, we assessed the associations between clinical parameters, prognosis, and *CXCR2* expression in a Chinese patient cohort with AML. To overcome problems related to the small sample size, we verified our findings with a well-documented public database (TCGA).

Patients with AML showed higher *CXCR2* expression levels compared with healthy controls, which was consistent with the results of a previous study.[16] We also found a significant association between *CXCR2* levels and the FAB subtypes. In patients with M4 and M5 subtypes, the *CXCR2* levels were similar and higher than those with other FAB subtypes in both cohorts. Previous study reported that CXCR2 played a critical role in the development of monocytes/macrophages, and CXCR2 deficiency reduces the ability of granulocyte and macrophage

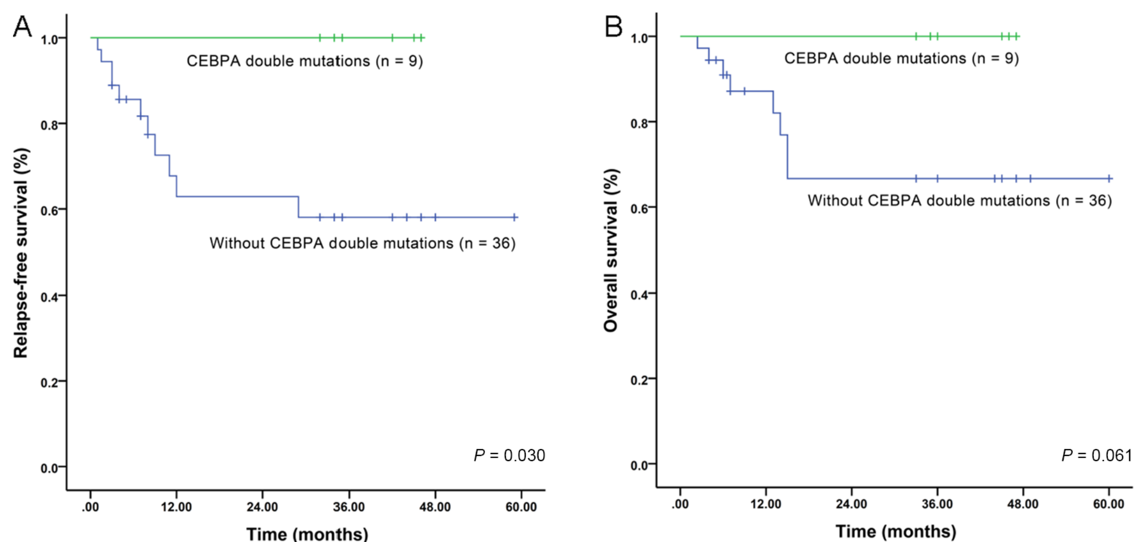

Figure 5. Influence of *CEBPA* double mutations on survival of AML patients from our cohort. (A) RFS of patients with and without *CEBPA* double mutations; (B) OS of patients with and without *CEBPA* double mutations.
AML, acute myeloid leukemia; OS, overall survival; RFS, relapse-free survival.

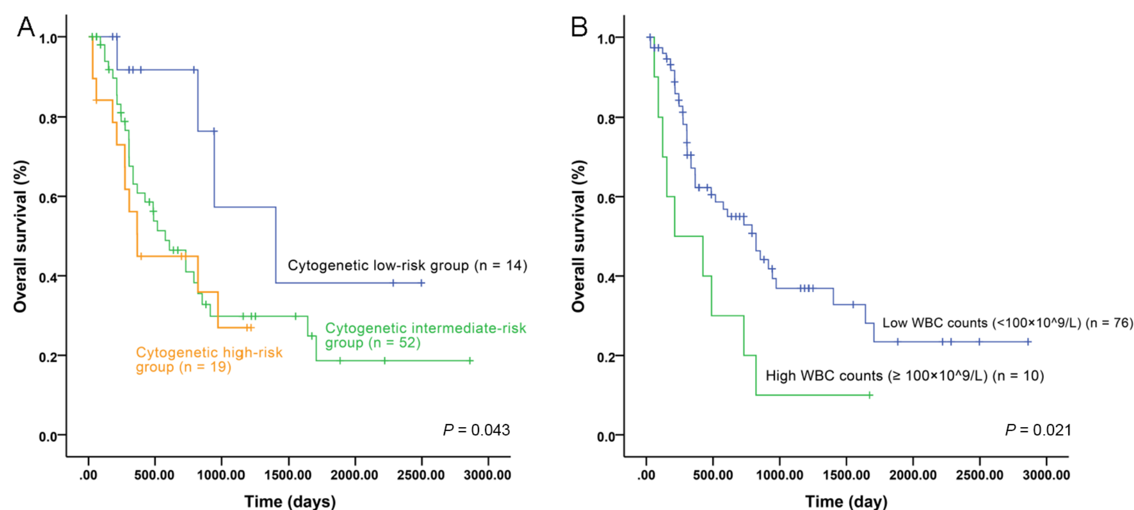

Figure 6. Influence of cytogenetic risk groups and WBC counts on survival of AML patients from TCGA cohort. (A) OS of patients with different cytogenetic risk groups; (B) OS of patients with high ($\geqslant 100 \times 10^9$/L) and low ($<100 \times 10^9$/l) WBC counts.
AML, acute myeloid leukemia; OS, overall survival; TCGA, the Cancer Genome Atlas; WBC, white blood cell.

progenitor cells to give rise to macrophage and dendritic cell progenitor cells.[24] Therefore, high *CXCR2* expression in patients with monocytic lineage of AML indicate that CXCR2 may contribute to leukemogenesis. Moreover, high *CXCR2* levels in patients with the monocytic lineage of AML may partly contribute to the high incidence of leukemic cell infiltration to extramedullary sites. The CXCR2 receptor is expressed in monocytes and it may account for their enhanced migration to the lungs in chronic obstructive pulmonary disease.[25] Similarly, during the early phases of nephrotoxic nephritis, CXCR2 promotes glomerular monocyte recruitment.[26] In our patient cohort, we found that high *CXCR2* levels were related with EMLI.

Table 3. The relationships between clinical factors and *CXCR2* expression.

	Our cohort		TCGA	
	CXCR2 levels	***p***	**CXCR2 levels**	***p***
Gender		0.318		0.927
Male	1.614 ± 0.769		1.682 ± 0.968	
Female	1.775 ± 0.691		1.663 ± 0.961	
FAB classification*		0.007		<0.001
M0	–		1.129 ± 0.640	
M1	1.093 ± 0.427		1.295 ± 0783	
M2	1.474 ± 0.713		1.478 ± 1.002	
M4	1.872 ± 0.663		2.257 ± 0.871	
M5	2.042 ± 0.752		2.528 ± 0.630	
Cytogenetic risk groups		0.841		0.259
Low-risk	1.696 ± 0.488		1.408 ± 0.636	
Intermediate-risk	1.665 ± 0.809		1.786 ± 1.038	
High-risk	1.467 ± 0.661		1.582 ± 0.905	
***NPM1* mutation**		0.390		0.163
Yes	1.821 ± 0.627		1.858 ± 1.069	
No	1.658 ± 0.760		1.600 ± 0.913	
***FLT3* mutations**		0.299		0.009
Yes	1.829 ± 0.638		2.022 ± 1.048	
No	1.644 ± 0.763		1.539 ± 0.899	
***c-kit* mutations**		0.884		0.508
Yes	1.740 ± 0.569		1.687 ± 0.970	
No	1.694 ± 0.744		1.38 ± 0.861	
***CEBPA* mutations**		<0.001		0.494
Yes	1.174 ± 0.609		1.499 ± 0.928	
No	1.842 ± 0.697		1.692 ± 0.969	

*Due to limited patient number, the *CXCR2* expression level was not calculated in patients with M6, M7 and others.
AML, acute myeloid leukemia; FAB, French-American-British; TGCA, the Cancer Genome Atlas.

Table 4. Cox proportional hazard models for RFS and OS in our cohort of patients.

	RFS		OS	
	CEBPA^{dm}	CXCR2 levels ⩾ 2.099	*CEBPA*^{dm}	CXCR2 levels ⩾ 2.099
B	11.902	−2.509	12.389	−1.918
SE	296.253	0.690	374.877	0.740
Wald	0.002	13.231	0.001	6.720
Exp (B)	147513.441	0.081	240051.89	0.147
CI	0.000–UD	0.021–0.314	0.000–UD	0.034–0.626
p	0.968	0.000275	0.974	0.010

B, regression coefficient; *CEBPA*^{dm}, *CEBPA* double mutations; CI, confidence interval; Exp (B), odds ratio; OS, overall survival; RFS, relapse-free survival; SE, standard error; UD, undetermined; Wald, statistical value.

Table 5. Cox proportional hazard model for OS in TCGA dataset.

	B	SE	Wald	Exp (B)	CI	*p*
CXCR2 levels ⩾ 2.082	−0.864	0.296	8.494	0.422	0.236–0.754	0.004
Low-risk cytogenetics			4.754			0.093
Intermediate-risk cytogenetics	−1.234	0.581	4.512	0.291	0.093–0.909	0.034
High-risk cytogenetics	−0.482	0.362	1.771	0.618	0.304–1.133	1.256
High WBC counts (⩾ 100×10^9/l)	−0.800	0.413	3.753	0.449	0.200–1.009	0.053

B, regression coefficient; CI, confidence interval; Exp (B), odds ratio; OS, overall survival; SE, standard error; TGCA, the Cancer Genome Atlas; Wald, statistical value; WBC, white blood cell.

Furthermore, the frequency of EMLI in patients with monocytic lineage of AML was higher (44.19%, 19/43) than in those with other FAB subtypes (32.5%, 13/40), although no statistical difference was reached ($\chi^2 = 1.195$, $p = 0.274$). Correlation analysis showed that *CXCR2* levels associated with EMLI ($r = 0.217$, $p = 0.048$). However, when FAB subtype was set as a control variable, no correlation between EMLI and *CXCR2* levels could be observed ($r = 0.200$, $p = 0.071$). Collectively, these results indicated that CXCR2 may not only participate in leukemogenesis, but also may be associated with extramedullary infiltration in patients with monocytic lineage of AML.

Interestingly, we found that patients with *CEBPA* mutations had lower *CXCR2* levels than those without in our cohort. A previous study reported that the majority of patients with *CEBPA* double mutations had the M1 and M2 subtypes,[6] which also could be observed in our patients (7/10). This may account for the low *CXCR2* levels observed in patients with *CEBPA* mutations. In the TCGA cohort, patients with *FLT3* mutations had higher *CXCR2* levels than those without. Tendencies of high *CXCR2* expression also could be found in patients with *FLT3* mutations and without *CEBPA* mutation in our and TCGA cohorts, respectively. Thus, further studies are needed to confirm the correlation between *CXCR2* levels and the above AML marker mutations, and to elucidate its biological significance.

The prognostic significance of high *CXCR2* expression was controversial in two previous studies.[16,17] We also could not find an optimal cutoff value to predict survival with the *CXCR2*

levels in the whole cohort of TCGA dataset. Nonetheless, in patients younger than 65 years, high *CXCR2* levels were associated with an unfavorable outcome. In our patient cohort, high *CXCR2* levels were an indicator of poor prognosis. Moreover, multivariate analyses demonstrated that high *CXCR2* levels were an independent risk factor for poor prognosis. CXCR2 ligands were reported to be related with proliferation of leukemia cells.[27] Hence, the prognostic significance of these ligands was analyzed as well. High expression levels of *CXCL1* and *CXCL7* associated with poor prognosis could be observed with TCGA cohort. Accordingly, *CXCR2* expression is a potential marker for risk stratification of AML patients.

We could not observe significant associations between *NPM1* mutation, *FLT3*-ITD mutation, cytogenetic risk groups, and survival in our patient cohort. Furthermore, the TCGA dataset lacks the information for *FLT3*-ITD or *FLT3*-TKD mutations, and *CEBPA* single or double mutations. Therefore, further research on the AML marker mutations and a larger sample are needed to verify our findings.

CSCs are rare immortal cells within a tumor that can both self-renew and give rise to many cell types that constitute the tumor, and can therefore form tumors. The regulatory effect of CXCR2 on CSCs activity have been reported in previous studies.[16,23] With *in vitro* and *in vivo* models, inhibition of CXCR2 by genetic and pharmacologic means leads to decreased viability in AML/MDS stem cells.[16] Reparixin is a small molecular weight antagonist of CXCR1/2. In a phase Ib study [ClinicalTrials.gov identifier: NCT02001974], 33 patients with metastatic breast cancer were treated with paclitaxel plus escalating doses of reparixin.[28] Finally, 27 patients were evaluated for antitumor activity, and 8 patients had a confirm RECIST response. The median time to progression in low (400 mg), intermediate (800 mg), and high dose (1200 mg) of reparixin groups were 58 days, 67 days, and 162 days, respectively. Three patients achieved long-term remission.[28] In another clinical trial [ClinicalTrials.gov identifier: NCT02001974], patients with untreated operable breast cancer not eligible for neoadjuvant treatment were treated with reparixin for 21 consecutive days. Signal of activity was defined as a ⩾20% reduction of CSCs in tumor tissue from baseline values determined by flow cytometry. A total of 20 patients were enrolled, and signal of activity was detected in the majority of patients.[29] In patients with metastatic castration-resistant prostate cancer, the safety and toxicity of CXCR2 antagonist AZD5069 in combination with enzalutamide are evaluated in a multicenter Phase I/II trial [ClinicalTrials.gov identifier: NCT03177187]. Therefore, clinical study is needed to explore the efficacy of CXCR2 blockade in AML.

In summary, using two independent AML patient cohorts, we found that *CXCR2* expression is associated with FAB subtypes. Patients with the monocytic lineage of AML showed higher *CXCR2* levels than those with other FAB subtypes. High *CXCR2* expression was associated with extramedullary leukemia infiltration and an independent risk factor for poor prognosis in AML patients. Accordingly, CXCR2 may be used as a prognostic indicator or therapeutic target for AML.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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Supplemental material

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