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ORIGINAL ARTICLE

Association of CXCR4 Expression and Clinical Outcome in Different Subsets of De Novo Acute Myeloid Leukemia Patients

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SUMMARY

Background: **Acute myeloid leukemia is a heterogeneous group of diseases characterized by the uncontrolled proliferation of hematopoietic stem cells (HSCs) and progenitor cells with a reduced capacity to differentiate into mature cells. CXC chemokine receptor (CXCR4) and its ligand stromal derived factor-1 (SDF-1/CXCL12) are important players involved in cross-talk between leukemia cells and the bone marrow (BM) microenvironment. The aim to study the association between the immuno-histochemical CXCR4 expression and the clinical outcome of AML in adult Egyptian patients.**

Methods: **Fifty-eight patients suffering from AML were recruited for this study, with an age range from 18 to 60 years and presenting from January 2013 to March 2017. All patients were subjected to complete blood count, BM aspiration, immunophenotyping, BM trephine biopsy, immunohistochemical staining with CXCR4 McAb and cytogenetics when feasible.**

Results: **CXCR4 was widely expressed (55.2%) among the studied patients. There was a significant relationship between CXCR4 and patients' outcomes. Fifteen (71.4%) patients who died were CXCR4 positive. The estimated mean time until death among CXCR4 negative cases was 37.6 ± 4.04 months which was longer than that of CXCR4 positive cases who had mean of 20.04 ± 4.9 months p = 0.016. The risk for death among CXCR4 positive cases was higher than CXCR4 negative cases with hazard ratio (HR) = 2.147 (p = 0.048).**

Conclusions: **These results suggest that CXCR4 was expressed in a subset of AML patients and was associated with poor prognosis. CXCR4 expression appears to be an independent prognostic factor for survival in a heterogeneous group of AML patients.**

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INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous group of diseases characterized by abnormal proliferation of the myeloid line of blood cells, in which there is an accelerated growth of abnormal cells that increase in the bone marrow and blood and inhibit normal blood cells [1].

The main symptoms and signs of AML are caused by

the substitute of normal blood cells with abnormal myeloid cells [2].

The WHO 2008 classification of acute myeloid leukemia attempts to be more clinically useful and to produce more meaningful prognostic information than the FAB (French-American-British (FAB) classification) [3].

Several interactions between ligands and corresponding receptors regulate bone marrow microenvironment by controlling migration, adhesion, quiescence, and proliferation of leukemia cells [4].

The chemokine receptor CXCR4, a transmembrane receptor with a unique high expression level in the hematopoietic system is activated by stromal cell-derived factor1 (SDF-1 or CXCL12). The CXCL12-CXCR4 pathway plays a pivotal role in migration and homing of hematopoietic stem cells (HSCs) to the BM [5].

It was found that CXCR4 expression is associated with poor prognosis in patients with AML. Specifically, CXCR4 expression is common in normal-karyotype AML and is a marker of more aggressive disease in this population. CXCR4 expression could be incorporated into the risk assessment of patients with AML [6].

The aim of this research was to study the association between the immuno-histochemical CXCR4 expression and the clinical outcome in adult Egyptian patients with AML.

MATERIALS AND METHODS

This prospective study was carried out in the Clinical Pathology Department of the National Cancer Institute (NCI), Cairo University after the approval of the institutional review board during the period between January 2013 and March 2017.

After informed written consent was obtained from patients or their guardians, 58 consecutive patients with de novo acute myeloid leukemia presenting to the outpatient clinic of the Medical Oncology Department at NCI were included in the study.

The eligibility criteria were: newly diagnosed adult AML, age between 18 - 60 years, ECOG ≤ 2, all FAB subtypes, no medical contraindication, no other malignancy and no prior chemotherapy or radiotherapy. Pregnant or lactating females were excluded.

All patients were subjected to full history and laboratory investigations including CBC, BM aspiration and examination (for cyto-morphological and cyto-chemical examinations), and assessment of response on day 28 after induction therapy. Patient cases were followed up for a period of at least one and a half years to calculate their overall survival.

Assessment of CXCR4 expression was done using paraffin embedded bone marrow biopsy sections, hematoxylin and eosin standard staining method for bone marrow biopsy sections, and with immunohistochemistry CXCR4 antibody staining for bone marrow biopsy sections (Novus Biologicals, USA).

CXCR4 expression in de novo AML patients were considered as CXCR4 positive when the percentage of stained blasts per case was above 20% [7].

Statistics

Data were analyzed using IBM advanced SPSS statistical package version 20 (SPSS Inc., Armonk, NY, USA). For quantitative data, either parametric or non-parametric *t*-test was used. Chi-square test (Fisher's exact test) was used to examine the relationship between qualitative variables. Correlation between the expression of CXCR4 and other numeric factors was done using Cox regression test. OS was estimated using the Kaplan-Meier analysis, log rank test was used to compare survival curves. All tests of hypotheses were conducted at the alpha of 0.05 level, with a 95% confidence interval.

RESULTS

General characteristics of the studied AML cases

The age of the studied patients ranged from 18 to 60 years with a mean ± SD of 42.98 ± 12.81 years and a median of 43 years. They included 32 (55.2%) males and 26 (44.8%) females with male to female ratio 1.2 to 1.

Laboratory data of the studied AML cases

Peripheral blood findings

1. Initial total leucocytic count ranged from 0.5 - 266.3 x 10^9/L with a mean ± SD of 48.02 ± 71.16 x 10^9/L and median of 15.4 x 10^9/L. The absolute neutrophilic count ranged from 0.02 - 90 x 10^9/L with a mean and ± SD of 12.05 ± 20.88 x 10^9/L and median of 2.05 x 10^9/L.
2. Hemoglobin concentration ranged from 2.3 - 12.1 gm/dL with a mean ± SD of 7.12 ± 1.75 gm/dL and median of 7.3 gm/dL.
3. Platelet count ranged from 6 - 735 x 10^9/L, with a mean ± SD of 67.95 ± 103.59 x 10^9/L and median of 35 x 10^9/L.
4. Peripheral blood blasts percentage ranged from 0 to 90 with a mean ± SD of 17.29 ± 23.39 and median of 12.5.

Bone marrow findings

The percentage of bone marrow aspirate blasts at the time of diagnosis ranged between 20 - 90% with a mean ± SD of 56.26 ± 21.67% and median of 55%.

Initial bone marrow aspirate cellularity was hypercellular in 41 cases (70.7%), normocellular in 14 cases (24.1%), and hypocellular in 3 cases (5.2%).

1. Initial BM trephine biopsy was hypercellular in 52 cases (89.7%) and normocellular in 6 cases (10.3%).
2. Fifty-eight cases (100%) had myeloid precursors in BM biopsy which was demonstrated by hematoxylin and eosin standard staining method for bone marrow biopsy section and positive MPO by immunohistochemistry.

Table 1. Relationship between CXCR4 expression and laboratory findings and age of AML patients.

	CXCR4	Mean	SD	p-value
TLC (10^9/L)	Positive	59.70	78.51	0.079
	Negative	33.63	59.26	
ANC (10^9/L)	Positive	15.35	24.99	0.025
	Negative	7.99	13.71	
HGB (gm/dL)	Positive	7.29	1.76	0.797
	Negative	6.91	1.75	
Platelets (10^9/L)	Positive	50.53	42.93	0.788
	Negative	53.63	41.83	
Peripheral blood blasts	Positive	16.56	24.54	0.794
	Negative	18.19	22.33	
BMA blast % at presentation	Positive	56.50	23.05	0.261
	Negative	55.96	20.29	
Day 14 (BMA blast %)	Positive	7.03	12.03	0.643
	Negative	8.54	14.26	
Day 28 (BMA blast %)	Positive	8.56	12.30	0.893
	Negative	7.15	15.29	
Age	Positive	43.81	12.57	0.494
	Negative	41.36	12.79	

Table 2. Risk classification of AML patients and its relationship to CXCR4.

		CXCR4		p-value
		Positive	Negative	
Risk group (n = 51) N.B: 7 missed patients considered as unclassified	Intermediate	15 (46.9%)	9 (34.6%)	0.574
	Poor	5 (15.6%)	6 (23.1%)	

Table 3. Relationship between CXCR4 expression and patients' outcomes.

		Outcome		p-value
		Alive (n:30)	Deceased (n:21)	
CXCR4	Positive		15 (71.4%)	0.015
	Negative		6 (28.6%)	

Table 4. Overall survival in AML patients and its relationship to CXCR4.

	Mean	Std. Error (SE)	95% Confidence Interval		p
			Lower Bound	Upper Bound	
CXCR4 (Positive)	20.047	4.931	10.383	29.711	0.016
CXCR4 (Negative)	37.640	4.044	29.714	45.566	

Table 5. Cox regression correlation analysis of AML patients.

	Exp (B) (hazard ratio)	95.0% CI for OR		p
		Lower	Upper	
CXCR4 positivity	2.147	0.816	5.643	0.048
Age	1.024	0.984	1.067	0.244
Gender	0.710	0.286	1.761	0.460
TLC	1.000	0.992	1.009	0.946
ANC	1.004	0.975	1.033	0.809
BM blasts at time of diagnosis	1.007	0.984	1.030	0.573
FLT3	1.525	0.323	7.208	0.595

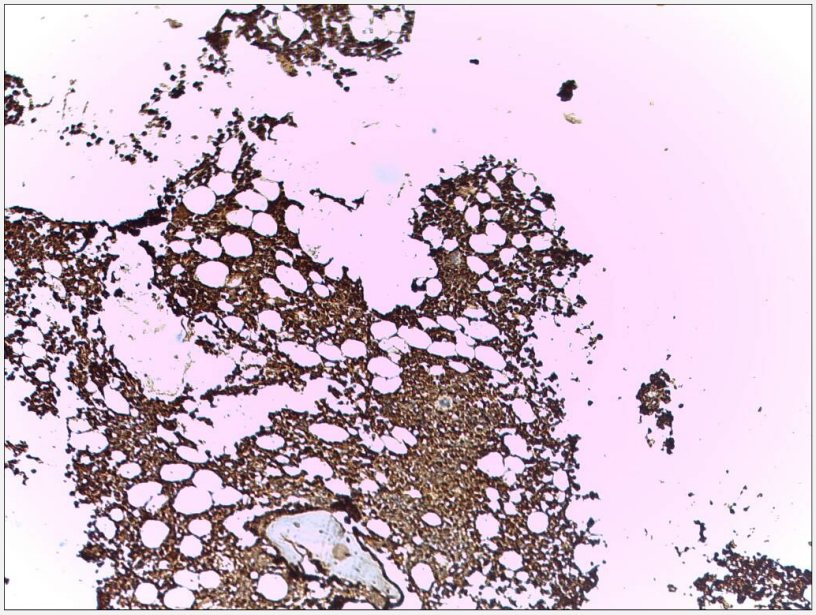

Figure 1. CXCR4 by IHC showing 100% positivity (10 x).

Immunophenotyping
All cases were positive for MPO, CD13, CD33, and CD117 while 37 cases (63.8%) were positive for HLA-DR.

The CXCR4% expression (by immunohistochemistry)
The expression ranged from 4 - 90% with a mean ± SD of 29.22 ± 23.73% and it was found that 32 (55.2%) were positive (the percentage of stained blasts per case was above 20%) and 26 (44.8%) were negative for CXCR4 expression (Figures 1, 2).

Relationship between CXCR4 expression and laboratory data (Table 1)
Analysis of CXCR4 expression with respect to laboratory findings showed that there was a statistically significant difference in CXCR4 expression with respect to ANC. Otherwise, there was no significant relationship between CXCR4 expression and other parameters ($p = 0.025$).

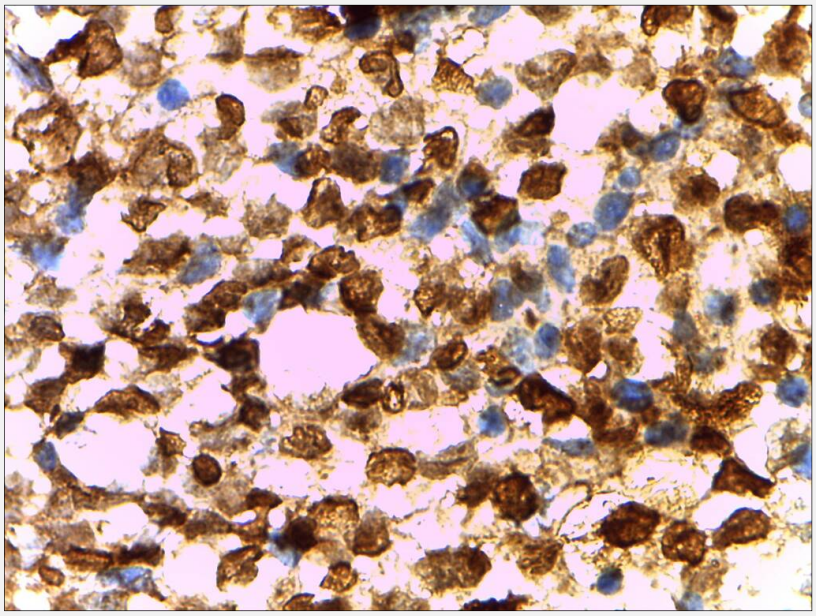

Figure 2. CXCR4 by IHC showing 90% positivity (100 x).

Relationship between CXCR4 expression and patients' outcomes (Figure 3 and Table 3)

Regarding living patient-cases, 11 (36.7%) out of 30 cases were CXCR4 positive and 19 cases (63.3%) were CXCR4 negative while among deceased cases, 15 (71.4%) out of 21 cases were CXCR4 positive and 6 cases (28.6%) were CXCR4 negative. Additionally, 7 out of 58 cases were lost to follow up, 6 of them being CXCR4 positive and one case was CXCR4 negative. There was a significant difference between patients' outcomes and expression of CXCR4 marker. Of the deceased patient-cases, 15 (71.4%) were CXCR4 positive and 6 (28.6%) were CXCR4 negative, while among living cases, only 11 (36.7%) were positive and 19 (63.3%) were negative, with a p-value = 0.015.

Overall Survival in AML patients and its relationship to CXCR4 (Tables 4, 5 and Figure 4)

In the present study the median overall survival was 11 months with a mean of 13.91 months. Thirty cases (51.7%) were living, 21 cases (36.2%) were deceased, and 7 cases (12.1%) were lost to follow up. Regarding CXCR4 expression and its relationship to survival, it was found that the estimated mean survival time among CXCR4 negative cases was 37.64 months (95% CI 29.71 - 45.57) which was significantly longer than that of CXCR4 positive cases who had mean of 20.04 months (95% CI 10.38 - 29.71) p = 0.016. Cox regression analysis shows the risk for death among CXCR4 positive cases was higher than among CXCR4 negative cases with hazard ratio of HR = 2.147 with significance of p = 0.048, while other prognostic parameters showed no significant relationship.

DISCUSSION

In the present study, the age range was 18 - 60 years, with a median of 43 years, and a male to female ratio of 1.2:1. This age range and male preponderance are in agreement with other investigators. In 2008, Wakui et al. studied 638 AML patients with an age range of 15 - 66 years, median of 45 years and male to female ratio was 1.6:1 [8]. While Konoplev et al. studied 122 AML patients with male to female ratio of 1.3:1, median age of 62 years, and age range of 22 - 82 years [9].

Regarding TLC, Hb, platelets, and BMA blast percent at time of diagnosis, in the present study they had the following medians respectively: 15.4 x 10^9/L, 7.3 gm/dL, 35 x 10^9/L, and 55%. These results correspond with Wakui et al. who had demonstrated the medians for TLC, Hb, platelets, and BMA blast percent at time of diagnosis as follows respectively: 13.7 x 10^9/L, 8.3 gm/dL, 52 x 10^9/L, and 56% [8]. Cignetti et al. had reported the percent of blasts in bone marrow at diagnosis as ranging between 44 - 95% while in the present study it ranged between 20 - 90% [10].

In 1998, Kanz et al. first published that leukemic blasts

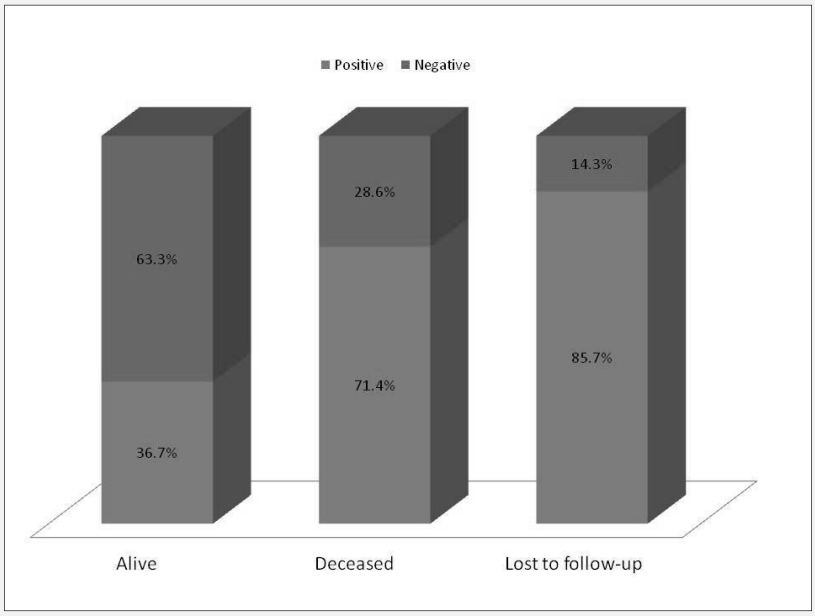

Figure 3. Relationship between CXCR4 expression and patients' outcomes.

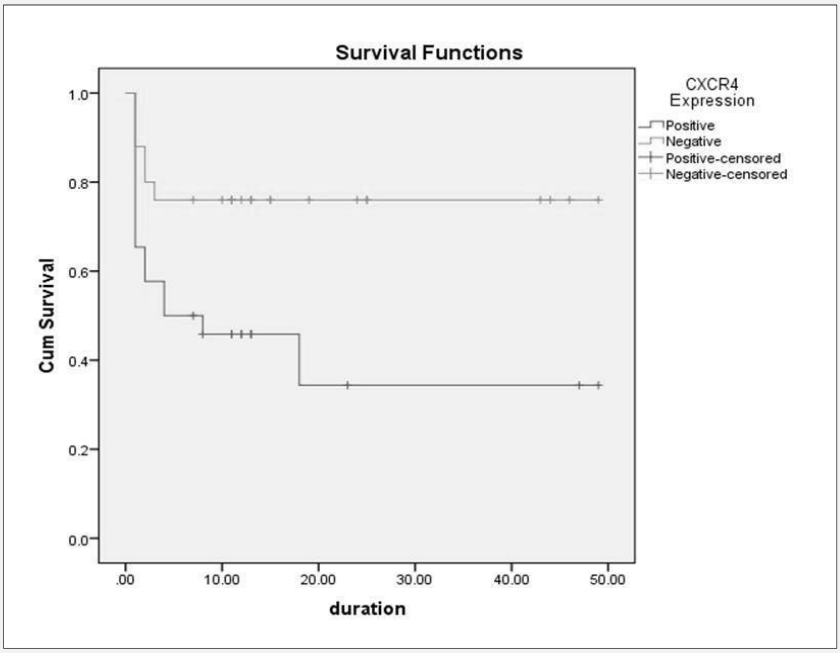

Figure 4. Overall survival in AML patients and its relationship to CXCR4.

(mostly CD34+) from patients with AML expressed variable amounts of CXCR4, and it was functionally active, as demonstrated by a positive correlation between the CXCL12-induced migration and the cell surface density of CXCR4 [11].

Regarding CXCR4 expression in the present study, it was found that CXCR4% expression ranged from 4% to 90% with a mean ± SD of 29.22 ± 23.73% and a median of 22.5%. Additionally, it was found that 32 patients (55.2%) were positive and 26 patients (44.8%) were negative for CXCR4 expression. This approximates the results reported by Ahn et al. who found that 26 out of 53 (49.1%) patients were CXCR4 positive [6]. In contrast to the present study, Cignetti et al. found that CXCR4 was widely expressed in 21 out of 25 cases of AML (84%). This difference may be due to his small sample size [10].

In this study, there was no significant correlation of CXCR4 with platelet count, with $p = 0.788$. This is contrary to Francesco et al. who found high intensity of CXCR4 associated with high platelet counts. This disagreement may be due to the sample size difference as they recruited 142 AML patients while the present study included 58 AML patients [12].

Furthermore, the present study documented that high levels of CXCR4 is significantly correlated with high ANC. ANC mean among positive cases was 15.35 ± 24.99, while ANC mean among negative cases was 7.99 ± 13.71 with $p = 0.025$.

Regarding the relationship between patients' outcomes and expression of the CXCR4 marker, there was a significant difference between deceased cases, of whom 15 (71.4%) were CXCR4 positive and 6 (28.6%) were CXCR4 negative on the one hand, and living cases, of whom 11 (36.7%) were positive and 19 (63.3%) were negative ($p = 0.015$). This result is in agreement with studies demonstrating CXCR4 expression is associated with poor prognosis in patients with AML [6,12].

Regarding CXCR4 expression and its relationship to overall survival (OS), in the present study it was found that the estimated mean survival time among CXCR4 negative cases is 37.6 ± 4.04 months which is significantly longer than that of CXCR4 positive cases who had mean of 20.04 ± 4.9 months ($p = 0.016$). These results are also in agreement with that of Ahn et al. who found that patients with CXCR4 expression also demonstrated a relatively worse OS than those without CXCR4 expression ($p = 0.07$) [6]. Additionally, these results were in agreement with those of Rombouts et al., who found that patients with high CXCR4 expression in CD34 positive subset of AML blasts had significantly reduced survival and higher probability of relapse, resulting in median RFS of 8.3 months ($p \leq 0.01$).

Spoo et al. prospectively evaluated the prognostic implication of CXCR4 in 90 consecutive patients with AML by flow cytometry [13]. Patients were divided into groups with a low (n = 32), intermediate (n = 26), or high (n = 32) CXCR4 expression, as defined by CXCR4 mean fluorescence intensity ratio thresholds of less than 5, 5 to 10, or more than 10, respectively. Interestingly, low CXCR4 expression on AML cells correlated with a better prognosis, longer relapse-free and overall survival of 24.3 ± 2.9 months for low CXCR4-expressing patients, compared with 17.4 ± 3.4 months and 12.8 ± 2 months (mean ± SEM) for patients with intermediate or high expression, respectively.

The results of survival analysis in the present study are in agreement with those found by Spoo et al. who found that patients who have low CXCR4 expression had longer OS and better prognosis whereas patients with intermediate and high CXCR4 expression had shorter OS. Additionally, Spoo et al. found most patients with intermediate and high CXCR4 expression achieved complete remission after allogenic stem cell transplantation and not after induction therapy. Spoo et al. concluded that CXCR4 expression by AML cells is a significant predictive factor for OS, $p = 0.01$. Considering CXCR4 expression as a risk factor for relapse, a multivariate analysis by Spoo et al. revealed that CXCR4 is a prognostic marker that is independent of other previously established prognostic markers such as cytogenetic abnormalities, LDH, leukocytosis or age. It is hoped that studying CXCR4 expression and its relationship to OS and DFS in a larger series of patients may lead to the incorporation of this marker into the initial diagnostic workup and into the risk-stratified treatment strategies [13]. In other words, positive cases would be provided with CXCR4 antagonists to enhance the efficacy of regular chemotherapy.

Furthermore, Tavernier-Tardey et al. had analyzed blasts from 36 patients by flow cytometry, and they demonstrated a significant relationship indicating OS is negatively influenced by higher CXCR4 expression ($p = 0.01$) [14].

Cox regression analysis of the present study showed that the risk for death among CXCR4 positive cases is higher than CXCR4 negative cases, hazard ratio HR = 2.147 (95% confidence interval, 0.816 - 5.643, $p = 0.048$). The hazard ratios of age, gender, TLC, ANC, BM blasts at diagnosis and FLT3 were 1.024, 0.710, 1.000, 1.004, 1.007, and 1.525, respectively, and the p-values were 0.244, 0.460, 0.946, 0.809, 0.573, and 0.595, respectively, so there is no evidence of an impact of these parameters on the risk of death among these groups.

CONCLUSION

The analysis of this study revealed CXCR4 expression is an independent prognostic factor for survival in AML patients. This is in agreement with the results found by Spoo et al., who worked on 90 cases of AML patients. They did univariate analysis showing that CXCR4 expression (as numerical MFI ratio values) and other prognostic markers as predictors of time from diagnosis to relapse or death; the hazard ratio associated with CXCR4 was 2.699 (95% confidence interval, 1.327 -

5.489) for OS, p = 0.006 and 3.853 (95% confidence interval, 1.298 - 11.442) for RFS, p = 0.015 [14]. This is in conformity with Ahn et al. that CXCR4 expression is an independent prognostic factor for survival in AML patients (p = 0.014) [6].

Declaration of Interest:
No conflicts are declared. All authors approved the manuscript and this submission.

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