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CD97 expression is associated with poor overall survival in acute myeloid leukemia

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Abstract

CD97, a member of the adhesion G-protein coupled receptor family, is normally expressed on leukocytes and smooth muscles. CD97 is also expressed in a variety of solid cancers, particularly those with aggressive metastatic phenotypes. Here we characterize the clinical significance of *CD97* in acute myeloid leukemia (AML). We analyzed 173 patients from the TCGA AML data set and found that *CD97* was higher in cytogenetically normal patients compared with cytogenetically abnormal patients ($p = 0.023$). High *CD97* was also associated with *NPM1* mutations ($p = 0.0033$). Patients with high *CD97* expression had shorter overall (median: 7.35 months vs. 24.1 months, $p = 0.0015$) and disease-free (median DFS: 8.2 months vs. 18.2 months, $p = 0.017$) survival. Importantly, we identified pathways involved in the leukemia stem cell interaction with the bone marrow niche, such as integrin, CXCR4, and interleukin-8, among the most upregulated signaling pathways in patients with high *CD97* expression. Our results suggest that high *CD97* expression is associated with poor clinical outcome and indicate a need for future functional and mechanistic studies to investigate the role of *CD97* in AML.

Acute myeloid leukemia (AML) is a heterogeneous, hematologic malignancy characterized by clonal proliferation of myeloid precursors [1]. It is the most common acute leukemia in adults. Overall survival of patients with AML remains dismal (<50% for younger patients and <10% for older patients) because of the high rate of relapse [2]. Cytogenetic and molecular genetic alterations provide significant prognostic information for determining the response to chemotherapy and survival outcome.

Adhesion G protein-coupled receptors (aGPCRs) were recently reported to be widely deregulated in AML [3,4]. Among aGPCRs, CD97 is particularly interesting; it is expressed predominantly in hematopoietic cells [5] and has been recently identified as a marker for leukemia stem cells [6]. Several ligands have been reported to bind CD97: CD55, which is a

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Authorship

VPV performed the analysis and generated the figures. SW performed the multivariate statistical analysis. JW performed the IPA analysis. VPV and HA wrote the article. HA conceived the project, designed the research, and supervised the project.

Conflict of interest disclosure

The authors declare no competing interests.

negative regulator of the complement cascade [7]; chondroitin sulfate, a component of the extracellular matrix (ECM) [8]; and the integrin $\alpha 5\beta 1$ [8]. The association with integrins is relevant to AML, as integrin and ILK signaling pathways play crucial roles in leukemia stem cell (LSC) survival and interaction with the bone marrow (BM) niche [9,10].

In solid cancers, CD97 association with integrin was found to regulate invasion, migration, and angiogenesis [11]. In undifferentiated anaplastic thyroid carcinoma, *CD97* overexpression was associated with metastatic lesions [12,13]. Additionally, high *CD97* expression was also reported to be associated with increased lymph vessel invasion and poor clinical outcome in colorectal carcinomas [14]. In pancreatic cancer, *CD97* expression significantly correlated with tumor aggressiveness [15]. More recently, CD97 was also shown to be the first member of the EGF-TM7 family to be directly involved in cell signaling. Ward et al. [16] demonstrated functional synergy and heterodimerization between CD97 and lysophosphatidic acid receptor 1 (LPAR1), resulting in increased RHO-GTP levels in a $G\alpha 12/13$-dependent signaling pathway in thyroid and prostate cancers, as well as correlating with increased tumor invasion.

The role of CD97 in invasion and signaling suggests that it is an important therapeutic target. In this study, we characterized *CD97* expression in patients with AML using publicly available data sets to determine its association with patient's clinical and molecular characteristics and clinical outcome. We also identified signaling pathways that are deregulated in patients with high *CD97*.

Methods

Patient data sets

Available data on 173 of 200 patients with previously untreated AML with complete clinical and RNA expression data were studied from the TCGA data set [17]. This data set includes patients with previously untreated AML, all of whom had been diagnosed and received treatment according to the National Comprehensive Cancer Network (NCCN) guidelines between November 2001 and March 2010. The risk group stratification of the patients was done according to NCCN guidelines. The patients were assigned subtype classifications according to the French–American–British (FAB) classifications. Patients included in the study were assessed for the most frequently found somatic mutations in AML, such as FLT3, NPM1, IDH1/2, and TET2. Patient's clinical, gene expression (Z score), mutation, gene methylation, and survival data were downloaded from the TCGA database available at cBioPortal on September 9, 2018. Metzeler-163 [18], Metzeler-79 [18], and Bullinger [19] data sets were downloaded from Oncomine.

Ethics approval and consent to participate were not necessary, as we studied public de-identified data.

Gene expression analyses

We downloaded publicly available and analyzed RNA sequencing data of 173 patients with acute myeloid leukemia and complete clinical and molecular data [20,21]. We dichotomized patients into two groups based on Z scores ≥ 1 (high) and <1 (low). We also validated the

associations using a *Z* score of 2 as the cutoff as well as the median of the mRNA $\log_2$ transformed data.

Statistical analyses

Overall survival (OS) was defined as the time between diagnosis and death from any reason, and disease-free survival (DFS) was defined as the time between diagnosis and removal from study because of relapse or death (two of the patients with missing information regarding their DFS were not included in the DFS analysis). We generated Kaplan–Meier survival curves for comparison of OS and DFS between patients with $Z \geq 1$ and $Z < 1$ *CD97* expression. Additionally, Kaplan–Meier survival curves were generated for patients with $Z \geq 1$ and $Z < 1$ *CD97* expression after stratification by age, cytogenetic status, transplant status, and *NPM1* mutation status. To determine the association between *CD97* expression and patients' clinical and molecular characteristics, we employed T test and Fisher's exact test for continuous and categorical variables, respectively, using the GraphPad Prism software package (Version 5.0, GraphPad Software Inc., La Jolla, CA). We used Stata SE 12.0 to perform a Cox proportional hazards model to conduct survival analysis of the effect of *CD97* expression on OS and DFS after adjusting for other clinical/molecular factors. A statistical cutoff of $p < 0.05$ was used for inclusion of variables into the multivariate survival analysis from univariate analysis.

Ingenuity pathway analysis (IPA)

Patients were grouped as *CD97* high ($Z > 2$) and *CD97* low ($Z < -1$). Pearson correlation scores of *CD97* versus all the available gene expression data in TCGA were calculated, and upregulated genes with a score >0.5 and downregulated genes with a score <–0.5 were exported into IPA. Signaling pathways were evaluated for CD97 high and CD97 low patients.

Results

CD97 *expression in AML*

CD97 mRNA (RNA Seq V2 RSEM) expression data were downloaded from the TCGA data set on cbioportal [20,21]. Histograms representing the distribution of *CD97* mRNA $\log_2$-transformed data and *CD97 Z*-scores are provided in Supplementary Figure E1A and B (online only, available at www.exphem.org). The scatterplot of *CD97* $\log_2$-transformed mRNA expression against *CD97 Z* score is shown in Figure E1C (Pearson's $r = 0.944$, $p \leq 0.001$). *CD97* mRNA levels were compared among patients classified according to the FAB. Although patients in the M5 subgroup had relatively higher *CD97* expression, there was no significant difference in *CD97* expression among the subgroups (Supplementary Figure E1D). When patients were classified based on cytogenetic status into cytogenetically normal AML (CN-AML) (N = 80) and cytogenetically abnormal AML (CA-AML) (N = 90), we found that patients with CN-AML had significantly higher *CD97* expression compared with patients with CA-AML (1.31-fold, $p = 0.023$) (Supplementary Figure E1E).

We also analyzed *CD97* expression according to the NCCN AML classification in which patients are grouped based on their molecular and cytogenetic risk status into favorable,

intermediate, and poor risk groups and found no significant difference between these groups (Supplementary Figure E1F).

Additionally, we used the Vizome data analysis tool, which contains data from the BEAT AML cohort [22], and examined the level of *CD97* expression in relapsed (N = 46) versus de novo AML (N = 288) samples and found no significant difference between the two groups (Supplementary Figure E1G).

We dichotomized the patients in the TCGA data set based on their *CD97* mRNA expression *Z* score (RNA Seq V2 RSEM) into *CD97* high (*Z* score ≥1, N = 26) and CD97 low (*Z* score <1, N = 147). Patients with high *CD97* expression had significantly higher white blood cell counts (WBCs) (median: 56.1 vs. 13.1 [× 10^9/L], $p = 0.004$) and higher percentages of bone marrow blasts (median: 83% vs. 71%, $p = 0.019$) (Table 1).

High CD97 *expression is associated with* NPM1 *mutations*

To understand potential molecular genetic aberrations that may lead to or be associated with high *CD97* in AML, we analyzed its expression with respect to the mutational status of patients with AML. *CD97* was significantly higher in patients with *NPM1* mutation (n = 48) than in patients with *NPM1* wild type (n = 125, 1.56-fold, $p < 0.0001$; Figure 1A). *CD97* was also significantly higher in patients with *FLT3* mutations (*ITD* and point mutations) (n = 49) than in patients carrying *FLT3* wild type (n = 124, 1.4-fold, $p = 0.0008$; Figure 1B). Additionally, *CD97* was significantly lower in the patients with *RUNX1* mutations (n = 17) than in patients with the wild-type *RUNX1* (n = 156) (42.1% lower, $p = 0.0002$; Figure 1C). No significant association was observed between *CD97* expression and mutations in *DNMT3A, IDH1, IDH2, TET2, TP53, CEBPA, WT1*, and *KIT*.

When we dichotomized patients according to *CD97 Z* scores, we found a higher frequency of *NPM1* mutations in high *CD97* (*Z* ≥1) patients than in low *CD97* (*Z* < 1) patients (52% vs. 30.6%, Fisher exact $p = 0.0065$). No association was found between *CD97* upregulation and mutations in other genes in the dichotomized analysis (Table 2).

High CD97 *expression is associated with poor clinical outcome*

The overall survival of patients in the *CD97* high group (*Z* ≥1) was significantly shorter than that of patients in the *CD97* low group (median OS: 7.35 vs. 24.1 months, $p = 0.0015$; Figure 2A). Patients with high *CD97* expression had significantly shorter DFS than patients with low *CD97* (median DFS: 8.2 months vs. 18.2 months, $p = 0.017$; Figure 2B). To further validate the association between high *CD97* and poor clinical outcome, we stratified patients into Z ≥ 2 and Z < 2 for survival analysis. Patients with high *CD97* expression had significantly shorter OS and DFS than patients with low CD97 (OS—median: 7.5 months vs. 20.5 months, $p = 0.04$; DFS—median: 7.3 months vs. 17 months, $p = 0.03$; Supplementary Figure E2A and B, online only, available at www.exphem.org). Because patients with APL or t(15, 17) are treated differently (with ATRA) and have better clinical outcome than the rest of patients with AML, we excluded these patients from the survival analysis. Yet, the OS of the *CD97* high group (*Z* ≥1) remained significantly shorter than that of the *CD97* low patients (median: 7.35 months vs. 19 months, $p = 0.003$; Figure 2C). A similar trend was observed in DFS (median: 8.2 months vs. 16.1 months, $p = 0.08$; Figure

2D). Among patients with APL, the OS of patients in the *CD97* high group ($Z \geq 1$) was significantly shorter than that of the *CD97* low patients ($p = 0.03$; Supplementary Figure E3A, online only, available at www.exphem.org). APL patients with high *CD97* expression had significantly shorter DFS than patients with low *CD97* ($p = 0.003$; Supplementary Figure E3B).

When patients were stratified according to their cytogenetic status, we found that among CA-AML patients, those with *CD97* high ($Z \geq 1$) had shorter overall survival than those with *CD97* low; however, this was not statistically significant (median OS: 7.2 months vs. 19.2 months, $p = 0.159$; Figure 3A). Yet the difference in OS between the two groups was statistically significant among the CN-AML patients (median OS: 7.5 months vs. 24.6 months, $p = 0.0045$; Figure 3B). Also, there was a significant decrease in the DFS of *CD97* high patients ($Z \geq 1$) compared with *CD97* low patients (median: 10.8 months vs. 39 months, $p = 0.043$; Figure 3C) in CA-AML but not in CN-AML patients (median: 8.2 months vs. 12.1 months, $p = 0.25$; Figure 3D).

In multivariate survival analysis, *CD97* high expression ($Z \geq 1$) was associated with shorter overall survival when adjusted for age, cytogenetic risk, and transplant status (HR = 1.96, 95% CI: 1.19–3.24, $p = 0.009$; Table 3). A similar trend was observed when patients with t(15,17) were excluded from the analysis: *CD97* expression ($Z \geq 1$) was associated with shorter overall survival (HR = 1.56, 95% Cl: 0.97–2.83, $p = 0.066$).

When patients were stratified according to whether they received a transplant or not, we found that only in patients who did not receive a transplant was *CD97* high expression ($Z \geq 1$) associated with significantly shorter OS and DFS (median OS: 5.5 months vs. 11.4 months, $p = 0.0078$; median DFS: 5.9 months vs. 32.7 months, $p = 0.007$) (Figure 4A, C).

No significant difference was observed in OS (Figure 4B) and DFS (Figure 4D) between the *CD97* high and low in patients who received a transplant.

We also analyzed the association of *CD97* expression with clinical outcome in additional data sets: Metzeler-163 [18] (N = 163 CN-AML patients), Metzeler-79 [18] (N = 79 CN-AML patients) and Bullinger (N = 105 CN-AML and CA-AML patients) [19] data sets (histograms representing the distribution of *CD97* mRNA expression are illustrated in Supplementary Figure E4A–C [online only, available at www.exphem.org]). Patients were dichotomized based on median *CD97* expression into *CD97* high (above median) and *CD97* low (below median). In the Metzeler-79 data set (which includes CN-AML only), patients with *CD97* high expression had shorter overall survival (median OS: 8.9 months vs. 42.01 months, $p = 0.0027$; Figure 5A). Similarly, in the Metzeler-163 data set (which includes CN-AML only), patients with high *CD97* expression survived for shorter times than patients with low *CD97* expression; however, the difference in survival was not statistically significant (median OS: 8.6 months vs. 14.2 months, $p = 0.23$; Figure 5B). On the contrary, in the Bullinger data set, which includes both CN and CA-AML, there was no significant association between *CD97* expression and clinical outcome (median survival: 13.15 months vs. 18.74 months, $p = 0.6$; Figure 5C). This discrepancy between the Bullinger data set survival analysis and those of other data sets is possibly due to the difference in the

microarray gene probe used to measure *CD97*. We also analyzed the TCGA data set using *CD97* median expression to dichotomize patients into high and low *CD97* expression groups. We found that patients with high *CD97* expression had shorter overall survival (median OS: months 11 vs. 27.4 months, p = 0.008; Figure 5D).

CD97 *expression is associated with survival status of patients with* NPM1 *mutation*

Because *CD97* expression was associated with *NPM1* mutation, which is a favorable prognostic marker in AML, we examined the association between *CD97* expression and clinical outcome in patients with *NPM1* mutation. We stratified patients according to *NPM1* mutational status and performed survival analysis in each group. We found that in patients with wild-type *NPM1*, high *CD97* expression was associated with a significantly shorter OS (median survival: 6.35 months vs. 22.3 months, $p = 0.0081$; Figure 6A) and DFS (median survival: 6.7 months vs. 22.2 months, $p = 0.0015$; Supplementary Figure E5A, online only, available at www.exphem.org). In patients with *NPM1* mutation, a similar trend but not statistically significant association was observed between high *CD97* expression and shorter OS (median survival: 7.5 months vs. 24.1 months, $p = 0.11$; Figure 6B) but not DFS (median survival: 10.8 months vs. 12.1 months, $p = 0.75$; Supplementary Figure E5B).

As patients with *NPM1* mutation have a better prognosis and patients with *FLT3* mutation have poor prognosis, we further analyzed the association between *CD97* expression and patients harboring *FLT3* and *NPM1* mutations to gain better insight. We identified 8 of 173 patients in the TCGA data that have both *FLT3* mutation, *NPM1* mutation, and *CD97* $Z \geq 1$. When we compared overall survival between patients with *CD97* $Z > 1$ (N = 8) and those with *CD97* $Z < 1$ (N = 18) among those with *FLT3* and *NMP1* mutations, we found that patients with *CD97* $Z \geq 1$ had significantly worse survival compared with the $Z \leq 1$ group ($p = 0.04$, median survival: 7.5 months vs. 24.6 months; Supplementary Figure E5C).

High CD97 expression is associated with poor survival in older patients

Because older patients with AML have significantly worse overall survival, we performed survival analysis in patients with AML stratified by their age into younger patients (<60) and older patients (≥60). In patients ≥60, high *CD97* ($Z \geq 1$) expression was associated with shorter OS compared with low *CD97* ($Z < 1$) (median OS: 3.3 months vs. 11 months, $p = 0.0019$; Figure 7A) and a decrease in DFS though not significant (median DFS: 5.9 months vs. 17 months, $p = 0.092$; Supplementary Figure E6A, online only, available at www.exphem.org). A similar trend but not statistically significant association was observed in younger patients (median OS: 27.75 months vs. 53.9 months; $p = 0.2$; Figure 7B) and (median DFS: 11.7 months vs. 20.8 months, $p = 0.1$; Supplementary Figure E6B).

Pathways involved in the leukemia stem cell interaction with the bone marrow niche are activated in patients with high CD97 expression

We further evaluated the CD97-associated cell signaling pathways through ingenuity pathway analysis (Supplementary Figure E7, online only, available at www.exphem.org). Patients were divided into *CD97* high ($Z > 2$, N = 11) and *CD97* low ($Z < -1$, N = 10). Pearson correlation scores of *CD97* versus all the available gene expression data in TCGA were calculated and analyzed in IPA. Our analysis revealed that the integrin signaling, IL-8

signaling, CXCR4 signaling, and ILK signaling pathways were all activated in patients with high *CD97* expression ($Z > 2$). Importantly, these signaling pathways were also downregulated in patients with low *CD97* expression ($Z < -1$). This further validates the association between *CD97* levels and activation of pathways involved in the LSC–BM niche interaction.

Discussion

CD97 has been reported to play a role in disease progression of a variety of malignancies by regulating cell invasion and metastasis. In glioma, high *CD97* expression is associated with worse survival [23]. In colorectal cancer, high *CD97* expression correlated with the cells' dedifferentiation ability [14]. With use of several cancer models including AML cells, CD97 knockdown was found to decrease cell migration and adhesion [16,23,24].

Here we report that high *CD97* mRNA expression is significantly associated with poorer overall survival in AML patients. This is particularly relevant in older patients: median overall survival of patients >60 years with high *CD97* was less than 4 months. This is significantly lower than the already dismal survival of these patients. In AML, the median age at diagnosis is 67 years, and more than 60% of newly diagnosed patients are older than 60 years [25]. Because of several factors, including comorbidities and disease complications, older patients are faced with challenging and not optimal therapeutic approaches compared with younger patients. Therefore, the identification of outcome predictors and possible viable targets in this subset of patients will greatly affect disease understanding and treatment outcome.

Interestingly, we found that patients with *FLT3* mutations had high *CD97* expression. Previous *in vitro* studies found that AML cell lines harboring a *FLT3*- ITD mutation had higher CD97 expression than cell lines with *FLT3-WT* [26]. Similarly, it was also reported that inhibiting FLT3-ITD in AML cell lines with PKC412, a FLT3-ITD inhibitor, resulted in reduced *CD97* expression [26]. The mechanism by which FLT3-ITD and CD97 pathways interact remains unclear. Additionally, our analysis also reveals a positive association between *CD97* and *NPM1* mutations. *NPM1* mutations are associated with a favorable outcome in AML [27]. Contrastingly, there is a trend for patients with *NPM1* mutation and high *CD97* expression ($Z \geq 1$) to have a worse survival outcome compared with patients with *NPM1* mutation and low *CD97* expression. Additionally, patients with *NPM1* wild type and high *CD97* expression ($Z \geq 1$) have significantly worse survival outcome compared with patients with *NPM1* wild type and low *CD97* expression. This suggests a benefit to including *CD97* expression in prediction of survival in patients with *NPM1* mutation. The mechanistic interplay between CD97 and *NPM1* is yet to be determined.

The identification of integrin and ILK signaling pathway to be activated in *CD97* high groups is of particular interest, as CD97 was previously reported to act as a chemoattractant for migration and invasion of human umbilical vein endothelial cells (HUVECs) in an integrindependent manner [11]. Importantly, integrin and ILK signaling pathways have been shown to contribute to leukemia stem cell survival [10]. Additionally, IL-8 and CXCR4 signaling pathways are among the most significant pathways in the high *CD97* group. IL-8

was previously reported to be regulated by hypoxia, as well as to influence the niche formation in the bone marrow by inducing the migration of mesenchymal stromal cells (MSC) [28]. Furthermore, high IL-8 expression also correlated with a worse clinical outcome in non-APL AML [28]. Interestingly, mAbCD97 inhibits IL-8-induced HSC/HPC mobilization [29], suggesting that CD97 may potentially regulate the IL-8 signaling pathway. Similarly, *CXCR4* expression is associated with poor prognosis of patients with AML [30]. CXCR4 plays an important role in the crosstalk between leukemic cells and the bone marrow niche [31,32]. The association between CXCR4 and CD97 is yet to be determined. Yet, knockdown of *CD97* in MV4-11 AML cells impaired cell adhesion on a MSC monolayer [26]. Additionally, the extracellular domain of CD97 binds to integrins α5β1 and αvβ3, stimulating endothelial cell migration and invasion [11]. Furthermore, the interaction of CD97 with Thy-1 plays a role in the regulation of leukocyte trafficking to inflammatory sites [33]. Because CD97 binding partners (integrins and Thy-1) are also present in the MSCs, it is plausible that CD97 upregulation may contribute to the interaction between LSCs and the bone marrow niche.

Conclusions

Patients with high *CD97* expression had shorter overall and disease-free survival. *CD97* was higher in cytogenetically normal patients compared with cytogenetically abnormal patients. Additionally, high *CD97* expression was also associated with *NPM1* mutation. Our findings demonstrate that *CD97* expression contributes to the clinical outcome of patients with AML, particularly older patients. This study provides a rationale for further functional and mechanistic studies aiming to understand the role of *CD97* in AML.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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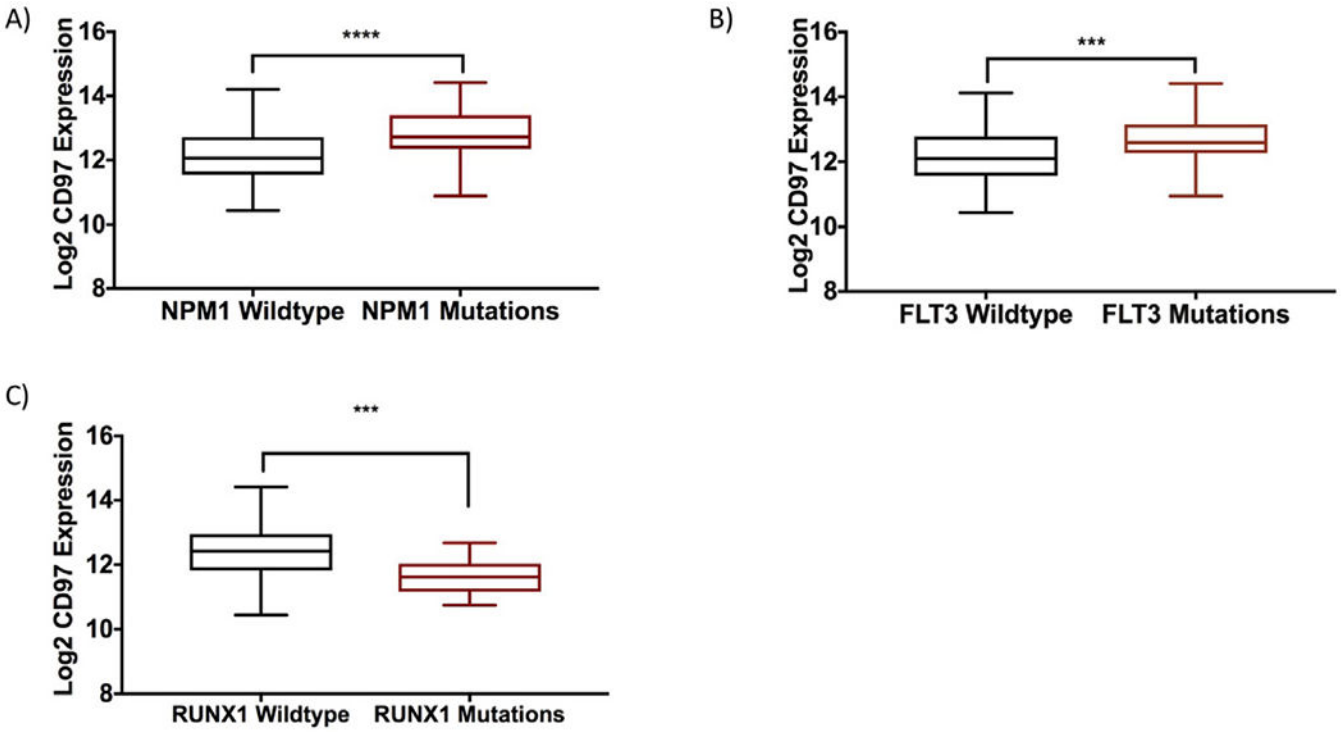

Figure 1.

Association of *CD97* mRNA expression with patient mutational status. Relative *CD97* $\log_2$ mRNA expression in (A) patients with *NPM1* mutations versus wild type; (B) patients with *FLT3* mutations versus wild type; and (C) patients with *RUNX1* mutations versus wild type. ***$p < 0.001$. ****$p < 0.0001$.

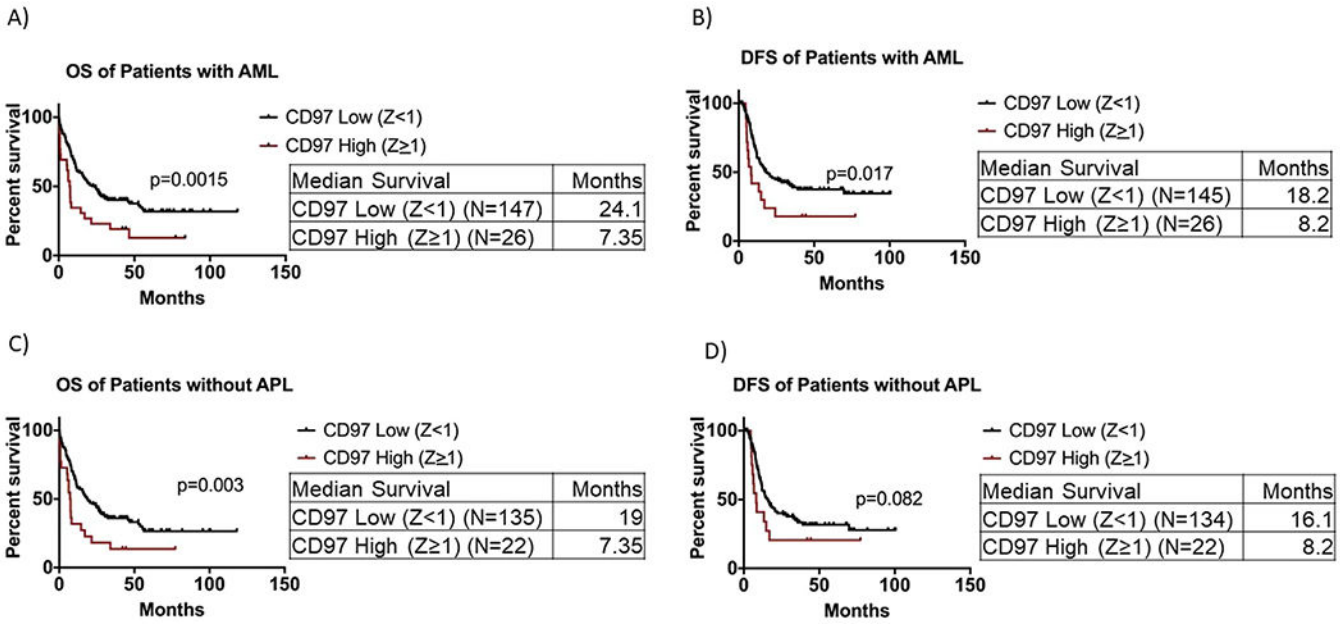

Figure 2.

Survival analysis of AML patients with respect to *CD97* expression. (A) Overall survival of 173 AML patients with *CD97 Z* score ≥1 and *CD97 Z* score <1. (B) Disease-free survival of 171 AML patients with *CD97 Z* score ≥1 and *CD97 Z* score <1. (C) Overall survival of patients without APL with *CD97 Z* score ≥1 and *CD97 Z* score <1. (D) Disease-free survival of patients without APL with *CD97 Z* score ≥1 and *CD97 Z* score <1.

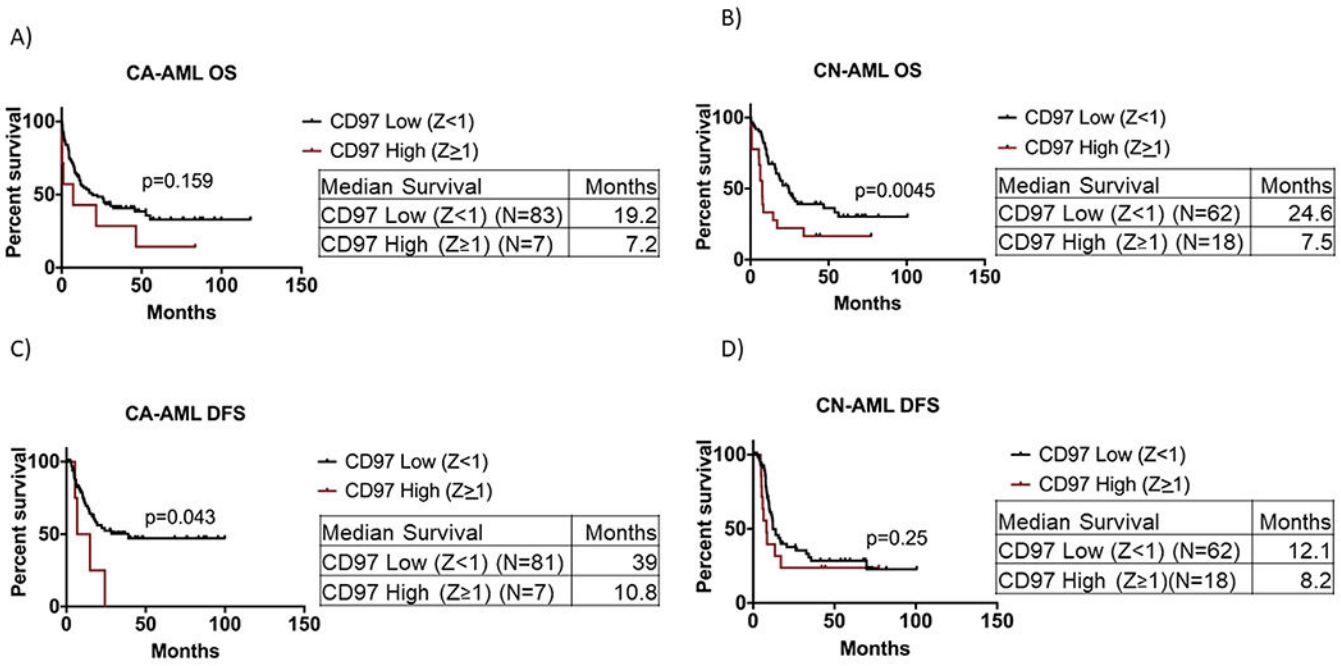

Figure 3.

Survival analysis of AML patients with respect to *CD97* expression after stratification based on cytogenetic status. (A) Overall survival of AML patients with *CD97* high (*Z* score ≥1) versus *CD97* low (*Z* score <1) in cytogenetically abnormal (CA-AML) patients. (B) Overall survival of AML patients with *CD97* high (*Z* score ≥1) versus *CD97* low (*Z* score <1) in cytogenetically normal (CN-AML) patients. (C) Disease-free survival of AML patients with *CD97* high (*Z* score >1) versus *CD97* low (*Z* score <1) in CA-AML patients. (D) Disease-free survival of AML patients with *CD97* high (*Z* score ≥1) versus *CD97* low (*Z* score <1) in CN-AML patients.

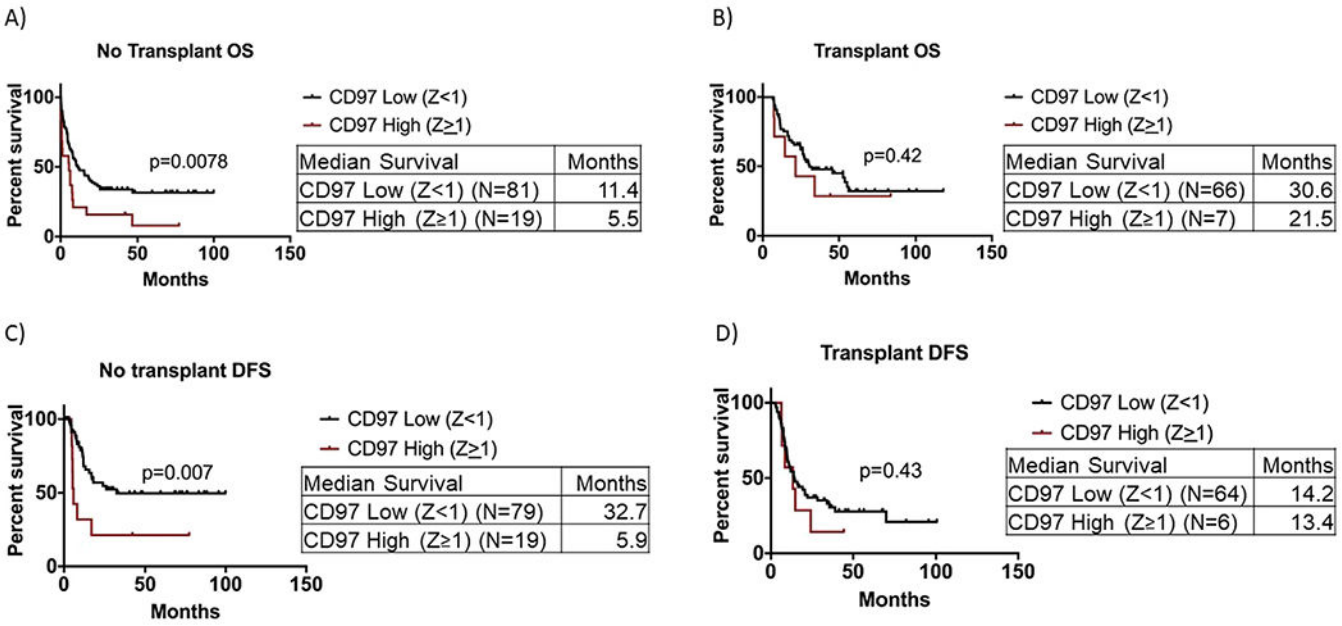

Figure 4.

Survival analysis of AML patients with respect to *CD97* expression after stratification based on patient transplant status. (A) Overall survival of AML patients with *CD97* high (*Z* score ≥1) versus *CD97* low (*Z* score <1) in patients who did not receive a transplant. (B) Overall survival of AML patients with *CD97* high (*Z* score ≥1) versus *CD97* low (*Z* score <1) in patients who received a transplant. (C) Disease-free survival of AML patients with *CD97* high (*Z* score ≥1) versus *CD97* low (Z score <1) in patients who did not receive a transplant. (D) Disease-free survival of AML patients with *CD97* high (*Z* score ≥1) versus *CD97* low (*Z* score <1) in patients who received a transplant.

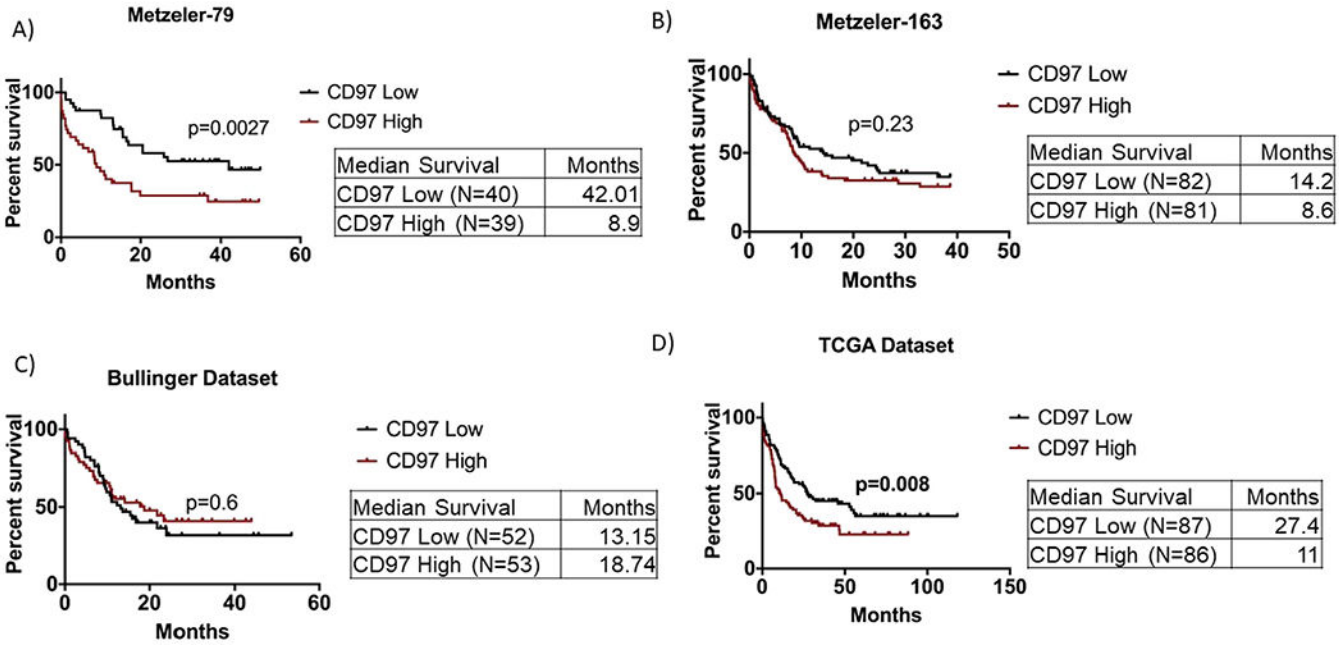

Figure 5.

Survival analysis of AML patients with respect to *CD97* expression in different data sets. Overall survival of patients with AML in four data sets, in which patients were dichotomized based on *CD97* median mRNA expression into *CD97* high and *CD97* low according to the $\log_2$ median-centered expression. (A) Metzeler-79 data set. (B) Metzeler-163 data set. (C) Bullinger data set. (D) TCGA data set.

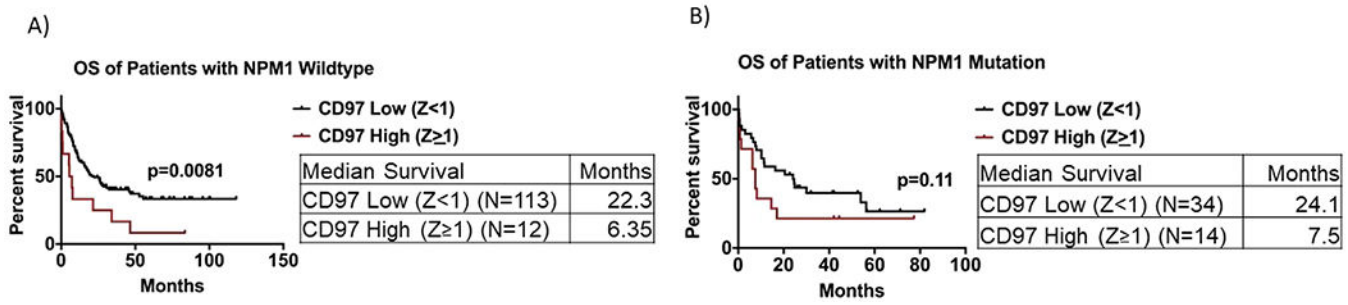

Figure 6.

Survival analysis of AML patients with respect to *CD97* expression after stratification based on *NPM1* mutation status. (A) Overall survival of patients with *CD97* high (*Z* score ≥1) versus *CD97* low (*Z* score <1) among patients with *NPM1* wild-type gene. (B) Overall survival of patients with *CD97* high (*Z* score ≥1) versus *CD97* low (*Z* score <1) among patients with *NPM1* mutated gene.

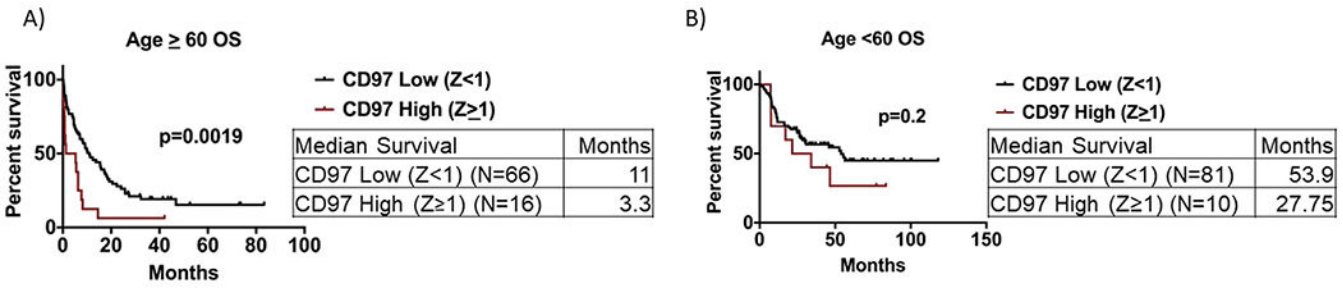

Figure 7.

Survival analysis of AML patients with respect to *CD97* expression after stratification based on age. (A) Overall survival of patients ≥60 years of age with *CD97* high (*Z* score ≥1) versus *CD97* low (*Z* score <1). (B) Overall survival of patients <60 years of age with *CD97* high (*Z* score ≥1) versus *CD97* low (*Z* score <1).

Table 1.

Clinical characteristics of the AML cohort in the TCGA data set with respect to *CD97* expression

	Total	*CD97* low (*Z* <1)	*CD97* high (*Z* ≥1)	*p* Value	
				T test	Fisher exact test
Sex, No. (%)					1
Female	81 (46.8)	69 (85.2)	12 (14.8)		
Male	92 (53.1)	78 (84.8)	14 (15.2)		
Age, y (range)				0.385	
Median	58	57	62.5		
Mean	55.2 ± 1.22	54.7 ± 1.32	57.7 ± 3.38		
WBC count $x10^9$/L				0.004	
Median	17	13.1	56.1		
Mean	36.63 ± 3.50	32.44 ± 3.81	60.30 ± 7.48		
PB blasts, %				0.25	
Median	39	34.5	49		
Mean	39.59 ± 2.44	38.38 ± 2.68	46.2 ± 5.85		
BM blasts, %				0.019	
Median	72	71	83		
Mean	69.08 ± 1.45	67.65 ± 1.59	77.15 ± 3.17		
NCCN subtype, No.					vs. favorable
Favorable	33	29	4		
Intermediate	92	76	16		0.5
Poor	45	40	5		0.6
FAB subtype, No.					vs. M5
M0	16	15	1		0.042
M1	44	39	5		0.028
M2	38	34	4		0.026
M3	16	12	4		0.476
M4	34	29	5		0.081
M5	18	11	7		
M6	2	2			
M7	3	3			

Table 2.

Association of *CD97* expression with patient mutational status in the TCGA data set

	Total	*CD97* low (*Z* <1)	*CD97* high (*Z* ≥1)	*p* Value	
				T test	Fischer exact test
FLT3, No. (%)					0.1005
Present	49 (28.3)	38 (77.5)	11 (22.4)	0.0008	
Absent	124 (71.67)	109(87.9)	15 (12.1)		
IDH1, No. (%)				0.09	0.4718
Mutated	16 (9.2)	15 (93.7)	1 (6.2)		
Wild type	157 (92.3)	132 (84.0)	25 (15.9)		
IDH2, No. (%)				0.77	0.7238
Mutated	17 (9.8)	14 (82.3)	3 (17.6)		
Wild type	156 (90.1)	133 (85.2)	23 (14.7)		
RUNX1, No. (%)				0.0002	0.070
Mutated	17 (9.8)	17 (100)	0 (0)		
Wild type	156 (90.1)	130 (83.3)	26 (16.6)		
TET2, No. (%)				0.10	0.1311
Mutated	15 (8.6)	15 (100)	0 (0)		
Wild type	158 (91.3)	132 (83.5)	26 (16.4)		
NRAS, No. (%)				0.57	1
Mutated	12 (6.9)	10 (83.3)	2 (16.6)		
Wild type	161 (93.0)	137 (85.0)	24 (14.9)		
CEBPA, No. (%)				0.54	0.6944
Mutated	13 (7.5)	12 (92.3)	1 (7.6)		
Wild type	160 (92.5)	135 (84.3)	25 (15.6)		
WT1, No. (%)				0.42	0.3624
Mutated	10 (5.7)	10 (100)	0 (0)		
Wild type	163 (94.2)	137 (84.0)	26 (15.9)		
DNMT3A, No. (%)				0.122	0.1453
Mutated	45 (26.0)	35 (77.7)	10 (22.2)		
Wild type	128 (73.9)	112 (87.5)	16 (12.5)		
NPM1, No. (%)				<0.0001	0.0033
Mutated	48 (27.7)	34 (70.8)	14 (29.1)		
Wild type	125 (72.3)	113 (90.4)	12 (9.6)		
TP53				0.34	
Mutated	14 (8.09)	13 (92.8)	1 (7.1)		0.6970
Wild type	159 (91.9)	134 (84.2)	25 (15.7)		

Table 3.

Cox proportional hazards model for overall survival in patients with AML comparing patients with high ($Z \geq 1$) *CD97* expression and patients with low ($Z < 1$) *CD97* expression.

Variable	Hazard ratio	95% Confidence interval		*p* Value
Age	1.03	1.01	1.04	0.002
Molecular risk				0.002
Intermediate	3.03	1.51	6.07	0.002
Poor	6.57	3.10	13.9	<0.001
Transplant status (Y/N)	0.47	0.29	0.74	0.001
***CD97* (*Z* ≥1)**	1.96	1.19	3.24	0.009