Higher Expression of WTI With Lower CD58 Expression may be Biomarkers for Risk Stratification of Patients With Cytogenetically Normal Acute Myeloid Leukemia

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Abstract

**Background:** Cytogenetics at diagnosis is the most important prognostic factor for adult acute myeloid leukemia (AML), but nearly 50% of AML patients who exhibit cytogenetically normal AML (CN-AML) do not undergo effective risk stratification. Therefore, the development of potential biomarkers to further define risk stratification for CN-AML patients is worth exploring. **Methods:** Transcriptome data from 163 cases in the GSE12417-GPL96 dataset and 104 CN-AML patient cases in the GSE71014-GPL10558 dataset were downloaded from the Gene Expression Omnibus database for overall survival (OS) analysis and validation. **Results:** The combination of Wilms tumor 1 (WT1) and cluster of diffraction 58 (CD58) can predict the prognosis of CN-AML patients. High expression of WTI and low expression of CD58 were associated with poor OS in CN-AML. Notably, when WTI and CD58 were used to concurrently predict OS, CN-AML patients were divided into three groups: low risk, WTI\textsuperscript{low} CD58\textsuperscript{high}; intermediate risk, WTI\textsuperscript{high}CD58\textsuperscript{high} or WTI\textsuperscript{low}CD58\textsuperscript{low}; and high risk, WTI\textsuperscript{high} CD58\textsuperscript{low}. Compared with low-risk patients, intermediate- and high-risk patients had shorter survival time and worse OS. Furthermore, a nomogram model constructed with WTI and CD58 may personalize and reveal the 1-, 2-, 3-, 4-, and 5-year OS rate of CN-AML patients. Both time-dependent receiver operating characteristics and calibration curves suggested that the nomogram model demonstrated good performance. **Conclusion:** Higher expression of WTI with lower CD58 expression may be a potential biomarker for risk stratification of CN-AML patients. Moreover, a nomogram model constructed with WTI and CD58 may personalize and reveal the 1-, 2-, 3-, 4-, and 5-year OS rates of CN-AML patients.

**Keywords**

WT1, CD58, biomarker, risk stratification, CN-AML

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Abbreviations
AML, acute myeloid leukemia; AUC, area under curve; CN-AML, cytogenetically normal AML; CM, costimulatory molecule; GEO, Gene Expression Omnibus; IC, immune checkpoint; OS, overall survival; ROC, receiver operating characteristic; WT1, Wilms tumor 1.

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Introduction
Acute myeloid leukemia (AML) is a genetically heterogeneous disease in which the accumulation of somatic mutations leads to uncontrolled cell proliferation and differentiation. Cytogenetics at diagnosis provides the most important prognostic information for adult AML, but 40% to 50% of AML patients present as cytogenetically normal AML (CN-AML) and do not have biomarkers for effective risk stratification.1–4 Currently, all such CN-AML cases are classified as intermediate risk.5,6 However, this group is quite heterogeneous, and the 4-year rate of overall survival (OS) is only 43%.7,8 With the development of transcriptome sequencing, many biomarkers are emerging that can also be used to further refine the molecular risk definition for CN-AML.

The Wilms tumor 1 (WT1) gene, located on chromosome 11p13, plays an important role in development, tissue homeostasis, and disease.9 Various studies have suggested that high expression of WT1 is significantly associated with the prognosis and relapse of AML patients, which has an important clinical value in guiding AML treatment.10,11 Recently, vaccines targeting WT1 and T-cells engineered to express a receptor specific for WT1 could stimulate specific immune responses and help prevent relapse in AML patients. However, not all patients benefit from the WT1 vaccine where only 64% of patients have an immune response.11,12 It is thought that there are other factors that may influence the effects of WT1 on immunotherapy and lead to a poor prognosis for AML patients. Recent studies have suggested that AML patients with high expression of immune checkpoint (IC) proteins predict poor prognosis.13–15 Moreover, AML patients have an immune response to IC inhibitors.16 In addition, various studies indicated that down-regulation of cluster of differentiation 3ζ, a T-cell costimulatory molecule (CM), is a reason for the decreased level of T-cell activation in leukemia patients. Thus, whether the pattern of higher WT1 and IC genes or lower CMs may contribute to the OS of CN-AML is worth exploring.

In this study, 2 large datasets in the Gene Expression Omnibus (GEO) database were used to explore the combination of WT1 and IC proteins or CMs as a potential biomarker for further refining risk stratification in CN-AML. In addition, we constructed a nomogram model to personalize and visualize the prediction of OS rates for CN-AML patients.

Materials and Methods

CN-AML Patients
The transcriptome data of 163 cases (see Figure 1) in the GSE12417-GPL96 dataset17 and 104 cases of CN-AML patients in the GSE71014-GPL10558 dataset18 were obtained from the GEO database (https://www.ncbi.nlm.nih.gov/geo/),19,20 which were designated as training and validation cohorts, respectively. The corresponding clinical information including age, sample source, survival time, and events were downloaded and listed in Supplemental Table S1. Moreover, the transcriptome data for WT1, IC genes, and CMs were obtained for data mining (Supplemental Table S2). Because the GEO database is publicly available, approval from the local ethics committee was not required.

Nomogram Model
A nomogram model is applied to individualize and visualize the clinical outcome of cancer patients.21–25 The “foreign” and “rms” packages in R software (version 4.0.2, https://www.r-project.org/) were used to construct a nomogram model to visualize the OS rate of CN-AML patients.26 First, each variable in the nomogram was given a weighted point. Then, the total points of all variables for each patient were summed and located on the total point scale. Finally, the OS rate was determined by drawing a vertical line on the total point scale. The time-dependent receiver operating characteristic (ROC) and calibration curves were used to evaluate the prediction performance of the nomogram model, and the judgment criterion included the following: (i) area under curve (AUC) >0.5 and (ii) the OS rate predicted by the nomogram model was significantly close to the actual OS rate.

Workflow of Data Analysis
The transcriptome data of 163 and 104 CN-AML patients in the GSE12417-GPL96 and GSE71014-GPL10558 datasets was downloaded from the GEO database, which was designated as training and validation cohorts, respectively. The expression patterns of WT1, IC, and CM genes were characterized, and the Spearman correlation between WT1 and IC and CM genes was further analyzed. Then, genes with P < .05 of Spearman correlation were selected for Kaplan–Meier curve analysis. Notably, genes with consistent prognostic characteristics in both the training and validation cohorts were used for the construction of risk stratification. Furthermore, univariate and multivariate Cox regression models were used to confirm that the combination of two genes had a better prediction of OS in CN-AML patients than a gene alone. Finally, the identified genes were used to visualize the 1-, 2-, 3-, 4-, and 5-year OS rates of CN-AML patients (Figure 1).
Statistical Analysis

All statistical analysis was conducted by R software (version 4.0.2, https://www.r-project.org/). The optimal cut-off for gene expression was determined by maximally selected rank statistics in the “maxstat” package. The “survival” package was used to plot the Kaplan–Meier curves, and comparison between groups was performed by the log-rank test. The AUC in the time-dependent ROC curve was obtained by the “survival ROC” package. The correlation coefficient between the two genes was determined by Spearman’s method. A two-tailed P value <.05 was considered statistically significant.

Results

Correlation Analysis of WT1 and IC or CM Gene Expression in CN-AML

The relationship between WT1 and IC or CM gene expression was evaluated based on the molecular profiles of the patients both in the training and validation datasets. Interestingly, the expression pattern of WT1, 5 IC genes programmed cell death 1, programmed cell death 1 ligand 2 (PD-L2), lymphocyte activating 3, indoleamine 2,3-dioxygenase 1 (IDO1), and human endogenous retrovirus-subfamily H long-terminal repeat-associating protein 2, and 12 CMs in both the training and validation cohorts were examined (Figure 2a and b). Spearman correlation analysis indicated that WT1 was negatively correlated with cluster of differentiation 86 (CD86), cluster of differentiation 58 (CD58), cluster of differentiation 40 (CD40), PD-L2, integrin subunit alpha L (ITGAL), CD40 ligand (CD40LG), intercellular adhesion molecule 1 (ICAM1), cluster of differentiation 2 (CD2), and Fas ligand (FASLG) in the training cohort, while WT1 had a negative correlation with ITGAL, CD58, CD86, cluster of differentiation 28 (CD28), ICAM1, IDO1, CD2, and inducible T-cell costimulator (ICOS) in the validation cohort (Correlation coefficient R < 0, P < .05, Figure 2c and d). However, only 5 CMs, including ITGAL, ICAM1, CD86, CD58, and CD2, were negatively correlated with WT1 in both the training and validation cohorts (R < 0, P < .05, Figure 2c and d).

OS Analysis of WT1, CMs, and ICs in CN-AML

As shown in Figure 3a, compared with the low expression of the WT1 group, high expression of WT1 was associated with poor OS for CN-AML patients in the training cohort (3-year OS rate: 19% vs 38%, P = .007). A similar result could be found in the validation cohort (3-year OS rate: 46% vs 68%, P = .026) (Figure 3b). Interestingly, CN-AML patients with high CD58 expression had favorable OS compared to those with low CD58 expression in the training cohort (3-year OS: 41% vs 22%, P = .012) (Figure 3c). This result could be confirmed in the validation cohort (3-year OS: 70% vs 43%, P = .018) (Figure 3d). However, the expression levels of CD2, CD86, ICAM1, and ITGAL had no significant correlation with OS in CN-AML (Supplemental Figure S1).

Higher WT1 Expression Concurrent with Lower CD58 Expression May Predict Poor OS for CN-AML Patients

To better understand the combination of WT1 and CD58 in predicting the OS of CN-AML patients, Spearman correlation...
Figure 2. Correlation of WT1 and IC genes or CMs in CN-AML. Expression levels of WT1 and IC genes and CMs in the training (a) and validation (b) cohorts. Genes in brown and green font represent IC genes and CMs, respectively. The gold color represents higher expression, and the blue color represents lower expression. WT1 was negatively correlated with IC genes and CMs in the training (c) and validation (d) cohorts. The selection criteria were $P < .05$. The red represents that WT1 correlated with IC and CM genes in both the training and validation cohorts. Abbreviations: WT1, Wilms tumor 1; IC, immune checkpoint; CM, costimulatory molecule; CN-AML, cytogenetically normal acute myeloid leukemia.

Figure 3. OS analysis of WT1 and CD58 in patients with CN-AML in the training (a, c) and validating (b, d) cohorts based on optimal cut-points. Optimal cut-points were obtained from the survminer package in R (version 4.0.2; https://www.r-project.org/) (left panel). The Kaplan–Meier curves were drawn by the survival package in R (version 4.0.2, https://www.r-project.org/) (right panel). Abbreviations: OS, overall survival; WT1, Wilms tumor 1; CN-AML, cytogenetically normal acute myeloid leukemia.
analysis was first conducted. As shown in Figure 4a, in the training cohort, *WT1* was negatively correlated with *CD58* ($R = -0.34$, $P < .001$). This result was confirmed in the validation cohort (*WT1*/*CD58*, $R = -0.43$, $P < .001$) (Figure 4c). When the combination of *WT1* and *CD58* was used to predict OS, CN-AML patients were divided into the following 3 groups: low risk, *WT1*low*CD58*high; intermediate risk, *WT1*high*CD58*high or *WT1*low*CD58*low; and high risk, *WT1*high*CD58*low. Compared with low-, intermediate-, and high-risk AML patients had a shorter survival time and worse OS in the training cohort (3-year OS rate, high vs intermediate vs low: 17% vs 26% vs 44%, $P < .001$) (Figure 4b). The results could be confirmed in the validation cohort (3-year OS rate, high vs intermediate vs low: 40% vs 50% vs 73%, $P = .004$) (Figure 4d).

To evaluate whether the combination of *WT1* and *CD58* is better than *WT1* or *CD58* alone in predicting OS in CN-AML patients, univariate and multivariate Cox regression analysis was performed. The results of univariate Cox regression analysis suggested that although high expression of *CD58* was associated with favorable OS in the training cohort (hazard ratio [HR] = 0.69, 95% confidence interval [CI]: 0.47-0.99, $P = .043$), this finding could not be confirmed in the validation cohort (HR = 0.69, 95% CI: 0.37-1.27, $P = .229$). What is more, there was no significant correlation between *WT1* and OS in both training and validation cohorts ($P > .05$). Interestingly, co-occurrence of high *WT1* expression and low *CD58* expression could predict unfavorable OS in CN-AML patients by univariate and multivariate Cox regression analysis (HR = 2.59, 95% CI: 1.25-5.38, $P = .010$). This result was confirmed in the validation cohort (HR = 3.01, 95% CI: 1.37-6.60, $P = .006$) (Table 1). These findings suggest that *WT1* and *CD58* may be biomarkers for risk stratification of CN-AML patients.
Construction of a Nomogram Model for CN-AML Patients

Based on the above findings, WT1 and CD58 were used to build a nomogram model to personalize and display the 1-, 2-, 3-, 4-, and 5-year OS rate of the 267 CN-AML patients included in the training and validation cohorts (Figure 5a). According to the nomogram model, high expression of WT1 and low expression of CD58 were assigned points of 73 and 100, respectively, while low expression of WT1 and high expression of CD58 were assigned 0 points. The detailed total points corresponding to the OS rates are shown in Supplemental Table S3. Next, the performance of the nomogram model was evaluated. The 1-, 2-, 3-, 4-, and 5-year AUC in the time-dependent ROC curve were all >0.60 (Figure 5b). Moreover, the calibration curves suggested that the 1-, 2-, 3-, 4-, and 5-year OS rates predicted by the nomogram model were significantly close to the actual OS rates (Figure 5c). Therefore, the results of the ROC and calibration curves demonstrated that the nomogram model constructed with WT1 and CD58 had better performance in predicting the OS rates of CN-AML patients.

Discussion

In this study, a total of 267 CN-AML patients from 2 datasets in the GEO database were used for OS analysis and validation. We

Table 1. Univariate and multivariate Cox regression analyses in CN-AML patients.

| Variables | Training cohort | Validation cohort | Training cohort | Validation cohort |
|-----------|-----------------|-------------------|-----------------|-------------------|
|           | Univariate Cox regression | Multivariate Cox regression | Univariate Cox regression | Multivariate Cox regression |
|           | HR (95% CI) | P value | HR (95% CI) | P value | HR (95% CI) | P value | HR (95% CI) | P value |
| WT1       | 1.22 (0.90-1.64) | .201 | 1.60 (0.79-3.25) | .195 | 1.14 (0.65-2.01) | .645 |
| CD58      | 0.69 (0.47-0.99) | .043 | 0.69 (0.37-1.27) | .229 | reference | reference |
| WT1/CD58  | Group I reference | reference | Group II 1.61 (1.05-2.49) | .031 | 1.67 (0.75-3.72) | .214 | 1.77 (0.99-3.16) | .054 | 1.67 (0.75-3.72) | .214 |
|           | Group III 2.31 (1.36-3.91) | .002 | 3.01 (1.37-6.60) | .006 | 2.59 (1.25-5.38) | .010 | 3.01 (1.37-6.60) | .006 |

Abbreviations: CN-AML, cytogenetically normal acute myeloid leukemia; CI: confidence interval; WT1, Wilms tumor 1; HR: hazard ratio; Group I: WT1lowCD58high; Group II: WT1lowCD5low or WT1highCD5high; Group III: WT1highCD5low. The bold values indicate that P values < .05 are statistically significant.

Figure 5. Nomogram model personalizing and displaying the OS of CN-AML patients. (a) The nomogram model is used to predict the 1-, 2-, 3-, 4-, and 5-year OS rate of CN-AML patients. (b) A time-dependent ROC curve was used to evaluate the performance of the nomogram model. (c) Calibration curves were used to describe the consistency between the OS rate predicted by the nomogram and the actual OS rate. The gray line shows the ideal OS rate predicted by the nomogram.

Abbreviations: OS, overall survival; CN-AML, cytogenetically normal acute myeloid leukemia; ROC, receiver operating characteristic; AUC, area under curve.
found that higher WT1 concurrent with lower CD58 expression was significantly associated with poor OS. Interestingly, the combination of WT1 and CD58 may further define risk stratification for CN-AML patients. Moreover, we developed a prediction nomogram of the OS rate for individual CN-AML patients.

High expression of WT1 predicts poor prognosis and relapse in AML patients.\textsuperscript{10,11} Furthermore, for CN-AML patients undergoing hematopoietic stem cell transplantation (HSCT), high expression of WT1 before HSCT is associated with a higher relapse rate and poor OS.\textsuperscript{27} However, more accurate risk stratification cannot be made for all CN-AML patients based on the expression of WT1 alone, and the development of targeted therapies for WT1 cannot benefit all patients.\textsuperscript{11,12,27} Although the survival curves suggested that the high expression of WT1 was associated with poor OS in CN-AML patients, there was no significant correlation between WT1 and OS in univariate Cox analysis, which was consistent with the publication of the validation dataset.\textsuperscript{18} Therefore, it is important to combine WT1 and other genes for the risk stratification of CN-AML patients.

CMs can enhance the immune response of T-cells to tumor cells, and down-regulating these CMs will lead to immune escape for tumor cells.\textsuperscript{28} In several clinical trials, CM agonists have been used as adjuvants for immunotherapy to enhance the antitumor effects of solid tumors and hematological malignancies, and many patients have benefited from them.\textsuperscript{29,30} These findings suggest that CMs may be potential biomarkers for risk stratification and a promising immunotherapy strategy for patients with hematological tumors. One study has found that CD58/CD2 is the main costimulatory pathway that can activate CD28–CD8 + T-cells to exert antitumor effects.\textsuperscript{31} In addition, another study indicated that high expression of CD58 predicts favorable clinical outcomes in acute lymphoblastic leukemia.\textsuperscript{129,132} These findings are in line with the results of our study. Furthermore, it is worth noting that WT1 had a negative relationship with CD58, suggesting that these genes may potentially serve as a biomarker for risk stratification of CN-AML patients. Interestingly, when WT1 and CD58 were combined, it was found to predict OS in CN-AML patients. Moreover, the combination of WT1 and CD58 was better than WT1 or CD58 alone in predicting OS in CN-AML patients.

A reliable estimation of the OS rate and risk stratification for patients is important for guiding doctors in choosing a therapeutic strategy. Because nearly half of AML patients have a normal karyotype, which is called CN-AML and classified as an intermediate risk group,\textsuperscript{1} it is difficult for clinicians to choose treatment. Thus, it is important to further define risk stratification for these patients. In this study, we show that WT1 and CD58 may be potential biomarkers for further defining CN-AML patients as low-, intermediate-, and high-risk. Moreover, nomogram models constructed with WT1 and CD58 may personalize and display the 1-, 2-, 3-, 4-, and 5-year OS rates for CN-AML patients. Notably, both time-dependent ROC and calibration curves indicated that the nomogram model had good performance in predicting prognosis. However, the limitations of this study include the following: (1) we do not have quantitative real-time polymerase chain reaction and immunohistochemical data for WT1 and CD58 from the clinical center to further validate our findings and (2) construction of the nomogram model was only based on the transcriptome sequencing data in the GEO database. Although the nomogram was internally validated by the calibration and time-dependent ROC curves, further study in a clinical center is needed to externally validate the proposed nomogram.

**Conclusion**

We demonstrate that higher WT1 concurrent with lower CD58 expression may predict poor OS for CN-AML patients. Importantly, WT1 and CD58 may be potential biomarkers for the risk stratification of CN-AML patients and the construction of a nomogram model that personally and visualy predicts the OS rate of each CN-AML patient.

**Authors’ Note**

The transcriptome data in the GSE12417-GPL96 and GSE71014-GPL10558 datasets were downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**Declaration of Conflicting Interests**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Supplemental Material**

Supplemental material for this article is available online.

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