Technology in Cancer Research & Treatment
Volume 20: 1-8

DOI: 10.1177/15330338211052152
journals.sagepub.com/home/tct
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Higher Expression of *WT1* 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 *WT1* and low expression of *CD58* were associated with poor OS in CN-AML. Notably, when *WT1* and *CD58* were used to concurrently predict OS, CN-AML patients were divided into three 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-risk patients, intermediate- and high-risk patients had shorter survival time and worse OS. Furthermore, a nomogram model constructed with *WT1* 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 *WT1* with lower *CD58* expression may be a potential biomarker for risk stratification of CN-AML patients. Moreover, a nomogram model constructed with *WT1* 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.

Received: January 17, 2021; Revised: August 13, 2021; Accepted: September 22, 2021.

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%.[1,7,8] 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 diffraction 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 dataset[17] and 104 cases of CN-AML patients in the GSE71014-GPL10558 dataset[18] 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).

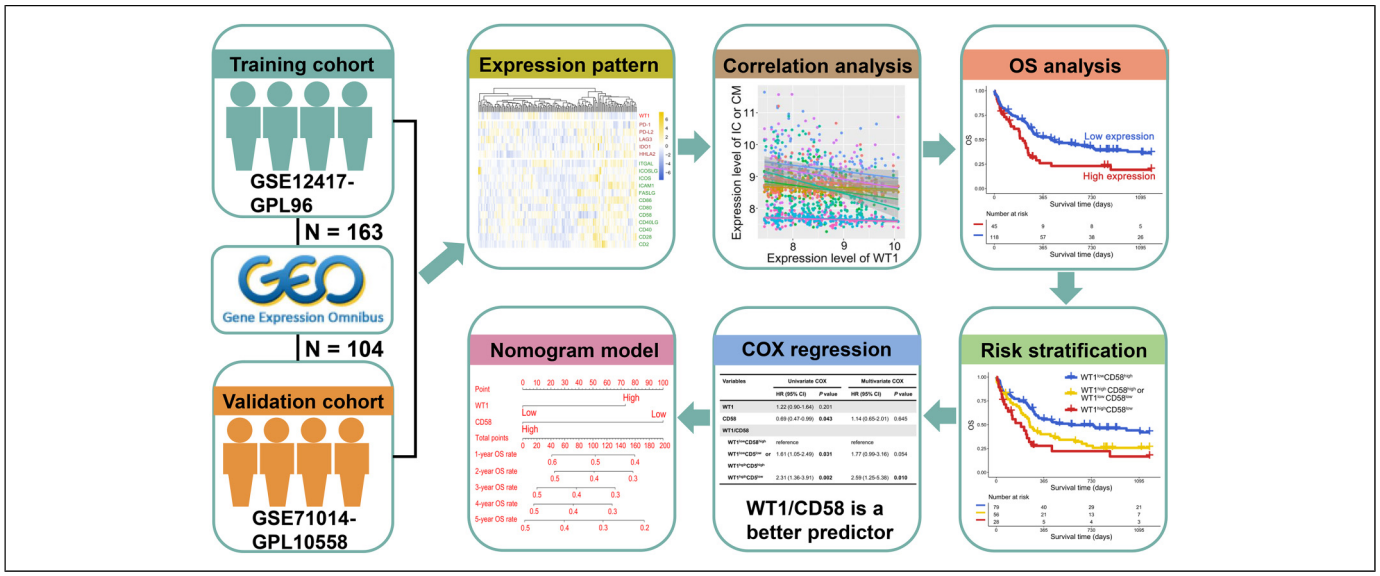

Figure 1. Study workflow. The transcriptome data of 163 and 104 patients with CN-AML in the GSE12417-GPL96 and GSE71014-GPL10558 datasets were designated as training and validation cohorts, respectively. After characterized the expression patterns of *WT1* and IC or CM genes, genes with $P < .05$ of Spearman correlation were selected for OS analysis. Then, *WT1* and IC gene or CMs with a log-rank test $P < .05$ in Kaplan–Meier curves in both training and validation cohorts were selected for risk stratification, univariate and multivariate Cox regression analysis, and construction of a nomogram model.

Abbreviations: CN-AML, cytogenetically normal acute myeloid leukemia; IC, immune checkpoint; CM, costimulatory molecule; OS, overall survival; WT1, Wilms tumor 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 diffraction 86 (*CD86*), cluster of diffraction 58 (*CD58*), cluster of diffraction 40 (*CD40*), *PD-L2*, integrin subunit alpha L (*ITGAL*), CD40 ligand (*CD40LG*), intercellular adhesion molecule 1 (*ICAM1*), cluster of diffraction 2 (*CD2*), and Fas ligand (*FASLG*) in the training cohort, while *WT1* had a negative correlation with *ITGAL*, *CD58*, *CD86*, cluster of diffraction 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

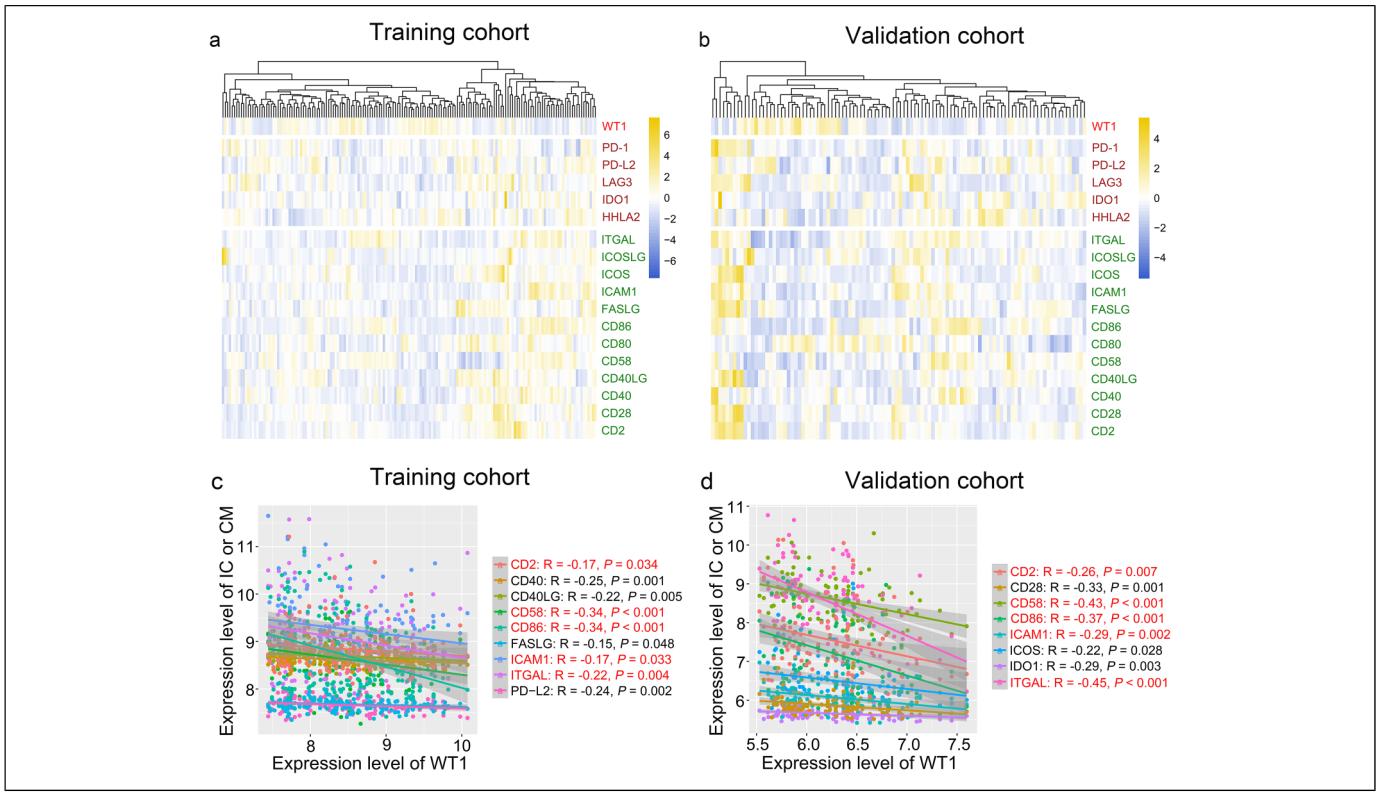

Figure 2. Correlation of *WT1* and IC genes or CMs in CN-AML. Expression levels of *WT1* and IC genes and CMs in the training (a) and validation (b) cohorts. Genes in brown and green font represent IC genes and CMs, respectively. The gold color represents higher expression, and the blue color represents 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 CMs 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.

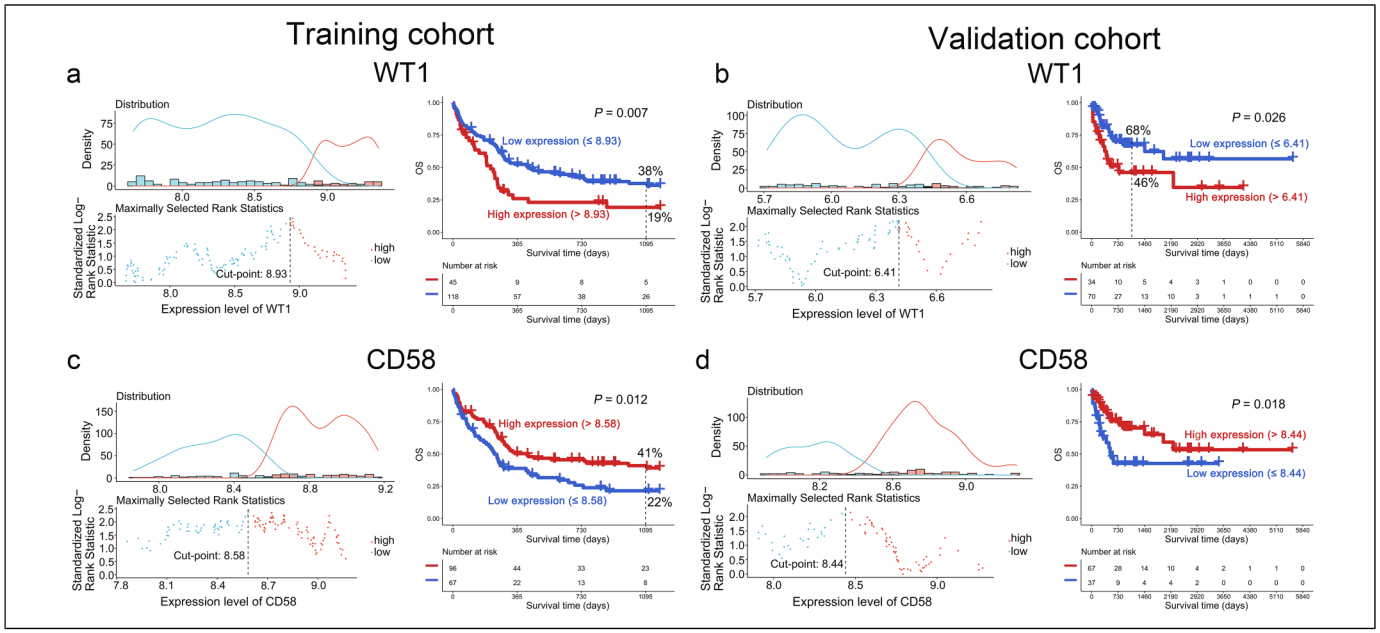

Figure 3. OS analysis of *WT1* and *CD58* in patients with CN-AML in the training (a, c) and validating (b, d) cohorts based on optimal cut-points. Optimal cut-points were obtained from the survminer package in R (version 4.0.2; https://www.r-project.org/) (left panel). The Kaplan–Meier curves were drawn 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.

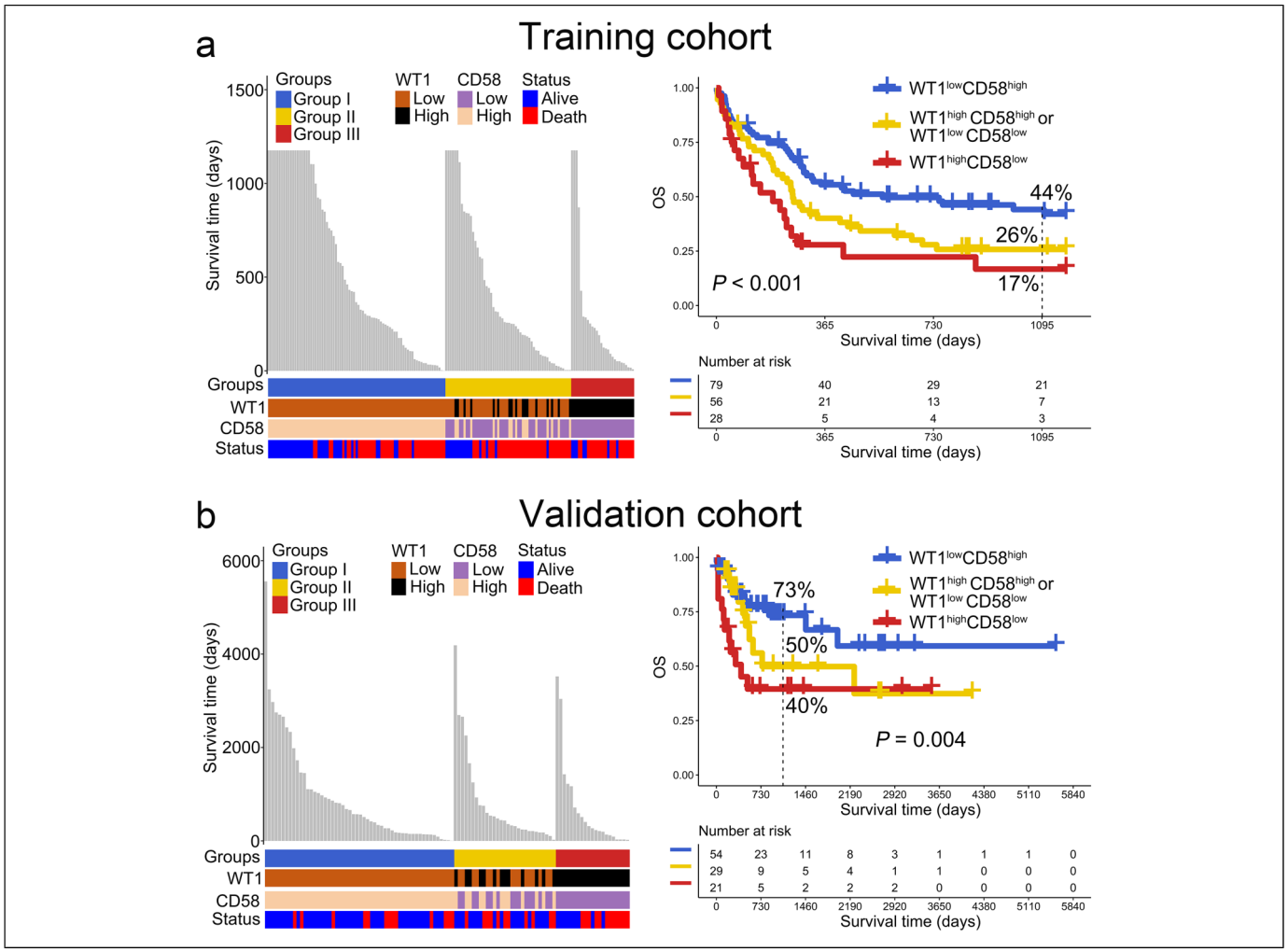

Figure 4. Higher *WT1* concurrent with lower *CD58* expression predicted poor OS in patients with CN-AML. Higher *WT1* concurrent with lower *CD58* expression was associated with poor OS in both the training (a) and validation (b) cohorts. Distribution of survival time (left panel) and survival curves (right panel) based on the expression levels of *WT1* and *CD58*. Group I: $WT1^{low}CD58^{high}$; Group II: $WT1^{low}CD5^{low}$ or $WT1^{hgih}CD5^{high}$; Group III: $WT1^{hgih}CD5^{low}$.

Abbreviations: WT1, Wilms tumor 1; OS, overall survival; 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* ($R = -.34$, $P < .001$). This result was confirmed in the validation cohort (*WT1*/*CD58*, $R = -.43$, $P < .001$) (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 in the training cohort 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.

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

	Univariate Cox regression				Multivariate Cox regression			
	Training cohort		Validation cohort		Training cohort		Validation cohort	
Variables	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				
CD58	0.69 (0.47-0.99)	**.043**	0.69 (0.37-1.27)	.229	1.14 (0.65-2.01)	.645		
WT1/CD58								
Group I	reference		reference		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: $WT1^{low}CD58^{high}$; Group II: $WT1^{low}CD5^{low}$ or $WT1^{hgih}CD5^{high}$; Group III: $WT1^{hgih}CD5^{low}$. The bold values indicate that *P* values < .05 are statistically significant.

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

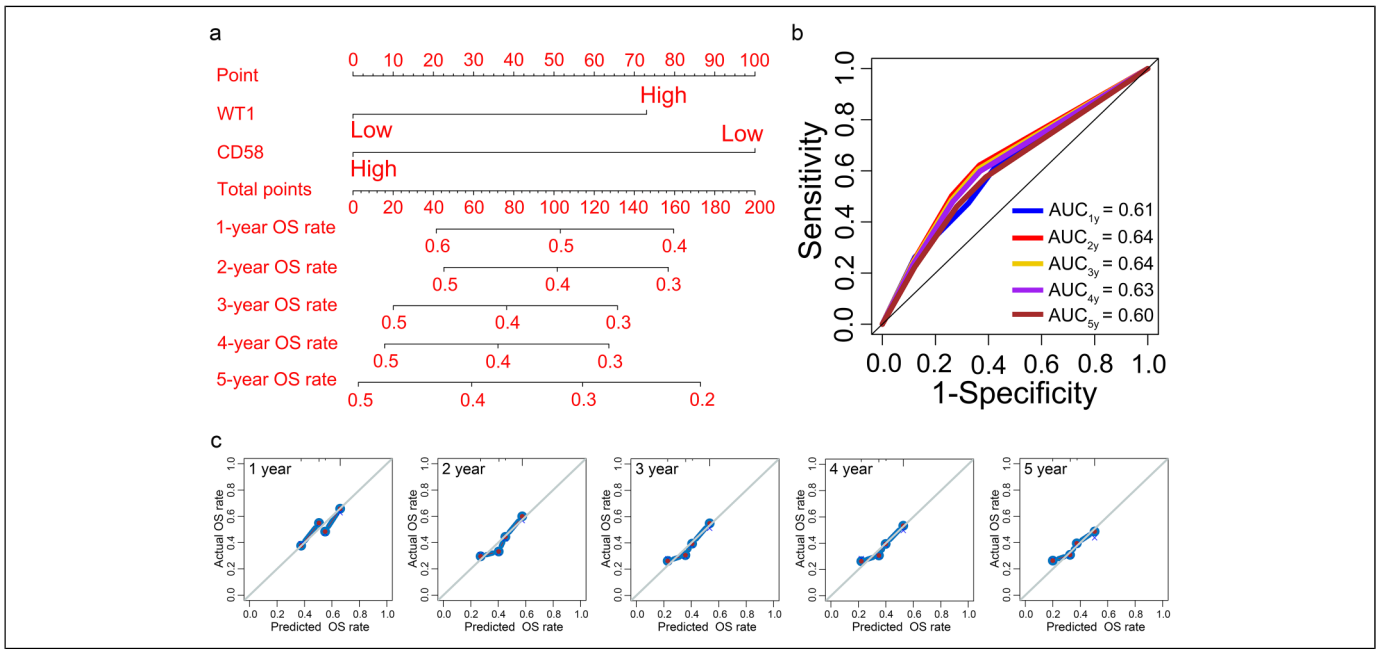

Figure 5. Nomogram model personalizing and displaying the OS of CN-AML patients. (a) The nomogram model is used to predict the 1-, 2-, 3-, 4-, and 5-year OS rate of CN-AML patients. (b) A time-dependent ROC curve was used to evaluate the performance of the nomogram model. (c) Calibration curves were 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.[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.[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.[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.[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.[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.[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.[31] In addition, another study indicated that high expression of *CD58* predicts favorable clinical outcomes in acute lymphoblastic leukemia.[32] 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,[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 visually 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.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by grants from the National Natural Science Foundation of China (No. 82070152), the Guangzhou Science and Technology Project (Nos. 201807010004 and 201803040017), and the Science and Technology Development Fund, Macau SAR (File nos. FDCT/0015/2018/A1 and FDCT/0043/2021/A1).

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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References

1. Schlenk R, Döhner K, Krauter J, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. *N Engl J Med*. 2008;358(18):1909-1918. doi: 10.1056/NEJMoa074306
2. Mrózek K, Heerema NA, Bloomfield CD. Cytogenetics in acute leukemia. *Blood Rev*. 2004;18(2):115-136. doi: 10.1016/s0268-960x(03)00040-7
3. Byrd JC, Mrózek K, Dodge RK, et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from cancer and leukemia

group B (CALGB 8461). *Blood*. 2002;100(13):4325-4336. doi: 10.1182/blood-2002-03-0772
4. Chen C, Chio CL, Zeng H, et al. High expression of CD56 may be associated with favorable overall survival in intermediate-risk acute myeloid leukemia. *Hematology*. 2021;26(1):210-214. doi: 10.1080/16078454.2021.1880734
5. Becker H, Marcucci G, Maharry K, et al. Favorable prognostic impact of NPM1 mutations in older patients with cytogenetically normal de novo acute myeloid leukemia and associated gene- and microRNA-expression signatures: a cancer and leukemia group B study. *J Clin Oncol*. 2010;28(4):596-604. doi: 10.1200/jco.2009.25.1496
6. Pastore F, Kling D, Hoster E, et al. Long-term follow-up of cytogenetically normal CEBPA-mutated AML. *J Hematol Oncol*. 2014;7(1):1–8. doi:10.1186/s13045-014-0055-7
7. Therneau T. A package for survival analysis in R. *R package version 32-7*, 2020. https://CRANR-projectorg/package=survival
8. Löwenberg B, Griffin JD, Tallman MS. Acute myeloid leukemia and acute promyelocytic leukemia [J]. *Hematology Am Soc Hematol Educ Program*. 2003;2003(1):82-101.
9. Hastie ND. Wilms' tumour 1 (WT1) in development, homeostasis and disease. *Development*. 2017;144(16):2862-2872. doi: 10.1242/dev.153163
10. Yoon JH, Kim HJ, Kwak DH, et al. High WT1 expression is an early predictor for relapse in patients with acute promyelocytic leukemia in first remission with negative PML-RARa after anthracycline-based chemotherapy: a single-center cohort study. *J Hematol Oncol*. 2017;10(1):30. doi: 10.1186/s13045-017-0404-4
11. Maslak PG, Dao T, Bernal Y, et al. Phase 2 trial of a multivalent WT1 peptide vaccine (galinpepimut-S) in acute myeloid leukemia. *Blood Adv*. 2018;2(3):224-234. doi: 10.1182/bloodadvances.2017014175
12. TCR Gene therapy improves AML prognosis. *Cancer Discov.* 2019;9(9):1148-1149. doi: 10.1158/2159-8290.Cd-nb2019-083
13. Chen CC, Liang CF, Wang SQ, et al. Expression patterns of immune checkpoints in acute myeloid leukemia. *J Hematol Oncol*. 2020;13(1):28. doi: 10.1186/s13045-020-00853-x
14. Huang JY, Tan JX, Chen YC, et al. A skewed distribution and increased PD-1 + Vβ + CD4 + /CD8 + T cells in patients with acute myeloid leukemia. *J Leukoc Biol*. 2019;106(3):725-732. doi: 10.1002/JLB.MA0119-021R
15. Chen C, Xu L, Gao R, et al. Transcriptome-based co-expression of BRD4 and PD-1/PD-L1 predicts poor overall survival in patients with acute myeloid leukemia. *Front Pharmacol*. 2020;11:582955. doi: 10.3389/fphar.2020.582955
16. Stahl M, Goldberg AD. Immune checkpoint inhibitors in acute myeloid leukemia: novel combinations and therapeutic targets. *Curr Oncol Rep*. 2019;21(4):37. doi: 10.1007/s11912-019-0781-7
17. Metzeler K, Hummel M, Bloomfield C, et al. An 86-probe-set gene-expression signature predicts survival in cytogenetically normal acute myeloid leukemia. *Blood*. 2008;112(10):4193-4201. doi: 10.1182/blood-2008-02-134411
18. Chuang M, Chiu Y, Chou W, et al. An mRNA expression signature for prognostication in de novo acute myeloid leukemia patients with normal karyotype. *Oncotarget*. 2015;6(36):39098-39110. doi: 10.18632/oncotarget.5390
19. Zhang SW, Zhang N, Wang N. Role of COL3A1 and POSTN on pathologic stages of esophageal cancer. *Technol Cancer Res Treat*. 2020;19:1533033820977489. doi: 10.1177/1533033820977489
20. Liu AG, Pang YY, Chen G, et al. Downregulation of miR-199a-3p in hepatocellular carcinoma and Its relevant molecular mechanism via GEO, TCGA database and in silico analyses. *Technol Cancer Res Treat*. 2020:19:1533033820979670. doi: 10.1177/1533033820979670
21. Wang PP, Liu SH, Chen CT, et al. Circulating tumor cells as a new predictive and prognostic factor in patients with small cell lung cancer. *J Cancer*. 2020;11(8):2113-2122. doi: 10.7150/jca.35308
22. Liao PY, Dong ZY, Huang CT, et al. Development and validation of a prognostic nomogram based on residual tumor in patients with non-disseminated nasopharyngeal carcinoma. *Technol Cancer Res Treat*. 2020;19:1533033820957035. doi: 10.1177/1533033820957035
23. Huang Z, Xing S, Zhu Y, et al. Establishment and validation of nomogram model integrated with inflammation-based factors for the prognosis of advanced non-small cell lung cancer. *Technol Cancer Res Treat*. 2020;19:1533033820971605. doi: 10.1177/1533033820971605
24. Wang PP, Fu Y, Chen YY, et al. Nomogram personalizes and visualizes the overall survival of patients with triple-negative breast cancer based on the immune genome. *Biomed Res Int*. 2020;2020:4029062. doi: 10.1155/2020/4029062
25. Chen C, Liu S, Jiang X, et al. Tumor mutation burden estimated by a 69-gene-panel is associated with overall survival in patients with diffuse large B-cell lymphoma. *Exp Hematol Oncol*. 2021;10(1):20. doi: 10.1186/s40164-021-00215-4
26. Wang P, Chen Y, Long Q. Increased coexpression of PD-L1 and TIM3/TIGIT is associated with poor overall survival of patients with esophageal squamous cell carcinoma. *J Immunother Cancer*. 2021;9(10):e002836. doi:10.1136/jitc-2021-002836
27. Yoon JH, Kim HJ, Jeon YW, et al. Outcome of allogeneic hematopoietic stem cell transplantation for cytogenetically normal AML and identification of high-risk subgroup using WT1 expression in association with NPM1 and FLT3-ITD mutations. *Genes Chromosomes Cancer*. 2015;54(8):489-499. doi: 10.1002/gcc.22260
28. Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell*. 2015;161(2):205-214. doi: 10.1016/j.cell.2015.03.030
29. Marin-Acevedo JA, Dholaria B, Soyano AE, et al. Next generation of immune checkpoint therapy in cancer: new developments and challenges. *J Hematol Oncol*. 2018;11(1):39. doi: 10.1186/s13045-018-0582-8
30. Omid H, John A. T, Adi D, et al. First in human (FIH) study of an OX40 agonist monoclonal antibody (mAb) PF-04518600 (PF-8600) in adult patients (pts) with select advanced solid tumors: preliminary safety and pharmacokinetic (PK)/pharmacodynamic results. *J Clin Oncol*. 2016;34(15_suppl):3079. doi: 10.1200/JCO.2016.34.15_suppl.3079
31. Leitner J, Herndler-Brandstetter D, Zlabinger GJ, et al. CD58/CD2 is the primary costimulatory pathway in human CD28–CD8 + T cells. *J Immunol.* 2015;195(2):477-487. doi: 10.4049/jimmunol.1401917
32. Archimbaud E, Thomas X, Campos L, et al. Expression of surface adhesion molecules CD54 (ICAM-1) and CD58 (LFA-3) in adult acute leukemia: relationship with initial characteristics and prognosis. *Leukemia*. 1992;6(4):265-271.