

Identification of *STAB1* in Multiple Datasets as a Prognostic Factor for Cytogenetically Normal AML: Mechanism and Drug Indications

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Cytogenetically normal acute myeloid leukemia (CN-AML) presents with diverse outcomes in different patients and is categorized as an intermediate prognosis group. It is important to identify prognostic factors for CN-AML risk stratification. In this study, using the TCGA CN-AML dataset, we found that the scavenger receptor stabilin-1 (*STAB1*) is a prognostic factor for poor outcomes and validated it in three other independent CN-AML datasets. The high *STAB1* expression (*STAB1*^{high}) group had shorter event-free survival compared with the low *STAB1* expression (*STAB1*^{low}) group in both the TCGA dataset (n = 79; p = 0.0478) and GEO: GSE6891 dataset (n = 187; p = 0.0354). Differential expression analysis between the *STAB1*^{high} and *STAB1*^{low} groups revealed that upregulated genes in the *STAB1*^{high} group were enriched in pathways related to cell adhesion and migration and immune responses. We confirmed that *STAB1* suppression inhibits cell growth in KG1a and NB4 leukemia cells. Expression correlation analyses between *STAB1* and cancer drug targets suggested that patients in the *STAB1*^{low} group are more sensitive to the BCL2 inhibitor venetoclax, and we confirmed it in cell lines. In conclusion, we identified *STAB1* as a prognostic factor for CN-AML in multiple datasets, explored its underlying mechanism, and provided potential therapeutic indications.

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous disease characterized by the expansion of undifferentiated myeloid precursors, resulting in impaired hematopoiesis.¹ Recurrent cytogenetic abnormalities are well-established markers for the diagnosis and prognosis of AML.² Cytogenetically normal AML (CN-AML) accounts for approximately 50% of all AML cases. Currently, CN-AML is considered an intermediate risk disease because some patients respond well to normal treatment and some others do not.³ Therefore, identifying effective prognostic factors for CN-AML is important for providing optimal care to patients.

Recently, various DNA and RNA markers have been proposed as prognostic factors for CN-AML. Mutations in *NPM1* and *CEBPA* have been widely used as prognostic factors for good outcomes in patients

with CN-AML;⁴ in contrast, patients with mutations in *FLT3*, *RUNX1*, *ASXL1*, and *TP53* have been associated with adverse outcomes. Furthermore, increased expression of *WT1*,⁵ *BAALC*,⁶ and *ERG*⁷ is reportedly associated with outcomes in patients with CN-AML. Based on gene expression, multiple prognostic factors have been suggested to define CN-AML subgroups. Early in 2008, Metzeler et al.⁸ developed an 86-probe-set gene expression signature for predicting overall survival (OS) in patients with CN-AML. A stem-cell-associated gene expression signature involving 44 genes was proposed as an indicator of negative prognostic outcomes in patients with primary CN-AML.⁹ More recently, an integrative prognostic risk score based on molecular markers for gene expression (*BAALC*, *ERG*, *MNI*, and *WT1*) and mutation (*NPM1*, *FLT3-ITD*, *CEBPA*, and *SNP rs16754*) was proposed for predicting outcomes in patients with CN-AML.¹⁰ However, these prognostic factors lack consistency in different cohorts and have thus not been introduced into clinical practice. In addition, the molecular mechanisms and appropriate therapeutic strategies underlying the groups identified by those markers remain unclear. Therefore, new prognostic factors validated in different CN-AML datasets are still required. Identification of the molecular mechanisms underlying the groups and their potential therapeutic strategies will be of considerable value for academic and clinical studies.

The scavenger receptor stabilin-1 (*STAB1*), which acts as a scavenging receptor, is induced during chronic inflammation and cancer

Received 12 May 2019; accepted 13 September 2019;
<https://doi.org/10.1016/j.omtn.2019.09.014>

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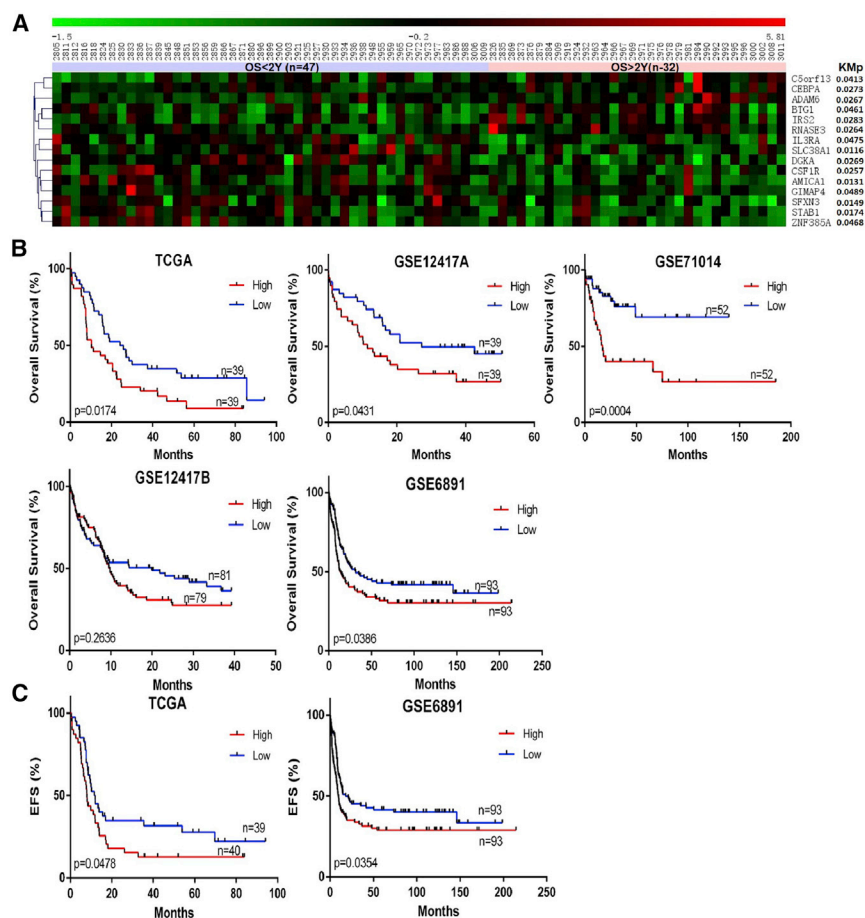


Figure 1. Selection of *STAB1* as a Prognostic Factor for CN-AML

(A) Heatmap of differentially expressed genes associated with OS in the TCGA dataset. (B) OS analysis of *STAB1* in five CN-AML datasets. (C) EFS correlation of *STAB1* in CN-AML in the TCGA and GSE6891 datasets.

analysis between the two groups was performed, and 84 differentially expressed genes—34 upregulated and 50 downregulated—were identified. Univariate survival analysis identified 15 genes associated with OS among the differentially expressed genes (Figure 1A). After univariate and multivariate analyses, the expression of six genes (*ADAM6*, *AMICA1*, *CSF1R*, *DGKA*, *IL3RA*, and *STAB1*) was associated with OS and was independent of other clinical factors, including age and mutation of *DNMT3A*, *RUNX1*, *FLT3-ITD*, *MT-CYB*, *WT1*, *IDH2*, *NPM1*, and *IDH1* ($p < 0.05$) (Tables S1 and S2).

To test whether these six genes are universal prognostic factors for CN-AML, we validated them using four other CN-AML datasets: GEO: GSE12417 test dataset (referred to as GSE12417A in this work; $n = 79$), GSE12417 training dataset (referred to as GSE12417B in this work; $n = 163$), GSE71014 ($n = 104$), and GSE6891 ($n = 187$). Only *STAB1* showed significance in three of the four datasets (Figure 1B; Figure S1). The GSE6891 dataset ($n = 187$)

was selected for further validation because of it having more clinical details. Our results showed that *STAB1* expression is a prognostic factor independent of the *FLT3*, *NPM1*, and *IDH2* mutation status (Table 1). Thus, *STAB1* may be a potential prognostic factor in patients with CN-AML.

Clinical Characteristics of Patients in the *STAB1*^{high} and *STAB1*^{low} Groups

To assess the correlation between *STAB1* expression and clinical factors in patients with CN-AML, we evaluated the patients' age, sex, white blood cell (WBC) counts, French-American-British (FAB) classification, mutations, and event-free survival (EFS) and OS status (based on the TCGA and GSE6891 datasets, respectively). In the TCGA cohort, patients in the *STAB1*^{low} group were more likely to survive for >2 years ($p = 0.0392$) (Table 2). However, clinical factors such as age, WBC counts, and gene mutation status were not significantly different between the *STAB1*^{high} and *STAB1*^{low} groups (Tables S3 and S4). In the GSE6891 dataset, compared with the *STAB1*^{high} group, the *STAB1*^{low} group contained fewer female patients ($p = 0.0276$) and carried fewer *FLT3-ITD* mutations ($p = 0.0255$), more *NPM1* mutations ($p = 0.0004$), and more *CEBPA* mutations ($p < 0.0001$) (Table 2). Moreover, the *STAB1*^{low} group had a longer EFS

progression.¹¹ The endocytic ligands of *STAB1* include SPARC, a protein that modulates progression of various cancers.^{12,13} Meanwhile, the cancer-promoting role of *STAB1* has been demonstrated in B16 melanoma and EL-4 lymphoma mouse models.¹⁴ Until now, the potential impact of *STAB1* expression on the prognosis of CN-AML has not been examined.

In the present study, we identified *STAB1* expression as a prognostic factor in multiple CN-AML datasets. We also investigated the potential molecular mechanisms and drug indicators underlying the CN-AML groups identified on the basis of *STAB1* stratification. Furthermore, we validated the effect of *STAB1* on leukemic cell growth and its sensitivity to the BCL2-targeting drug venetoclax. Our study identified a universal prognostic factor for CN-AML having potential applications in leukemia prognosis and treatment.

RESULTS

STAB1 Is a Potential Prognostic Factor Validated by Multiple CN-AML Datasets

To identify potential prognostic factors for CN-AML, we screened CN-AML samples ($n = 79$) from the TCGA AML dataset and classified them into two groups based on 2-year OS. Differential expression

Table 1. Multivariable Analysis of *STAB1* and Clinical Variables in TCGA and GSE6891 Cohort

Overall Survival Covariate	TCGA Dataset		GSE6891 Dataset	
	HR (95% CI)	p Value	HR (95% CI)	p Value
<i>STAB1</i>	1.98 (1.01–3.03)	0.0473	0.43 (1.05–2.24)	0.027511
Age	2.44 (1.15–3.62)	0.0147	NA	NA
<i>DNMT3A</i>	2.20 (1.07–3.50)	0.028	NA	NA
<i>RUNX1</i>	2.04 (1.03–3.37)	0.0417	NA	NA
<i>FLT3-ITD</i>	1.59 (0.89–3.07)	0.1124	0.57 (1.20–2.60)	0.004015
<i>MT-CYB</i>	1.33 (0.66–8.75)	0.185	NA	NA
<i>WT1</i>	1.16 (0.66–5.02)	0.2461	NA	NA
<i>IDH2</i>	0.47 (0.56–2.59)	0.638	–0.05 (0.49–1.83)	0.879925
<i>NPM1</i>	–0.05 (0.53–1.85)	0.963	–0.45 (0.43–0.94)	0.022836
<i>IDH1</i>	–1.28 (0.16–1.4)	0.2016	NA	NA

The model was generated from a COX regression model that included age, gene mutation of *DNMT3A*, and *RUNX1*, *FLT3-ITD*, *MT-CYB*, *WT1*, *IDH2*, *NPM1*, *IDH1*, and expression level of *STAB1*. CI, confidence interval; HR, hazard ratio; NA, not available.

in both the TCGA ($p = 0.0478$) and GSE6891 ($p = 0.0354$) datasets (Figure 1C). Thus, lower expression of *STAB1* could predict better outcomes in patients with CN-AML.

Potential Mechanisms Affecting CN-AML Groups Stratified by *STAB1* Based on Differential Expression and Regulation

To explore the mechanisms underlying CN-AML groups stratified by *STAB1*, we identified 353 differentially expressed genes (284 upregulated and 69 downregulated) by comparing the *STAB1*^{high} and *STAB1*^{low} groups in the TCGA dataset. Functional analysis of these differentially expressed genes showed enrichment of several gene ontology (GO) functions and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Functions associated with lysosomes, exosomes, and phagosomes, cell adhesion and migration, and immune-related responses were overrepresented. The enriched GO functions and KEGG pathways corroborate the known functions of *STAB1*, which are induced in macrophages during inflammation, are involved in cell-cell contact, and may contribute to integrin-mediated leukocyte migration and adhesion (Figure 2A). Several infectious and inflammatory disease-related pathways, such as those involved in legionellosis, toxoplasmosis, and rheumatoid arthritis, were considerably enriched, thereby justifying the reported role of *STAB1* as an immune modulator.¹⁵ We also identified four microRNAs (miRNAs) that were differentially expressed between the *STAB1*^{low} and *STAB1*^{high} groups: hsa-miR-21-5p, hsa-miR-181a-5p, hsa-miR-181-2-3p, and hsa-miR-126-3p. We constructed a miRNA-transcription factor (TF) co-regulatory network combining enriched functional categories to explore the mechanism of *STAB1* (Figure 2B). The resulting network included 275 nodes and 2,492 edges comprising four miRNAs and 21 TFs whose abnormal expression contributed to altered gene expression. The downregulation of the cancer suppressor miR-181 family is reportedly associated with good outcomes in AML.¹⁶ miR-21-5p is an onco-miRNA and is

Table 2. Significant Clinical Characteristics in the TCGA Cohort and GSE6891 Dataset between the *STAB1*^{high} and *STAB1*^{low} Group

Clinical Factor	<i>STAB1</i> ^{low}	<i>STAB1</i> ^{high}	p Value
TCGA Dataset	(n = 40)	(n = 39)	
OS (≥ 2 years)	21	11	0.0392
OS (< 2 years)	19	28	
M5	1	10	0.003
Non-M5	39	29	
GSE6891 Dataset	(n = 93)	(n = 93)	
Female	38	54	0.0276
Male	55	39	
EFS (≥ 1 year)	53	37	0.0275
EFS (< 1 year)	40	56	
<i>FLT3-ITD</i> negative	62	46	0.0255
<i>FLT3-ITD</i> positive	31	47	
<i>NPM1</i> negative	53	28	0.0004
<i>NPM1</i> positive	40	65	
<i>CEBPA</i> negative	74	91	<0.0001
<i>CEBPA</i> positive	19	2	
FAB			<0.0001
M1	41	11	<0.0001
Non-M1	46	75	
M2	24	11	0.0154
Non-M2	63	75	
M4	6	22	0.0008
Non-M4	81	64	
M5	11	41	<0.0001
Non-M5	76	45	

consistently upregulated in AML.¹⁷ Collectively, these TFs and miRNAs may help explain the better outcomes in patients with CN-AML in the *STAB1*^{low} dataset.

Potential Drug Indications for Groups Stratified by *STAB1*

To explore the drug indications for groups stratified by *STAB1*, we studied the expression correlation between drug target genes and *STAB1*. First, we identified genes that act as direct targets or transporters of the following 11 clinical leukemia drugs: cytarabine, daunorubicin, idarubicin, mitoxantrone, etoposide, vincristine, dexamethasone, aclarubicin, decitabine, fludarabine, and venetoclax. Expression correlation analyses showed that five (linked with red edges in Figure 3A) and ten genes (linked with blue edges in Figure 3A) were positively and negatively correlated with *STAB1*, respectively ($p < 0.05$). The cytarabine-sensitive genes *DCK*, *POLB*, and *RRM1* were negatively correlated with *STAB1*, whereas the cytarabine-resistant genes *CDA* and *SAMHD1* were positively correlated with *STAB1*. These data suggest that patients in the *STAB1*^{low} group are sensitive to cytarabine. DNA topoisomerase II alpha (TOP2A), a transporter of the drugs etoposide, idarubicin, daunorubicin, and



Figure 2. miRNA-TF Regulation and Its Correlation with *STAB1*

(A) Enriched GO/KEGG terms of differential genes in the *STAB1*^{high} versus *STAB1*^{low} groups. Numbers in brackets are the numbers of differential genes in each category. The enrich ratio represents the proportion of enriched genes accounting for the terms. (B) The miRNA-TF co-regulatory network for the enriched terms in (A). Triangles, TFs; circles, target genes; rounded rectangles, miRNAs; red nodes, upregulation in the *STAB1*^{high} group; green nodes, downregulation.

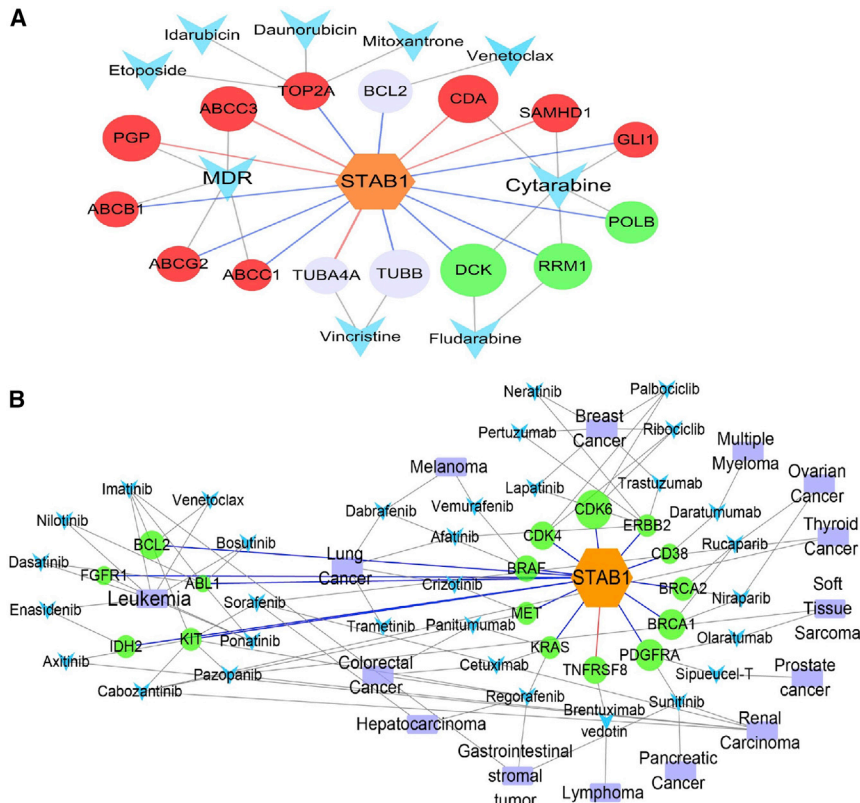


Figure 3. Expression Correlation of *STAB1* and Drug Targets in the *STAB1*^{high} and *STAB1*^{low} Groups

(A) Correlation of *STAB1* expression with gene targets of leukemia drugs. Hexagons, *STAB1*. (B) Correlation of *STAB1* expression with gene targets of FDA-approved cancer drugs. Circles, targeted genes; arrowheads, drugs. The red nodes represent genes resistant to drugs in the *STAB1*^{high} group; the green nodes represent genes sensitive to drugs in the *STAB1*^{high} group. The colors of the edges represent different expression correlations (red, positive; blue, negative) between *STAB1* and the drug targets. The size of the circle nodes represents the correlation strength between *STAB1* and the target genes.

mitoxantrone, was negatively correlated with *STAB1*. Considering the role of multidrug resistance (MDR)-related proteins in AML cells and the correlation of their expression with resistance to chemotherapy *in vitro*,¹⁸ we investigated the expression correlation between *STAB1* and MDR-related genes; *PGP* and *ABCC3* showed a positive correlation, whereas *ABCB1*, *ABCC1*, and *ABCG2* were negatively correlated with *STAB1* expression. This finding indicates the complexity of the interactions of *STAB1* with MDR drugs.

We performed expression correlation analyses between *STAB1* and the target genes of 34 US Food and Drug Administration (FDA)-approved cancer drugs ($p < 0.05$) (Figure 3B). All significant target genes except for *TNFRSF8* (also known as *CD30*), which is the target of brentuximab vedotin used to treat lymphoma, were negatively correlated with *STAB1* expression.¹⁹ *CDK6* ($R = -0.52$), *PDGFR* ($R = -0.42$), *BRCA1* ($R = -0.41$), *BCL2* ($R = -0.38$), and *CDK4* ($R = -0.38$) were the top five genes that were negatively correlated with *STAB1* expression.

***STAB1* Suppression May Inhibit Leukemia Growth and Induce Cell Apoptosis**

To investigate the role of *STAB1* in AML and the mechanism underlying *STAB1* stratification, we chose two leukemia cell lines, KG1a and NB4; both of these cell lines have relatively high levels of *STAB1* mRNA expression (Figure 4A), and they are suitable *in vitro* models for loss-of-function experiments. KG1a cells were sta-

bly transfected with three si-*STAB1*s compared with negative control (NC) small interfering RNA (siRNA)-transfected cells and resulted in the most efficient *STAB1* downregulation by the si-*STAB1*-2 (Figure 4B), which was used in the further experiments. Using western blotting, in both the KG1a and NB4 cell lines, the protein level of *STAB1* in the si-*STAB1* group was found to be downregulated compared with that in the NC group (Figure 4C). CCK8 assays revealed that compared with NC, si-*STAB1* led to a marked decrease in KG1a cell proliferation and a decreased tendency toward NB4 cell proliferation (Figure 4D). Fluorescence-activated cell sorting (FACS) analysis showed an increase in the apoptosis of KG1a cells with si-*STAB1* transfection compared with NC (Figure S2) (p value not significant). These results suggest that *STAB1* suppression inhibits leukemia growth and induces cell apoptosis.

The *STAB1*^{low} Group Showed Greater Sensitivity to Venetoclax in KG1a and NB4 Cells

Because *BCL2* is inhibited by venetoclax and *BCL2* expression is strongly correlated with sensitivity to venetoclax in hematologic malignancies,^{20,21} we inferred that compared with those in the *STAB1*^{high} group, patients in the *STAB1*^{low} group may be more sensitive to venetoclax. To assess whether patients in the *STAB1*^{low} group were more sensitive to venetoclax as well as considering the promising potential of venetoclax, we treated the NC and si-*STAB1* cells with venetoclax. KG1a cell viability testing demonstrated that compared with the *STAB1*^{high} group, the *STAB1*^{low} group was more sensitive to venetoclax ($p = 0.0484$) after treatment for 72 h as monotherapy. Assessment of cell viability demonstrated a concentration-dependent decrease up to 5 nmol/mL (Figure 4E). The NB4 cells from the *STAB1*^{low} group were more sensitive to venetoclax ($p = 0.0026$) treatment for 48 h, and the assessment of cell viability demonstrated a concentration-dependent decrease of up to 2 nmol/mL (Figure 4E). These data support the prediction that patients in the *STAB1*^{low} group are sensitive to venetoclax (Figure 3); furthermore, the data corroborate the finding that venetoclax was only modestly effective as monotherapy in AML.²¹

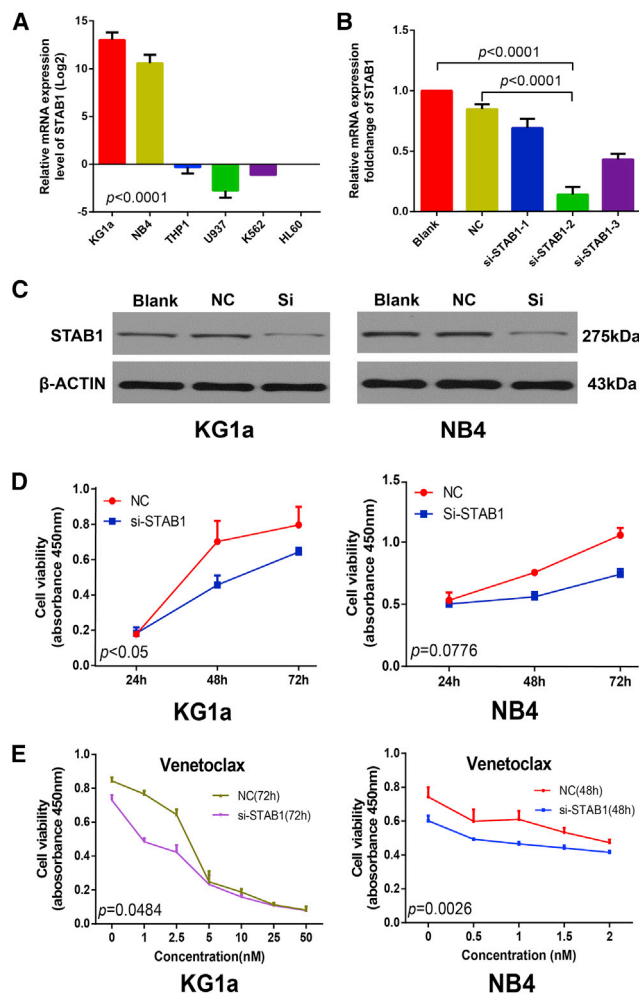


Figure 4. *STAB1* Suppression Inhibits Leukemic Cell Growth and Shows More Sensitivity to Venetoclax in KG1a and NB4 Cells

(A) Relative mRNA expression level (after log₂ conversion) of *STAB1* in six hematological cell lines analyzed by real-time qPCR. (B) Relative mRNA expression fold change of KG1a cells transfected with NC and three *STAB1*-specific siRNAs for 48 h. (C) Western blot analysis of KG1a and NB4 cells transfected with si-*STAB1*-2. (D) Cell proliferation of KG1a and NB4 cells transfected with NC or si-*STAB1* for 72 h tested using the CCK8 assay. (E) Cell proliferation graph of KG1a cells and NB4 cells transfected with NC or si-*STAB1* treated with venetoclax and tested using the CCK8 assay. Each experiment was repeated at least three times. Error bars represent SD.

DISCUSSION

To the best of our knowledge, this is the first report to show that *STAB1* expression may be a prognostic factor for CN-AML. Although previous studies have identified promising prognostic factors for CN-AML, none of the identified indicators has been validated using independent datasets.²² Using the TCGA CN-AML data, we identified that compared with higher *STAB1* expression, lower *STAB1* expression was associated with better survival in patients with CN-AML; furthermore, we validated our findings using three other independent datasets. The GSE71014 dataset mainly includes Asians,²³ whereas

the other four cohorts (TCGA and the three GEO datasets) mainly include Europeans, indicating the universality of *STAB1* as a marker of poor prognosis in patients with CN-AML. Although age, FAB classification, mutations in *FLT3*, *NPM1*, and *IDH2*, and *EFS* and OS status may affect the survival of patients with CN-AML, we demonstrated *STAB1* expression to be an independent prognostic factor in two CN-AML datasets. The potential ease of testing of *STAB1* adds to its advantages for clinical applications.

Most previous studies have not explored the potential molecular mechanisms underlying prognostic genes and have not justified the regulatory mechanisms underlying leukemogenesis. In this study, we systematically surveyed genes and miRNAs with differential *STAB1* expression. Gene network analysis of *STAB1* using the GO and KEGG pathway databases demonstrated that genes in the *STAB1*^{high} group tended to be associated with leukemogenesis. However, the detailed mechanisms of action of *STAB1* in AML leukemogenesis require further studies.

Cancer drugs and inhibitors are being increasingly designed to target specific genes, and these drugs may be used on the basis of the gene expression patterns of cancers.²⁴ On the basis of gene expression, we constructed gene networks combined with drug-specific analyses and investigated the relationships between inhibitor drugs and targets to identify potential drug indicators based on *STAB1* expression stratification. *STAB1* expression stratification is a potentially valuable strategy for treating patients with CN-AML. Small-molecule inhibitor drugs offer a promising alternative to existing treatment for patients with CN-AML.²⁵ The correlation between *STAB1* expression and the gene targets of FDA-approved drugs for various cancers indicates a potential for drug repurposing, which is a promising approach for developing treatment for patients in the *STAB1*^{low} group. Although many FDA-approved drugs have not yet been applied for AML treatment, they may be clinically beneficial as part of a treatment strategy in patients with CN-AML.

Our study has some limitations. First, the study may be biased due to the presence of confounding factors. However, factors that could influence the results were controlled as much as possible using multivariate analyses; it is therefore unlikely that the survival benefit associated with low *STAB1* expression was an artifact. Second, because it is difficult to track patients and obtain clinical samples, we downloaded data from public datasets and validated our findings in cell lines. Third, the mRNA of *STAB1* is 7,700-bp long, making constructing plasmids for *STAB1* overexpression difficult. Thus, the replenishment experiment was not implemented in this study.

In summary, our study is the first to provide evidence that low *STAB1* expression is associated with better outcomes in patients with CN-AML, even after adjusting for known clinical factors. Multiple datasets were used to assess the consistency of our findings. *STAB1* overexpression may be a valuable new marker for risk stratification in patients with CN-AML. In addition, drug-specific analyses were applied to identify potential drug indicators based on *STAB1*

expression stratification. We validated *in vitro* the suppression of leukemia growth and induction of apoptosis by *STAB1* and showed that compared with the *STAB1*^{high} group, the *STAB1*^{low} group is more sensitive to venetoclax.

METHODS

Clinical Patient Information

We downloaded sequencing data (normalized at level-2 intensity) and clinical information of 79 patients with CN-AML from the TCGA LAML dataset (<https://tcga-data.nci.nih.gov/tcga/>). We also obtained four CN-AML microarray datasets: GSE12417 test dataset (GSE12417A; n = 79),⁸ GSE71014 (n = 104),²³ GSE12417 training dataset (GSE12417B; n = 163),⁸ and GSE6891 (n = 187).²⁶ The datasets were normalized as described by Metzeler et al.,⁸ and the OS data were obtained from their publications and the GEO database (<http://www.ncbi.nlm.nih.gov/geo/>). The GSE12417 dataset includes a test (referred to as GSE12417A herein) and training (referred to as GSE12417B herein) dataset. Based on clinical experience, the 79 TCGA patients with CN-AML from the TCGA LAML dataset were further classified into two groups on the basis of 2-year OS: OS <2 years (n = 47) and >2 years (n = 32).

Survival Analysis

In the two CN-AML cohorts, TCGA and GSE6891, the association between *STAB1* expression and clinical factors, such as age and FLT3 mutations, were analyzed using Fisher's exact test. High or low *STAB1* expression was defined as the median expression level of all CN-AML samples in that cohort. OS was defined as the time from AML diagnosis to death due to any cause or the last clinical follow-up. The EFS was defined as the time from AML diagnosis to an event or the last follow-up. Clinical factors, such as age, sex, WBC counts, mutation status, and FAB classification, were assessed using univariate analyses with the Kaplan-Meier method using GraphPad Prism (<https://www.graphpad.com>) software. Variables with $p < 0.1$ were retained for further analysis. After univariate analyses, the significant prognostic factors were combined using multivariate analyses. For multivariate analyses, the multivariable Cox proportional hazards model was used to assess the association between *STAB1* expression and OS or EFS in the presence of other known clinical factors such as age, sex, WBC counts, mutation status, and FAB classification. These analyses were performed using the R survival package.²⁷ Hazard ratios with relative 95% confidence intervals were shown in multivariate analyses.

Differential Expression Analysis

Patients with *STAB1* expression values greater than the median were classified as the high *STAB1* group (*STAB1*^{high}), whereas the others were considered as the low *STAB1* group (*STAB1*^{low}). The R survival package DESeq²⁸ was used to conduct differential expression analysis with the thresholds false discovery rate (FDR) <0.05 and |fold change| >1.5 between the *STAB1*^{high} and *STAB1*^{low} groups. An expression heatmap was constructed and clustering was performed using the MultiExperiment Viewer (MeV) software (<http://en.bio-soft.net/chip/MeV.html>). Functional enrichment an-

notations for GO and KEGG pathways were analyzed using the Database for Annotation, Visualization and Integrated Discovery online tool (<https://david.ncifcrf.gov/>). To further infer the regulatory role of miRNA, DIANA-miRPath v.3.0²⁹ and miRNA path³⁰ were used to identify miRNA-regulating pathways.

Construction of the Regulatory Network in CN-AML

We applied the method described in our review³¹ to merge the predicted and experimentally verified miRNA and TF targets³² (<http://bioinfo.life.hust.edu.cn/hTFtarget/>). miRNA-gene/TF and TF-gene/miRNA regulatory relations were clearly identified for differentially expressed genes and miRNAs in different CN-AML subgroups. Using in-house-developed scripts, we subsequently identified miRNA-TF-gene feed-forward loops and miRNA-TF feedback loops based on their regulation. These networks were visualized using Cytoscape v.3.5 (<https://cytoscape.org/>).

Correlation Analysis

We identified the standard AML drugs applied in clinical practice and obtained their drug targets from DrugBank (<https://www.drugbank.ca/>). Furthermore, we identified genes sensitive or resistant to cytarabine as well as MDR genes from the literature. The target genes of FDA-approved cancer drugs for 23 cancer types were identified using DrugBank.³³ Coexpression analysis was performed to identify the expression correlation between drug targets and *STAB1*. Genes with $p < 0.05$ were selected for further analysis conducted using the R survival package.

Cell Culture, Transfection with siRNA, and RNA Expression Quantification

KG1a, NB4, THP-1, K562, MOML13, HL60, and U937 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 100 U/mL ampicillin, and incubated in a humidified atmosphere containing 5% CO₂ and 95% air at 37°C. For transfection, the cultured cell lines were seeded in six-well plates and transfected with 100 nmol/L *STAB1* siRNAs or control oligonucleotides (NC) (RiboBio, Guangzhou, China). The sequences for si-*STAB1*-1, si-*STAB1*-2, and si-*STAB1*-3 were 5'-GGATCGTCTTCTACAA CCA-3', 5'-AGATCACCGTCACCTTTAA-3', and 5'-GGAACA ATGGTCACTTGTA-3', respectively.

After 48 h of transfection, total RNA was extracted from KG1a cells using RNAiso (Takara) following the manufacturer's protocol, and 1 µg of the RNA was reverse transcribed using PrimeScript RT Master Mix (Takara). Quantitative real-time PCR (real-time qPCR) of *STAB1* was performed using the cDNA as a template in the presence of SYBR Premix Ex Taq (Tli RNaseH Plus; Takara). The levels of mRNA were normalized to the level of actin.

Western Blotting Analysis

The KG1a and NB4 cells were seeded into six-well culture plates, and the cells were then treated with 100 nmol/L *STAB1* siRNA and NC and cultured for 48 h. Subsequently, total protein was extracted from the cells, separated using sodium dodecyl sulfate-polyacrylamide-gel

electrophoresis, and transferred onto a polyvinylidene fluoride membrane. A gel imaging analysis system (Bio-Rad) was used to detect protein bands. After incubation with secondary antibodies, the membranes were visualized using the software Quantity One (Bio-Rad). β -Actin was used as the standard internal reference. *STAB1* antibody was purchased from Bio-Techne.

Assays for Cell Proliferation, Apoptosis, and Drug Treatment

Cells transfected with the siRNA and NC were seeded into 96-well plates (5×10^4 cells/well) in medium containing 10% FBS and cultured for 24, 48, and 72 h. Cell viability testing was performed using the Cell Counting Kit-8 (CCK-8, Dojindo Laboratories, Kumamoto, Japan), following the manufacturer's instructions. For apoptosis assays, the cells were washed with PBS and resuspended in 100 μ L of binding buffer containing 5 μ L of annexin V and 5 μ L of propidium iodide (BD Pharmingen). After incubation for 20 min, fluorescence was quantified by flow cytometry on a FACSCalibur system. Venetoclax was purchased from Selleck (<https://www.selleck.cn/>). KG1a and NB4 cells were incubated for appropriate durations in RPMI 1640 medium supplemented with 10% FBS and titrated concentrations of venetoclax. Cell viability was assessed at an absorbance of 450 nm using a PerkinElmer EnSpire 2300 multi-mode plate reader.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.omtn.2019.09.014>.

AUTHOR CONTRIBUTIONS

S.-Y.L. analyzed the data and wrote the manuscript. F.-F.H. revised manuscript. F.-F.H., Y.-R.M., H.H., and Q.Z. participated in data analysis. Q.L. offered guidance on laboratory technique. Z.C., Q.L., and H.W. provided specific knowledge on AML. A.-Y.G. and Z.C. designed the study and revised the manuscript. All authors read and approved the final manuscript and submission.

CONFLICT OF INTERESTS

The authors declare no competing interests.

ACKNOWLEDGMENTS

We thank Dr. Peter J.M. Valk at Erasmus University Medical Center for providing clinical information on the GSE6891 dataset and members working for the TCGA AML group. This work was supported by the National Natural Science Foundation of China (NSFC grants 31822030 and 31771458) and the National Key Research and Development Program of China (grant 2017YFA0700403).

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OMTN, Volume 18

Supplemental Information

Identification of *STAB1* in Multiple Datasets as a Prognostic Factor for Cytogenetically Normal AML: Mechanism and Drug Indications

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Supporting information

Figure S1. Overall survival (OS) analysis of differentially expressed genes associated with OS screened from TCGA cohort in four independent CN-AML datasets, and A, B, C and D referred to GSE311602 (n=79), GSE71014 (n=104), GSE12417 (n=163) and GSE6891 (n=187), respectively.

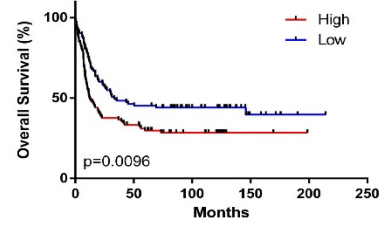
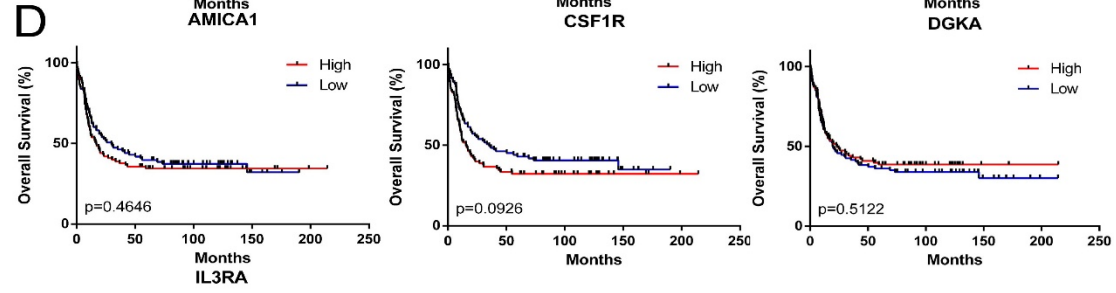
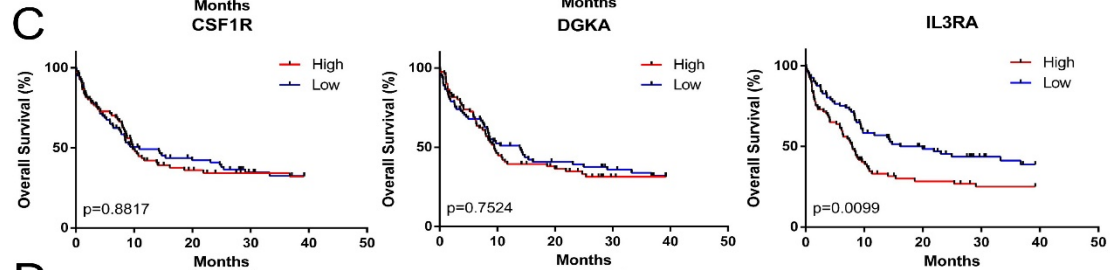
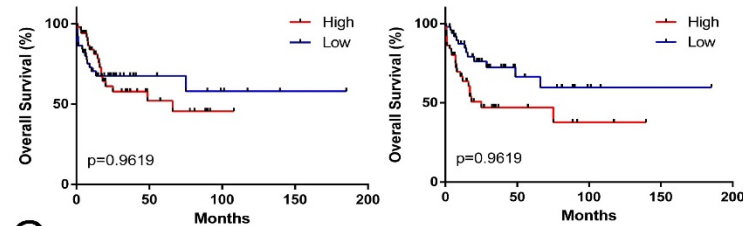
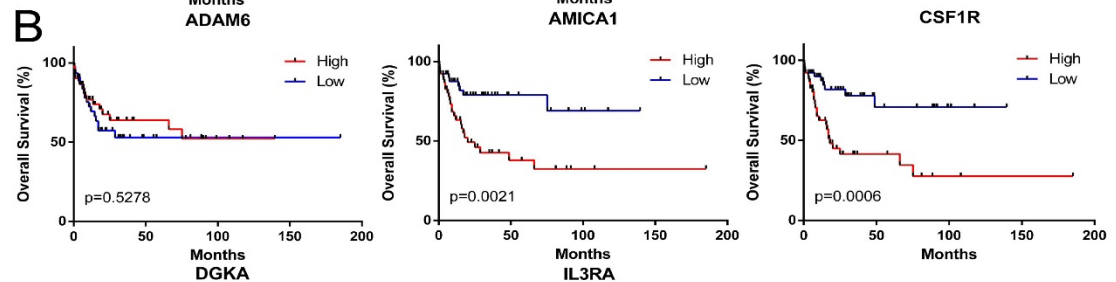
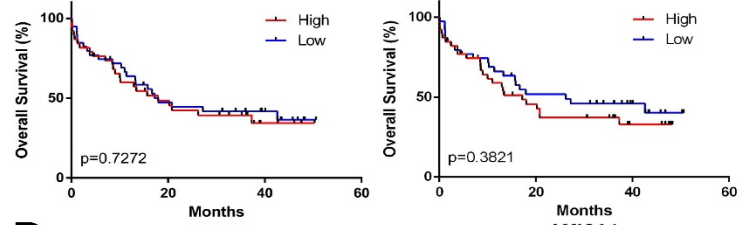
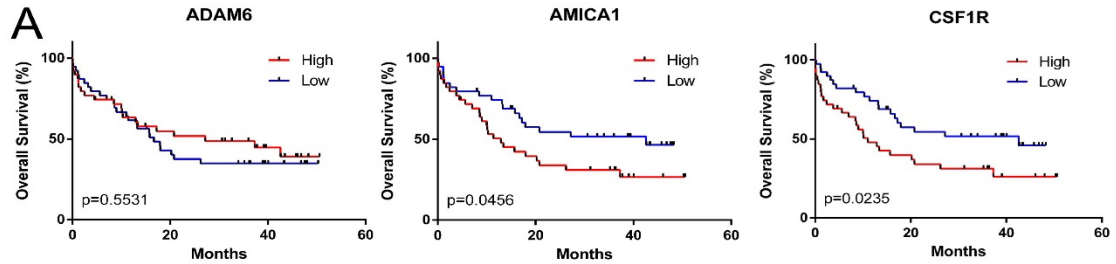


Figure S2. Cell apoptosis of KG1a cells transfected with NC or si-STAB1 for 48h and tested by FACS analysis.

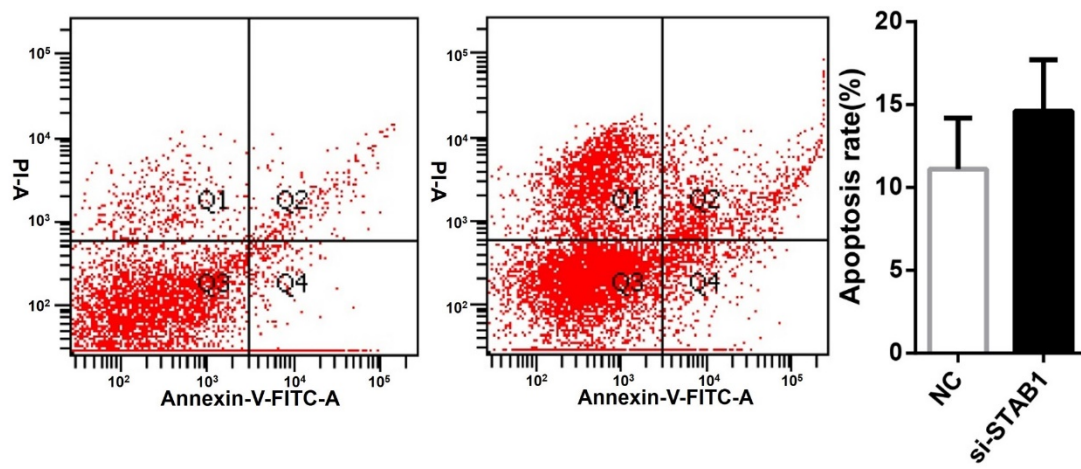


Table S1. Univariate analysis of clinical factors in the cohort of 79 CN-AML patients

Clinical Factor	group	beta	HR (95% CI for HR)	p-value
Sex.	Sex.M	-0.31	0.74 (0.45-1.2)	0.23
Age.	Age.over 60	0.75	2.1 (1.3-3.5)	0.0032
WBC.	WBC.over 70	0.24	1.3 (0.74-2.2)	0.38
MLL_PTD.	MLL_PTD.positive.f	-0.48	0.62 (0.29-1.3)	0.21
FLT3.	FLT3.positive.m	0.46	1.6 (0.95-2.6)	0.078
NPM1.	NPM1.positive.m	-0.15	0.86 (0.52-1.4)	0.54
DNMT3A.	DNMT3A.positive.m	0.44	1.6 (0.93-2.6)	0.093
IDH2.	IDH2.positive.m	0.39	1.5 (0.74-2.9)	0.27
IDH1.	IDH1.positive.m	-1.2	0.31 (0.11-0.87)	0.026
RUNX1.	RUNX1.positive.m	0.58	1.8 (0.9-3.6)	0.097
TET2.	TET2.positive.m	-0.45	0.64 (0.3-1.3)	0.23
NRAS.	NRAS.positive.m	-0.9	0.41 (0.13-1.3)	0.13
CEBPA.	CEBPA.positive.m	-0.14	0.87 (0.37-2)	0.74
WT1.	WT1.positive.m	0.1	1.1 (0.44-2.8)	0.83
PTPN11.	PTPN11.positive.m	0.21	1.2 (0.49-3.1)	0.66
SMC1A.	SMC1A.positive.m	0.7	2 (0.73-5.6)	0.18
SMC3.	SMC3.positive.m	0.74	2.1 (0.75-5.8)	0.16
STAG2.	STAG2.positive.m	-0.16	0.86 (0.31-2.4)	0.76
MT_CYB.	MT_CYB.positive.m	1.4	4.1 (1.2-14)	0.022
PHF6.	PHF6.positive.m	0.18	1.2 (0.37-3.8)	0.77
FAB.				0.5413
FAB.M1	FAB.M1	-0.843525	0.43 (0.14-1.3)	0.140695
FAB.M2	FAB.M2	-0.610884	0.54 (0.18-1.7)	0.284304
FAB.M3	FAB.M3	NA	NA (NA-NA)	NA
FAB.M4	FAB.M4	-0.250967	0.78 (0.26-2.3)	0.654013
FAB.M5	FAB.M5	-0.802819	0.45 (0.13-1.6)	0.211716
FAB.M6	FAB.M6	NA	NA (NA-NA)	NA
FAB.M7	FAB.M7	0.4083068	1.5 (0.17-14)	0.716368
FAB.nc	FAB.nc	-16.68037	5.7e-08 (0-Inf)	0.995119

Table S2. Multivariable analysis of ADAM6, AMICA1, CSF1R, DGKA, IL3RA and clinical variables in TCGA cohort.

Overall Survival Covariate	HR (95% CI)	p-value
AMICA1	2.02 (1.02-3.02)	0.0437
Age	2.47 (1.16-3.70)	0.0132
DNMT3A	1.58 (0.89-2.95)	0.1151
RUNX1	1.93 (0.98-3.26)	0.0541
FLT3-ITD	-1.52 (0.14-1.28)	0.1277
MT-CYB	1.79 (0.93-4.77)	0.0731
WT1	1.55 (0.77-10.11)	0.1203
IDH2	0.07 (0.47-2.26)	0.9475
NPM1	0.71 (0.52-3.99)	0.4757
IDH1	-0.28 (0.49-1.70)	0.7776
ADAM6	-2.14 (1.01-3.03)	0.0325
Age	2.69 (1.24-3.95)	0.0007
DNMT3A	2.59 (1.20-3.86)	0.0097
RUNX1	2.08 (1.05-5.62)	0.0378
FLT3-ITD	0.60 (0.63-2.38)	0.5506
MT-CYB	1.63 (0.80-10.74)	0.1038
WT1	0.72 (0.54-3.82)	0.4693
IDH2	0.09 (0.47-2.28)	0.093
NPM1	0.16 (0.56-1.96)	0.8766
IDH1	-1.45 (0.15-1.33)	0.1462
CSF1R	1.97 (1.00-2.88)	0.0489
Age	2.50 (1.17-3.71)	0.0124
DNMT3A	2.31 (1.11-3.53)	0.021
RUNX1	1.66 (0.88-4.59)	0.0973
FLT3-ITD	1.81 (0.96-3.25)	0.0697
MT-CYB	1.15 (0.58-8.11)	0.2484
WT1	0.72 (0.53-3.95)	0.4718
IDH2	0.60 (0.58-2.75)	0.5494
NPM1	0.19 (0.57-1.98)	0.849
IDH1	-1.40 (0.15-1.38)	0.1624
DGKA	2.48 (1.16-3.49)	0.0131
Age	2.30 (1.10-3.42)	0.0212
DNMT3A	2.75 (1.28-4.30)	0.0059
RUNX1	1.90 (0.98-5.05)	0.0574
FLT3-ITD	1.60 (0.89-3.11)	0.1103
MT-CYB	1.06 (0.55-7.45)	0.2905
WT1	0.60 (0.50-3.70)	0.5467
IDH2	0.53 (0.57-2.67)	0.5947
NPM1	0.28 (0.58-2.07)	0.7764
IDH1	-1.45 (0.14-1.33)	0.147

IL3RA	2.84 (1.29-3.99)	0.0045
Age	2.82 (1.29-4.15)	0.0048
DNMT3A	2.35 (1.12-3.64)	0.019
RUNX1	1.34 (0.77-3.99)	0.1787
FLT3-ITD	1.22 (0.79-2.71)	0.2208
MT-CYB	1.32 (0.66-8.57)	0.1858
WT1	0.33 (0.44-3.19)	0.7398
IDH2	-0.15 (0.43-2.07)	0.8817
NPM1	0.30 (0.58-2.09)	0.7615
IDH1	-1.74 (0.12-1.14)	0.082

The model was generated from a COX regression model that included Age, gene mutation of DNMT3A, and RUNX1, FLT3-ITD, MT-CYB, WT1, IDH2, NPM1, IDH1 and expression level of STAB1.

HR: Hazard Ratio

N/A: Not available

Table S3. Clinical characteristics in TCGA cohort between *STAB1*^{high} and *STAB1*^{low} group.

Clinical Factor	<i>STAB1</i> ^{low} group (n=40)	<i>STAB1</i> ^{high} group (n=39)	p-value
Female	20	20	1
Male	20	19	
WBC High(>70)	10	14	0.3348
WBC Low(≤70)	30	25	
Age (≥60)	16	20	0.3699
Age (< 60)	24	19	
OS (≥2year)	21	11	0.0392
OS (< 2year)	19	28	
EFS (≥1year)	20	25	0.258
EFS (< 1year)	20	14	
EFS (≥2year)	12	7	0.2933
EFS (< 2year)	28	32	
FLT3-	28	21	0.1681
FLT3+	12	18	
NPM1-	23	16	0.1793
NPM1+	17	23	
DNMT3A-	29	31	0.3746
DNMT3A+	11	18	
IDH2-	34	35	0.737
IDH2+	6	4	
IDH1-	35	35	1
IDH1+	5	4	
RUNX1-	32	37	0.0872
RUNX1+	8	2	
TET2-	32	36	0.1927
TET2+	8	3	
TP53-	39	39	1
TP53+	1	0	
NRAS-	36	37	0.6752
NRAS+	4	2	
CEBPA-	34	37	0.2633
CEBPA+	6	2	

WT1-	35	38	0.2007
WT1+	5	1	
PTPN11-	38	36	0.6752
PTPN11+	2	3	
KIT-	40	40	1
KIT+	0	0	
KRAS-	38	39	1
KRAS+	2	0	
MT-CO2-	38	39	1
MT-CO2+	2	0	
TTN-	37	36	1
TTN+	3	3	
U2AF1-	40	38	0.4937
U2AF1+	0	1	
SMC1A-	39	36	0.3589
SMC1A+	1	3	
SMC3-	38	37	1
SMC3+	2	2	
STAG2-	37	37	1
STAG2+	3	2	
MTCYB-	40	36	0.1156
MTCYB+	0	3	
PHF6-	37	39	0.2405
PHF6+	3	0	
ASXL1-	39	39	1
ASXL1+	1	0	
FAB			0.0326
M0	3	1	0.3171
	37	38	
M1	15	8	0.0966
	25	31	
M2	11	9	0.6513
	29	30	
M4	8	11	0.3936
	32	28	
M5	1	10	0.003
	39	29	
M6	0	0	
	40	39	1
M7	1	0	1
	39	39	

Table S4. Clinical characteristics in GSE6891 dataset between *STAB1*^{high} and *STAB1*^{low} group.

Clinical Factor	<i>STAB1</i> ^{low} group (n=93)	<i>STAB1</i> ^{high} group (n=93)	p-value
Female	38	54	0.0276
Male	55	39	
Age (≥50)	41	37	0.6559
Age (< 50)	52	56	
OS (≥3year)	45	35	0.1824
OS (< 3year)	48	58	
EFS (≥1year)	53	37	0.0275
EFS (< 1year)	40	56	
FLT3-ITD-	62	46	0.0255
FLT3-ITD+	31	47	
FLT3-TKD-	86	83	0.2379
FLT3-TKD+	7	13	
NPM1-	53	28	0.0004
NPM1+	40	65	
NRAS-	85	86	1
NRAS+	7	7	
KRAS-	93	92	1
KRAS+	0	1	
EVI1-	90	90	1
EVI1+	3	3	
CEBPA-	74	91	<0.0001
CEBPA+	19	2	
IDH2-	82	87	0.3089
IDH2+	11	6	
FAB			<0.0001
AML-M0	2	1	0.5671
No AML-M0	85	85	
AML-M1	41	11	<0.0001
No AML-M1	46	75	
AML-M2	24	11	0.0154
No AML-M2	63	75	
AML-M4	6	22	0.0008
No AML-M4	81	64	
AML-M5	11	41	<0.0001

No AML-M5	76	45	
AML-M6	3	0	0.0824
No AML-M6	84	86	
