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Loss of TRIM62 Expression Is an Independent Adverse Prognostic Factor in Acute Myeloid Leukemia

Alfonso Quintás-Cardama[#1], **Nianxiang Zhang**[2], **Yi Hua Qiu**[1], **Sean Post**[1], **Chad J. Creighton**[3], **Jorge Cortes**[1], **Kevin R. Coombes**[2], and **Steven M. Kornblau**[#1]

[1]Department of Leukemia, The University of Texas M.D. Anderson Cancer Center, Houston, TX

[2]Department of Bioinformatics, The University of Texas M.D. Anderson Cancer Center, Houston, TX

[3]Dan L. Duncan Cancer Center, Division of Biostatistics, Baylor College of Medicine, Houston, TX

[#] These authors contributed equally to this work.

Abstract

TRIM62 is a putative tumor suppressor gene. We investigated the levels of expression of TRIM62 protein in 511 patients with acute myeloid leukemia (AML) by reverse-phase protein array technology. Low TRIM62 levels were associated with markedly poorer outcomes and improved the prognostic impact of NPM1 and FLT3 mutations. Low TRIM62 levels, therefore, is an independent adverse prognostic factor in AML.

Background—Tripartite motif (TRIM)-62 is a putative tumor suppressor gene whose role in leukemia is unknown.

Materials and Methods—We evaluated the effect of TRIM62 protein expression in patients with acute myeloid leukemia (AML). We used reverse-phase protein array methodology to determine TRIM62 levels in leukemia-enriched protein samples from 511 patients newly diagnosed with AML.

Results—TRIM62 levels in AML cells were significantly lower than in normal CD34-positive cells, suggesting that TRIM62 loss might be involved in leukemogenesis, but was not associated with specific karyotypic abnormalities or Nucleophosmin (NPM1), Fms-like Tyrosine Kinase-3 (FLT3), or rat sarcoma viral oncogene (RAS) mutational status. Low TRIM62 levels were associated with shorter complete remission duration and significantly shorter event-free and overall survival rates, particularly among patients with intermediate-risk cytogenetics. In that AML subgroup, age and TRIM62 levels were the most powerful independent prognostic factors

Addresses for correspondence: Alfonso Quintás-Cardama, MD, Department of Leukemia, Unit 428, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030-4095, Fax: 713-794-1938; dragbona@gmail.com, Steven M. Kornblau, MD, Department of Leukemia, Unit 428, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030-4095, Fax: 713-794-1938; skornblau@mdanderson.org.

Disclosure

The authors have stated that they have no conflicts of interest.

Supplemental Data

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for survival. TRIM62 protein levels further refined the risk associated with NPM1 and FLT3 mutational status. TRIM62 loss was associated with altered expression of proteins involved in leukemia stem cell homeostasis (β-catenin and Notch), cell motility, and adhesion (integrin-β3, ras-related C3 botulinum toxin substrate [RAC], and fibronectin), hypoxia (Hypoxia-inducible factor 1-alpha [HIF1α], egl-9 family hypoxia-inducible factor 1 [Egln1], and glucose-regulated protein, 78kDa [GRP78]), and apoptosis (B-cell lymphoma-extra large (BclXL) and caspase 9).

Conclusion—Low TRIM62 levels, consistent with a tumor suppressor role, represent an independent adverse prognostic factor in AML.

Keywords

AML; Proteomics; Reverse phase protein array; RPPA; TRIM62

Introduction

The tripartite motif (TRIM) family of proteins, alternatively named “containing ring, B-box, and coil-coiled domains” proteins, share 3 functional domains including: a “really interesting new gene” (RING) domain in the N-terminus, and 1 or 2 B-boxes, and a coiled-coil motif.[1,2] Structurally, the main differences among different TRIM proteins reside in the number of B-boxes and the nature of the C-terminal domain.[1,3,4] Although more than 70 members of the TRIM family of proteins have been identified, the function of most remains unknown.[3] Group 1 TRIM proteins are highly conserved among vertebrate species and are present in invertebrates, and group 2 members are evolutionarily younger, absent in invertebrates, and contain a C-terminal SPRY domain.[3] *TRIM* genes have been implicated in cancer, several inherited disorders, and in cellular response to viral infection.[4] For instance, pyrin/marenostrin (TRIM20),[5] MID1 (TRIM18),[6] HT2A (TRIM32),[7] and MUL (TRIM37)[8] are mutated in familial Mediterranean fever, X-linked Opitz/GBBB syndrome, limb-girdle muscular dystrophy type 2H, and mulibrey nanism, respectively, and TRIM5α recognizes the HIV-1 core through its C-terminal PRY-SPRY domain and induces its degradation before reverse transcription.[9-11] The role of TRIM proteins in tumorigenesis was initially identified for promyelocytic leukemia gene (*TRIM19*),[12-14] Ret finger protein (*TRIM27*),[15] and transcriptional intermediary factor 1 (*TRIM24*),[16] which acquire oncogenic activity when fused to retinoic acid receptor alpha, Ret proto-oncogene, and B-raf, respectively. Recently, TRIM24, which has been shown to target p53 for degradation,[17] and estrogen responsive finger protein (*TRIM25*) have been shown to enhance breast tumor growth,[18,19] with the latter also being implicated as a tumor suppressor in the pathogenesis of hepatocellular carcinoma.[20] Hitherto, the implication of TRIM proteins in leukemia has been limited to the pathogenetic role of TRIM19 in acute promyelocytic leukemia (APL).

Tripartite motif-62, also referred to as ductal epithelium–associated RING chromosome 1, is a putative tumor suppressor gene that maps to chromosome 1p35.1, a genomic region frequently associated with loss of heterozygosity in human cancer.[21] Recently, TRIM62 has been found to be involved in the regulation of mammary gland polarity and morphogenesis, and loss or downregulation of expression has been demonstrated in 70% of ductal carcinoma in situ specimens, suggesting a potential role of TRIM62 as a tumor suppressor involved in

the early stages of breast cancer.[21] Furthermore, TRIM62 expression has been proposed as an independent predictor of recurrence-free survival in young patients with breast cancer.

In leukocytes, TRIM62 can be readily detected using Northern blot analysis as a predominant 4.4-kb band and using immunoblot analysis as a 54-kDa band, corresponding to the predicted full-length TRIM62 protein molecular weight.[21] No data are available regarding the role of TRIM62 in the pathogenesis of human leukemia. Because of the putative role of TRIM62 as a tumor suppressor, we investigated the effect of TRIM62 expression in a cohort of 511 cases of Acute myeloid leukemia (AML) and 21 cases of APL using reverse-phase protein array (RPPA). Herein, we report that TRIM62 protein expression is an independent prognostic factor for survival among patients with AML.

Materials and Methods

Patient Characteristics

The characteristics of the patients included in the AML719 array used for this analysis are shown in Table 1.[22] The training and the test sets built into the array were well balanced regarding known prognostic factors for response and survival in AML, when the sets were analyzed as a whole and when only patients who received AML therapy (n ¼ 415) were considered. The cohort of patients included a high percentage of patients with unfavorable cytogenetics (49%) and/or with an antecedent hematological disorder (40%). Among treated patients, 277 received high-dose ara-C (HDAC), 191 received an anthracycline, 49 received fludarabine either in the context of the FLAG (fludarabine, HDAC, and G-CSF) or the FA (fludarabine and HDAC) schemas, 28 received clofarabine, 8 were treated with other agents, and 1 patient received HDAC alone. Thirty-five patients received standard dose ara-C with clofarabine (n ¼ 33) or with daunorubicin and etoposide (n ¼ 2), and 8 received low-dose ara- C–based regimens. Idarubicin and troxacitabine was used in 2 cases and cloretazine was used in 25 cases. Hypomethylating agents and/or histone deacetylase inhibitors were used in 45 patients. Targeted agents were used in 13 patients, gemtuzumab ozogamicin (GO) in combination with interleukin-11 in 6, and investigational agents in phase 1 stages of development in 4 patients. Of the 21 patients with APL, 15 were treated with arsenic trioxide with all-*trans* retinoic acid (ATRA), including 6 who also received GO. Four received idarubicin and ATRA, 1 in conjunction with GO and 1 each received liposomal ATRA and GO with ATRA.

Patient Samples

Peripheral blood and bone marrow specimens were collected from 511 patients with newly diagnosed AML and 21 patients with newly diagnosed APL diagnosed and treated at M.D. Anderson Cancer Center (MDACC) between September 1999 and March 2007. Samples were acquired during routine diagnostic assessments in accordance with the regulations and protocols (Lab 01-473) approved by the MDACC Investigational Review Board (IRB). Informed consent was obtained and research was conducted in accordance with the Declaration of Helsinki. AML samples were analyzed according to an IRB-approved laboratory protocol (Lab05-0654). Samples were enriched for leukemic cells by performing Ficoll density gradient separation to yield the mononuclear fraction. This was followed by

CD3/CD19 depletion to remove contaminating T and B cells if they were deemed to represent > 5% based on the differential. Samples were then normalized to a concentration of 1×10^4 cells per microliter and a whole-cell lysate prepared as previously described.[23] Overall, 387 bone marrow and 283 peripheral blood samples were included in this study from the newly diagnosed cases, including 140 who had paired samples available. A paired relapse sample was available for 49 of the 142 AML and 1 of 4 APL cases that relapsed. All but 1 relapse sample were bone marrow specimens. The analysis of outcomes in this study was restricted to samples obtained from newly diagnosed patients who underwent treatment at MDACC.

Reverse Phase Protein Array Methodology

Proteomic profiling was performed on samples from patients with AML using RPPA with the custom-built AML719 array. The methodology and validation of this technique and the 207 antibodies used (Supplemental Table 1) have been fully reported previously.[24,25] Briefly, patient samples were printed in 5 serial dilutions onto slides along with normalization and expression controls. Then, slides were probed with a strictly validated primary antibody against TRIM62 (Abcam Cat# 102012) and a secondary antibody to amplify the signal, and finally a stable dye was precipitated.[26] Stained slides were analyzed using Microvigene software (Vigene Tech, Carlisle, MA) to produce quantified data.

Statistical Analysis

Supercurve algorithms were used to generate a single value from the 5 serial dilutions.[27] Loading control and topographical normalization procedures accounted for protein concentration and background staining variations.[28] Then, an analysis using an unbiased clustering protocol, perturbation bootstrap clustering, and principle component analysis was carried out as previously described[24] to quantify the levels of expression of total TRIM62. TRIM62 expression levels were divided into tertiles (low, medium, and high cohorts) based on the range of expression of all 511 samples.

Comparison of the protein levels between paired samples was done by performing a paired *t* test. The association between TRIM62 expression levels and categorical clinical variables were assessed using R with standard *t* tests, linear regression, or mixed-effects linear models. Associations between continuous variables and protein levels were assessed using Pearson and Spearman correlation and linear regression. Bonferroni corrections were performed to account for multiple statistical parameters for calculating statistical significance. The Kaplan–Meier method was used to plot the survival curves. Univariate and multivariate Cox proportional hazard modeling was performed to analyze the association between TRIM62 expression levels and survival as categorized variables using Statistica version 10 software (StatSoft, Tulsa, OK).

Results

Level of Expression of TRIM62 in AML Samples

Tripartite motif-62 protein expression was analyzed using RPPA in 511 samples obtained from patients with newly diagnosed AML (Supplemental Figure 1). To determine the effect

of sample source on protein levels, we first compared the expression in the 140 same-day paired peripheral blood and bone marrow samples (Figure 1A). No differences were observed between peripheral blood and bone marrow-derived AML samples ($P = .24$), thus samples from both sources were combined for the subsequent data analysis. The levels of TRIM62 expression did not change significantly between samples obtained from patients with newly diagnosed AML and those obtained from the same patients at relapse (median values, −0.04 for diagnosis and 0.04 for relapse fold change = 0.08 log 2, $P = .29$; Figure 1B). However, compared with the expression of TRIM62 in CD34-positive ($^{+}$) cells obtained from healthy volunteers, the levels of TRIM62 protein in AML samples were significantly lower (median = 0.82 for normal $CD34^{+}$ and −0.02 for newly diagnosed AML; net difference = −0.84 log 2; $P = .004$; Figure 1C).

Expression Level and Clinical Characteristics of TRIM62

Next, we analyzed the correlation between TRIM62 protein expression levels and several clinical characteristics (Supplemental Table 2). No differences in TRIM62 expression were detected on the basis of sex, performance status, antecedent malignancies, and previous chemotherapy and/or radiotherapy. Interestingly, low TRIM62 levels were associated with a greater rate of infectious complications ($P = .004$). Differences in TRIM62 expression were also observed across different AML subtypes according to the French-American-British (FAB) classification ($P = .03$), with AML M4 and M5 cases expressing lower levels of TRIM62 than other FAB subtypes. However, stratification according to specific cytogenetic abnormalities ($P = .92$) failed to show statistically significant differences (Supplemental Figure 2A and B). Similarly, stratification of patients according to standard cytogenetic risk criteria (favorable, intermediate, poor; $P = .68$) or according to the World Health Organization criteria ($P = .15$), failed to define patient subsets with differential levels of TRIM62 expression (Supplemental Figure 2C and D). Similar levels of TRIM62 expression were also observed between patients with and those without Fms-like Tyrosine Kinase-3 (FLT3) internal tandem duplication ($P = .09$) or FLT3-D835 ($P = .12$), or between those with or without mutations in Nucleophosmin (NPM1) ($P = .5$), p53 ($P = .56$), rat sarcoma viral oncogene (RAS) ($P = .63$), isocitrate dehydrogenase (IDH1) ($P = .65$), or IDH2 ($P = .61$) (Supplemental Figure 3). A trend toward lower TRIM62 protein levels was observed among patients carrying the $IDH1^{G105G}$ mutation (vs. others, $P = .06$). The protein levels of TRIM62 correlated negatively with white blood cell (WBC), percentage of bone marrow blasts, percentage of bone marrow and peripheral blood monocytes, serum albumin, CD13, and CD34 (Supplemental Figure 4).

Correlation Between TRIM62 Expression and Clinical Outcomes

Next, we investigated the potential effect of TRIM62 protein expression levels on clinical outcomes of patients with AML. Clinical outcome analyses were based on 415 patients who received therapy at MDACC and therefore had outcome and long-term follow-up data available. Patients were divided in tertiles according to TRIM62 protein expression levels and outcomes of patients in the upper tertile (high TRIM62) were compared with those of patients in the lower 2 tertiles (low TRIM62). Patients with high levels of TRIM62 expression were more likely ($\chi = 4.0$; $P = .045$; degrees of freedom = 1) to achieve remission (62.3%) compared with patients with low or mid levels (52%). The median

complete remission (CR) duration was 52.9 weeks (95% confidence interval [CI], 41.1-97.6). Low TRIM62 levels were associated with a shorter CR duration compared with patients with high levels of TRIM62 protein expression (38 vs. 63 weeks; $P = .06$; Supplemental Figure 5A). A similar trend was observed among patients with cytogenetically normal AML (CN-AML; 47.7 vs. 58.1 weeks; $P = .09$).

We then investigated the effect of TRIM62 levels in overall survival (OS). The median OS for the entire cohort was 47.6 weeks (95% CI, 37.9-55.9). In the training set, the OS of patients with AML was markedly different in those with low TRIM62 compared with those expressing intermediate or high TRIM62 (47.7 vs. 46 vs. 67.4 weeks; $P = .033$). This finding was confirmed in the independent test set, obtaining almost identical results (32 vs. 43 vs. 79 weeks; $P = .008$) (Supplemental Figure 5B and C). Similar survival curves were therefore obtained when the training and the test sets were combined (38 vs. 39 vs. 76 weeks; $P = .00038$) (Supplemental Figure 5D). Because no differences were observed between the outcomes of patients with low and intermediate TRIM62 levels, these 2 groups were combined for the remainder of the analysis and considered as expressing “low TRIM62” (Figure 2 and Supplemental Figure 6). The discriminatory effect of TRIM62 levels was even more significant when only patients with CN-AML were included in the analysis, with patients expressing low TRIM62 having remarkably shorter survival compared with those expressing high TRIM62 (49 vs. 124 weeks; $P = .00004$; Figure 3). TRIM62 levels, however, failed to segregate patients with distinct OS in the favorable (200 vs. 191 weeks; $P = .49$) and the unfavorable (34.2 vs. 36.8 weeks; $P = .35$) cytogenetic risk groups (Supplemental Figure 7). Age is one of the most important risk factors among patients with AML. Importantly, TRIM62 levels segregated prognostically distinct groups among patients younger than 60 years of age ($P = .002$) and among those 60 years of age and older ($P = .012$) (Figure 4A and B). In aggregate, these data confirm TRIM62 protein levels as an important prognostic factor for survival among patients with AML, particularly for those with CN-AML. We next investigated the effect of TRIM62 levels on clinical outcomes according to treatment modality. When patients were divided according to whether they received cytarabine-based chemotherapy or epigenetic therapy (ie, hypomethylating agents and/or histone deacetylase inhibitors) we observed that those with high TRIM62 levels had significantly improved OS compared with those with the low TRIM62 level, for patients receiving cytarabine-based therapy ($P = .00003$) and epigenetic therapy ($P = .021$; Figure 4C and D).

Prognostic Effect of TRIM62 Expression in Subsets of Patients With CN-AML

Cytogenetically normal AML is genetically heterogeneous; mutations and/or expression deregulation has been demonstrated in a variety of genes in this subset of patients. Several gene mutations have been identified as discriminators of distinct outcomes among patients with CN-AML. Mutations in *FLT3* and *NMP1* genes, alone but particularly in combination, have been consistently shown to be prognostically important among patients with CN-AML, with the former being associated with poorer and the latter with more favorable outcomes.[29-36] Because TRIM62 levels segregated prognostically distinct groups in patients with intermediate risk cytogenetics, we next analyzed the prognostic power of TRIM62 levels among patients with CN-AML in the context of other prognostically relevant gene

mutations. Notably, low levels of TRIM62 expression discriminated a subset of patients with a distinct poor prognosis compared with high TRIM62 expression among patients with *NPM1* mutated (36 weeks vs. not reached; $P = .01$), *NPM1* wild type (51 vs. 102 weeks; $P = .017$), *FLT3* mutated (32 vs. 99 weeks; $P = .008$), and *FLT3* wild type (68 vs. 127 weeks; $P = .005$; Figure 5), and *RAS* wild type (46 vs. 110 weeks; $P = .0002$), but not *RAS* mutated (72 vs. 102 weeks; $P = .07$; Supplemental Figure 8). Therefore, TRIM62 levels represent an important prognostic marker among patients with CN-AML independent from *FLT3* and *NPM1* mutational status. However, approximately 40% of patients with *NPM1* mutations also carry *FLT3* internal tandem duplication and patients with CN-AML with wild type *FLT3* and mutant *NPM1* (*FLT3*^{wt}*NPM1*^{mut}) have been shown to have a particularly favorable outcome.[29,33-36] Similar results were also observed in our cohort of patients with CN-AML (Supplemental Figure 9). This favorable prognosis notwithstanding, a significant proportion of patients with *FLT3*^{wt}*NPM1*^{mut} CN-AML perish because of their disease. On these grounds, we next studied whether TRIM62 levels could further stratify patients according to their FLT3 and NPM1 mutational status. For all but the mutant *FLT3-NPM1* wild type group, low TRIM62 expression segregated significantly distinct prognostic groups of patients. Among patients with *FLT3*^{wt}*NPM1*^{mut} CN-AML (the best prognosis of all CN-AML), those with the highest TRIM62 levels had a much longer survival than those with the lowest levels (245 vs. 90 weeks; $P = .2$) after more than 4 years of follow-up (Figure 6), however, small sample size precluded statistical significance. These results indicate that *FLT3*^{wt}*NPM1*^{mut} TRIM62 high expression level defines a subset of patients with AML with excellent long-term outcome after standard chemotherapy for whom allogeneic stem cell transplantation might not be indicated. Similarly, when patients with *FLT3*^{wt}*NPM1*^{mut} were excluded from the analysis, TRIM62 levels also stratified 2 distinct groups of patients with AML regarding OS ($P = .03$). These differences were even more notable when the analysis was restricted to patients with CN-AML after exclusion of *FLT3*^{wt}*NPM1*^{mut} cases ($P = .0006$; Supplemental Figure 10).

Protein Level of TRIM62 Is an Independent Predictor of Survival in AML

We next performed a Cox proportional hazard model to identify prognostic factors that were independent predictors of OS. Initially, a series of variables that were univariate predictors in this data set, or that have been reproducibly confirmed in multiple analyses to be prognostic (eg, age, cytogenetics, performance status, antecedent hematologic disorder) were used to perform a stepwise iterative multivariate analysis designed to reduce the list of potential prognostic variables strictly to those statistically significant ($P < .05$; Supplemental Table 3). This was followed by sequential add-back of all previously removed variables, 1 by 1, until a final model was obtained in which only significant variables remained. This type of analysis was first performed in the whole patient population. A final model was obtained containing 8 variables: age, cytogenetics (favorable and unfavorable), mutations of the *FLT3* gene, platelet and white blood cell count, albumin, and high TRIM62 levels (Table 2).

Based on the high prognostic effect of TRIM62 levels among patients with CN-AML, we next performed the same analysis but included only patients with intermediate cytogenetics. A final model was reached that included only 7 variables: age ($P < .00001$), performance

status ($P < .0001$), creatinine ($P = .0014$), FLT3 mutation ($P = .00132$), NPM1 mutation ($P = .012$), hemoglobin ($P = .00088$), and high TRIM62 levels ($P = .0014$). Overall, these results demonstrate that TRIM62 protein level is a powerful an independent predictor of survival in AML, particularly among patients with CN-AML.

Correlation Between the Expression of TRIM62 and Other Proteins

To gain insight into the pathogenetic role of TRIM62 in AML, we investigated the correlation of TRIM62 protein expression levels with that of proteins shown to be important in AML. To that end, the same AML719 array used to determine the levels of TRIM62 protein expression was also probed with 206 other antibodies and identified a series of strong protein/protein correlations (ie, Pearson $R > 0.25$; Supplemental Figure 11). We then compared 146 patients with the highest TRIM62 levels (top quartile) and 146 with the lowest TRIM62 levels (low quartile) and identified a signature of 40 differentially expressed proteins (Figure 7). Among the proteins most strongly downregulated in patients with low TRIM62 expression were Notch1 and TCF4 (involved in stem cell homeostasis), integrin-β3, ras-related C3 botulinum toxin substrate (RAC), and fibronectin (associated with cell motility and adhesion), HIF1α, Egln1, and GRP78 (involved in hypoxia), and several proteins involved in apoptosis (B-cell lymphoma-extra large [BclXL] and caspase 9). Upregulated were proteins like PTEN, Beclin, and GSK3p21.9 (involved in the WNT signaling pathway). When the same analysis was limited to the set of patients with CN-AML, a similar pattern of protein downregulation was observed among patients in the lowest quartile of TRIM62 expression with the addition of the tumor suppressor p53 (accompanied by upregulation of mouse double minute 2 homolog (Mdm2), a negative regulator of p53).

Discussion

Tripartite motif 62 has been proposed as a regulator of polarity in mammary epithelium.[21] In such a context, *TRIM62* has been found mutated and its expression diminished, which suggests that it might function as a tumor suppressor. Because TRIM62 is highly expressed in leukocytes, we investigated whether this protein might play a tumor suppressor role in AML. To that end, we assayed the expression of TRIM62 using an RPPA in 511 samples from patients with newly diagnosed AML. We found that the expression of TRIM62 protein in AML specimens was significantly lower than in normal $CD34^+$ cells, in consonance with the notion of TRIM62 as a tumor suppressor. Interestingly, no differences in TRIM62 levels were observed among patients with different cytogenetic abnormalities and between those with or without mutations in genes prognostically important in AML such as *FLT3, NPM1, p53, RAS, IDH1*, and *IDH2*. However, low TRIM62 expression was linked to a higher proliferative potential, supported by the correlation between low TRIM62 levels with higher WBC, bone marrow blasts, and monocytes.

Low TRIM62 protein level was an adverse prognostic factor for OS among patients with AML. The remission rate was statistically significantly greater in patients with higher TRIM62 levels and, the remission duration of patients with low levels of TRIM62 was shorter than that of patients with high TRIM62 (38 vs. 63 weeks; $P = .06$). Importantly, loss

or downregulation of TRIM62 segregated a distinct population of patients with a particularly short OS, which was particularly notable among those with CN-AML, again supporting a role of TRIM62 as a tumor suppressor. Cytogenetic abnormalities at diagnosis assessed using standard techniques are among the most powerful risk factors for patients with AML. However, approximately 40% to 50% of patients with AML will have a normal karyotype. In recent years, it has been established that 80% to 85% of all cases of CN-AML can be further characterized according to gene mutations, including *NPM1* (50%), *FLT3* (40%), mixed-lineage leukemia (*MLL*)-*PTD* (10%), *CEBPA* (15%), and/or *N-RAS* (10%). *NMP1* and *FLT3* mutations have been established as powerful markers of good and poor prognosis in AML, respectively. Patients with the *FLT3* internal tandem duplication and wild type *NPM1* AML are considered as having high-risk AML and are customarily recommended to undergo allogeneic stem cell transplantation. Importantly, the prognostic power of TRIM62 levels was independent of *NMP1* and *FLT3* mutational status. Thus, TRIM62 expression further refines the risk of death for patients with CN-AML associated with NMP1 and FLT3 mutations.

In patients with CN-AML, low expression of TRIM62 is associated with low p53 levels and high levels of the p53 negative regulator MDM2. The transcription factor p53 activates an array of genes involved in apoptosis, cell cycle arrest, and senescence. Mutations in the *p53* gene occur in 30% of human cancers. However, other elements of the p53 pathway are altered during leukemogenesis, contributing to the functional inactivation of the p53 pathway.[37] *MDM2* encodes an E3 ubiquitin ligase that targets p53 for degradation, thus impairing the ability of leukemic cells to undergo cell cycle arrest and apoptosis. In addition, low TRIM62 levels were correlated with upregulation of GSK3 and PKC. Cytokine-dependent GSK3 phosphorylation has been shown to regulate hematopoietic cell proliferation, and the PKC family of kinases appears to induce GSK3 phosphorylation.[38] Furthermore, GSK3 is required in the maintenance of leukemias carrying mutations of the MLL proto-oncogene, inducing cell proliferation and transformation through destabilization of the cyclin-dependent kinase inhibitor p27(Kip1).[39] In the presence of Wnt activity, the proteolytic activity of the GSK3/Axin/APC complex is inhibited leading to stabilization β-catenin, which is critical for self-renewal of AML stem cells.[40] Interestingly, TCF4, a key transcription factor in this pathway that physically interacts with β-catenin and promotes specific gene expression, was also found to be highly correlated with TRIM62 protein levels, thus suggesting an important role of TRIM62 in AML stem cell maintenance.

The adverse prognostic effect of low TRIM62 expression is associated with low levels of hypoxia response genes (HIF1α, VHL, and Egln) and with decreased adhesion markers (integrin β3 and fibronectin). These interactions are important because AML stem cells have been shown to be maintained by HIF1α through the repression of a negative feedback loop in the Notch pathway.[41] TRIM62 levels were strongly correlated with members of the Notch family in our RPPA array. Taken together, our results unveil an intricate protein network that links TRIM62 with proteins involved in hypoxia, proliferation, cell adhesion, homing, and motility, and leukemia stem cell (eg, Wnt/β-catenin and Notch) pathways.

Conclusion

Low TRIM62 protein level is an adverse prognostic factor in AML that is associated with short survival and appears to be involved in the regulation of proliferative survival signals and in leukemic stem cell pathways. TRIM proteins are involved in many cellular processes and constitute the largest group of RING-containing putative ubiquitin E3 ligases.[42] Indeed, ubiquitin E3 ligase activity has been demonstrated for a small subset of them.[19,43-46] However, little information is available regarding the sets of proteins targeted by each specific TRIM E3 ligase.[42] It is tempting to speculate that TRIM62 regulates the levels of proteins involved in leukemogenesis through its putative ubiquiting E3 ligase activity. Investigation into the mode of action of TRIM62 at the molecular level in AML and the potential oncogenic consequences of losing TRIM62 expression in vivo is under way.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Clinical Practice Points

- Tripartite motif (TRIM)-62 is a putative tumor suppressor gene but its role in leukemogenesis and its role as a potential prognostic marker are unknown.
- We evaluated the clinical impact of TRIM62 protein expression in a large cohort of patients with newly diagnosed acute myeloid leukemia (AML) by using reverse-phase protein array methodology.
- We found that TRIM62 levels in AML cells were significantly lower than in normal CD34-positive cells, suggesting that TRIM62 loss might be involved in leukemogenesis.
- Low TRIM62 levels were associated with shorter complete remission duration and significantly shorter event-free and overall survival rates.
- TRIM62 further refined the prognostic impact of NPM1 and FLT3 mutations.
- Low TRIM62 levels represent an independent adverse prognostic factor in AML and might aid in risk stratification strategies for patients with that malignancy.

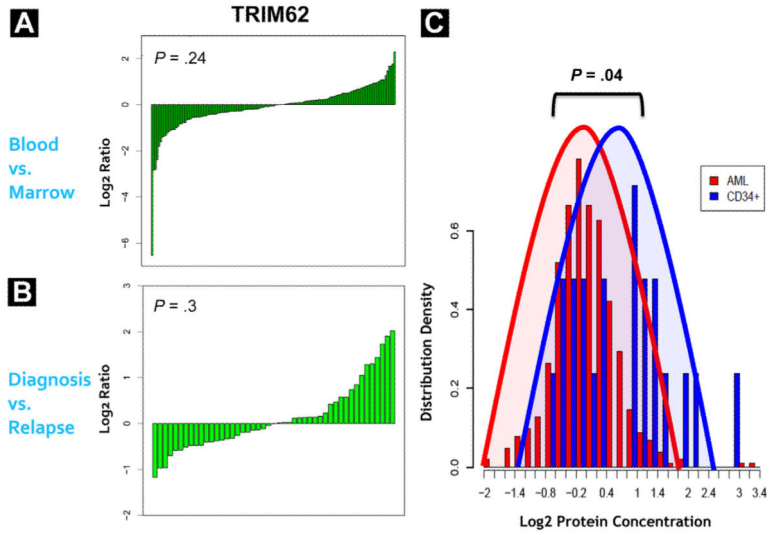

Figure 1.

Tripartite Motif (TRIM)-62 Protein Expression in AML Samples. (A) The Levels of TRIM62 Expression Did Not Differ Significantly Between Samples Obtained From Patients At Diagnosis and Those Obtained From the Same Patients At Relapse. (B) No Significant Differences Were Observed Between Peripheral Blood and Bone Marrow-Derived Acute Myeloid Leukemia (AML) Samples, Thus Samples From Both Sources Were Combined for the Subsequent Data Analysis. (C) Distribution Histogram of AML Cells Versus Normal CD34D+ Cells. When Compared With the Expression of TRIM62 in CD34D+ Cells Obtained From Healthy Volunteers, the Levels of TRIM62 Protein in AML Samples Were Significantly Lower

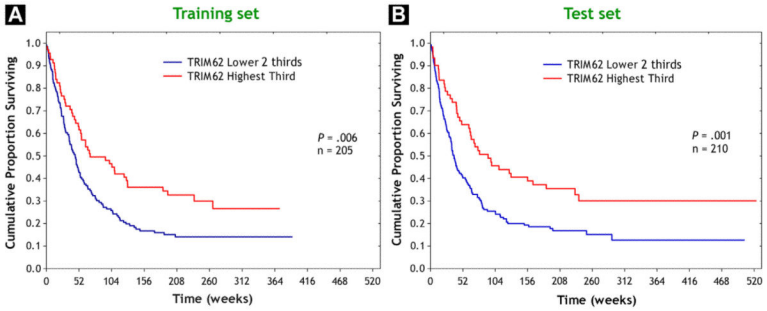

Figure 2.

Overall Survival According to the Level of Expression of Tripartite Motif (TRIM)-62 Protein in the Training and the Test Sets of the AML719 Array. (A) Overall Survival of Patients Expressing High Levels of TRIM62 Protein (ie, Highest Tertile) and Those With Low Expression of TRIM62 Protein (ie, Lower Two Tertiles) in the Training Set (A; N = 205), in the Test Set (B; N = 210). The Survival of Patients With Low TRIM62 Expression was Significantly Reduced Compared With That of Patients Expressing High TRIM62 Protein Levels

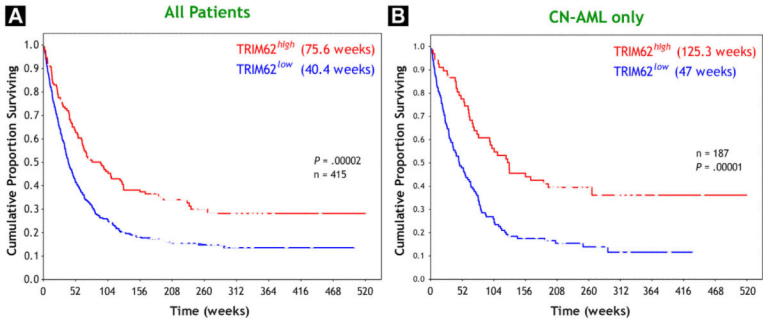

Figure 3.

Overall Survival According to the Level of Expression of Tripartite Motif (TRIM)-62 Protein in the Whole Cohort of Patients and in Those With Cytogenetically Normal (CN) Acute Myeloid Leukemia (AML) in the AML719 Array. (A) Overall Survival of Patients Expressing High Levels of TRIM62 Protein (Highest Tertile, Solid Curve) and Those With Low Expression of TRIM62 Protein (Lower 2 Tertiles, Dotted Curve) in the Entire Cohort of Patients in the Array That Received AML-Directed Therapy (A; N = 415) and in Those With CN-AML Alone (B; N = 177). In Both Groups of Patients, Low Levels of Expression Were Associated With Significantly Shorter Median Overall Survival

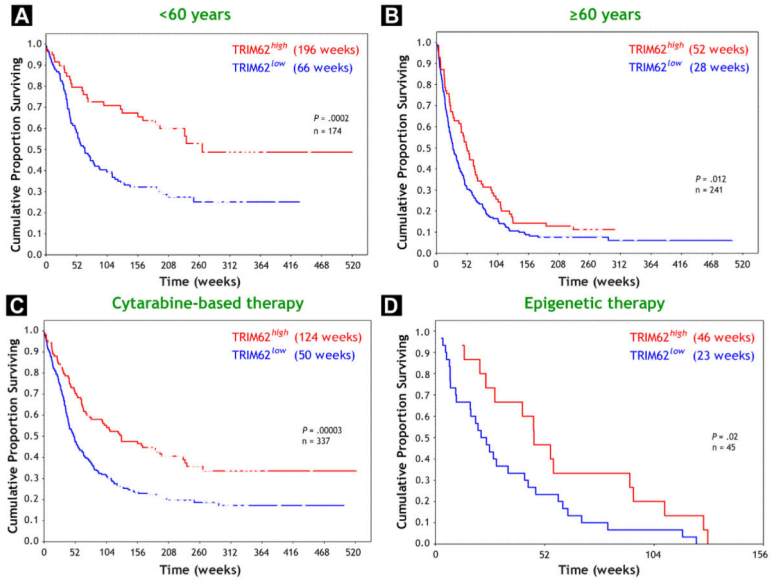

Figure 4.

Overall Survival According to the Level of Expression of Tripartite Motif (TRIM)-62 Protein and to Age and Acute Myeloid Leukemia Therapy. Overall Survival of Patients Expressing High and Low Levels of TRIM62 Protein (Solid and Dotted Curves, Respectively) Younger Than 60 Years of Age (A) or 60 Years of Age and Older (B). Effect of TRIM62 Protein Levels on Overall Survival According to Whether Patients Received Cytarabine-Based Therapy (C) or Epigenetic-Based Therapy (D)

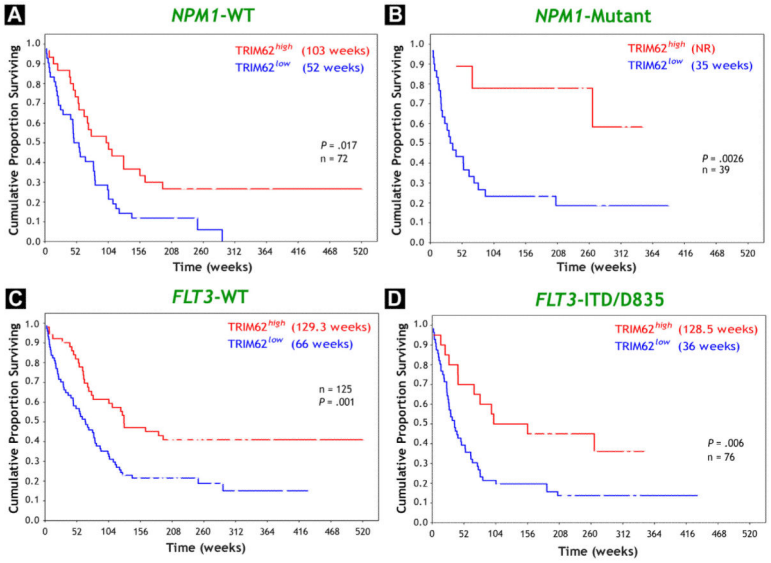

Figure 5.

Overall Survival According to the Level of Expression of Tripartite Motif (TRIM)-62 Protein and *NPM1* or *FLT3* Mutational Status in Patients With Cytogenetically Normal Acute Myeloid Leukemia. High Levels of TRIM62 Expression (Solid Curves) Resulted in Significantly Improved Survival of Patients Carrying Wild Type (WT) or Mutant *NPM1* Alleles (A and B, Respectively) and Between Those Carrying WT or Mutant *FLT3* Alleles (C and D, Respectively)

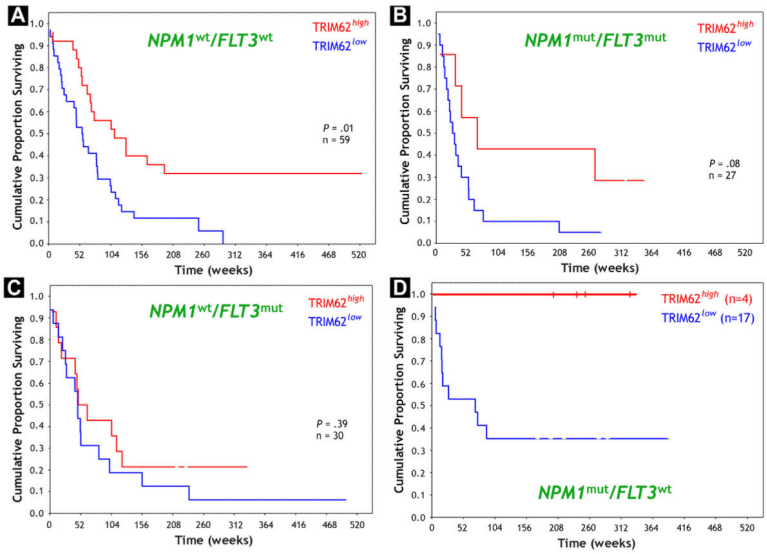

Figure 6.

Overall Survival According to the Level of Expression of Tripartite Motif (TRIM)-62 Protein Among Patients With Cytogenetically Normal Acute Myeloid Leukemia Stratified According to *NPM1* and *FLT3* Mutational Status. When *FLT3* and *NPM1* Mutational Status Were Combined, Patients With Higher Levels of TRIM62 Protein Again Exhibited a Longer Overall Survival (Solid Curves) Compared With That of Patients With Low TRIM62 (Dotted Curves). These Differences Were Significant for Patients With Wild Type (wt) *NPM1* and *FLT3* (A), and Displayed a Trend for Patients With Mutant *NPM1* and *FLT3* Alleles (B), for Those With WT *NPM1* and Mutant *FLT3* (C), and for Those With Mutant *NPM1* and WT *FLT3* Alleles (D)

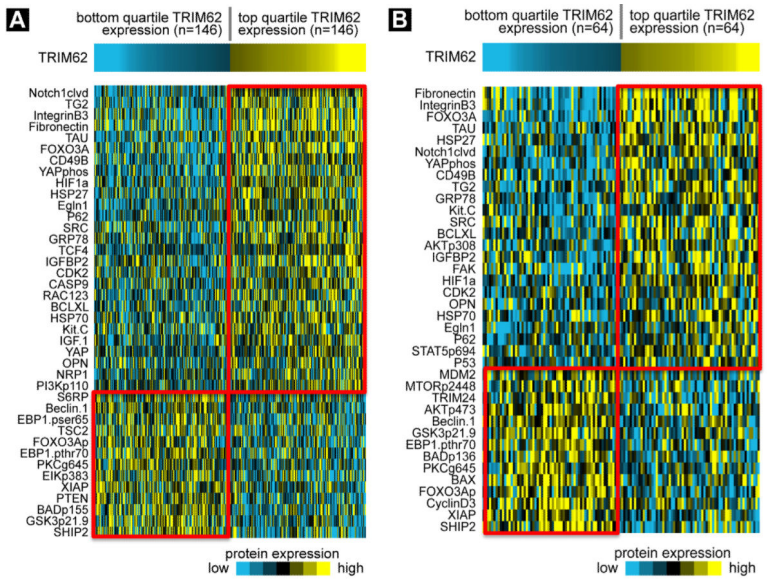

Figure 7.

Heat Map of the Protein Expression Signature Associated With High and Low Levels of Tripartite Motif (TRIM)-62 Protein in Patients With Acute Myeloid Leukemia (AML) in the AML719 Array. Differential Protein Expression was Studied in the Whole Cohort of Patients (A) and in Those With Cytogenetically Normal AML (B). For Both Cohorts, Patients in the Bottom and Those in the Top Quartile of TRIM62 Expression Were Studied. Rows Represent Probe Sets and Columns Represent Individual Patients. Patients Were Grouped According to Level of TRIM62 Expression and Proteins Were Ordered by Hierarchical Cluster Analysis. Expression Values of the Probe Sets are Represented by Color: Blue Represents Expression < the Median Value for the Given Probe Set Whereas Yellow Represents Expression > the Median Value for the Given Probe Set. Significantly Upregulated or Downregulated Proteins are Annotated on the Left Margin. TRIM62 Levels Were Significantly and Positively ($R \geq 0.2$) Correlated With CD49b ($R = 0.23$), CDK2 ($R = 0.28$), CDK4 ($R = 0.2$), egl-9 family hypoxia-inducible factor 1 (Egln1) ($R = 0.33$), Fibronectin ($R = 0.23$), Integrin B3 ($R = 0.21$), FOXO3a ($R = 0.25$), Notch1 ($R = 0.33$), transcription factor (TCF4) ($R = 0.27$), VHL ($R = 0.21$), Hypoxia-inducible factor 1-alpha (HIF1α) ($R = 0.33$), HSP27 ($R = 0.24$), OPN ($R = 0.2$), P21 ($R = 0.21$), P62 ($R = 0.32$), TAU ($R = 0.38$), TG2 ($R = 0.26$), Phosphor-Yap ($R = 0.23$); All With $P < .00001$ and Significantly and Negatively ($R = -0.2$) Correlated With Beclin1 ($R = -0.2$), Glycogen synthase kinase (GSK3p21.9 ($R = -25$), Protein kinase C alpha (Pkc)g645 ($R = -22$), SHIP2 ($R = -0.23$); All $P < .0001$). When Cases With Intermediate-Risk AML Were Analyzed Separately, TRIM62 Protein Levels Were Also Strongly Correlated With glucose-regulated protein, 78kDa (GRP78) ($R = 0.22$), Kit.C ($R = 0.23$), Notch3 ($R = 0.29$), STAT1p701 ($R = 0.23$), STAT5p694 ($R = 0.21$), and XIAP ($R = -0.25$); All $P < .0001$)

Table 1

Characteristics of the Training (n = 256) and Test (n = 255) Cohorts of Patients and Patients With Newly Diagnosed AML Included in the Study

Characteristic	All Cases			Treated		
	Training	Test	*P*	Training	Test	*P*
n	256	255		205	210	
Sex						
Male	56.6%	57.3%	.88	52.2%	52.9%	.99
FAB						
M0	6.6%	4.7%	.91	4.9%	5.2%	.94
M1	10.5%	11.0%		12.2%	11.0%	
M2	33.6%	32.5%		39.0%	34.8%	
M4	18.0%	22.4%		18.0%	22.4%	
M4Eos	2.7%	2.4%		3.4%	2.9%	
M5	3.5%	6.7%		2.4%	6.7%	
M5a	3.9%	3.1%		4.4%	2.9%	
M5b	3.1%	0.4%		2.9%	0.5%	
M6	4.7%	5.9%		4.9%	5.2%	
M7	2.0%	2.0%		1.5%	2.4%	
RAEBT	7.8%	5.9%		5.4%	5.7%	
NA	3.5%	3.1%		1.0%	0.5%	
2008 WHO						
AML with characteristic genetic abnormalities	9.8%	8.6%	.43	11.2%	9.5%	.366
AML with multilineage dysplasia	24.2%	18.8%		21.5%	16.2%	
AML not otherwise categorized	52.0%	58.0%		53.7%	61.9%	
Therapy-related	14.1%	14.5%		13.7%	12.4%	
Performance Status						
3 or 4	4.3%	2.4%	.71	3.9%	2.4%	.83
AHD						
>2 Months	41.0%	38.8%	.56	37.6%	36.7%	.82
Cytogenetics						
Favorable[a]	7.4%	5.9%	.72	8.8%	7.1%	.822
Intermediate	44.5%	43.5%		44.4%	45.7%	
Unfavorable	48.0%	50.6%		46.8%	47.1%	
FLT3						
ITD	14.9%	18.3%	.30	17.2%	19.9%	.48
D835	4.8%	5.2%	.84	5.6%	6.3%	.704
NPM1						
Mutant	19.5%	14.5%	.23	23.2%	15.0%	.096

Characteristic	All Cases			Treated		
	Training	Test	*P*	Training	Test	*P*
Treatment						
Anthracycline and HDAC	34.8%	40.0%	.19	43.4%	48.6%	.19
Anthracycline and other	0.0%	0.8%		0.0%	1.0%	
Demethylating-histone DAC	10.5%	7.1%		13.2%	8.6%	
Fludarabine and HDAC	9.0%	10.2%		11.2%	12.4%	
HDAC and nonanthracycline	8.6%	5.9%		10.7%	7.1%	
MYLO and IL11	0.4%	2.0%		0.5%	2.4%	
Standard dose AraC and other	7.8%	9.4%		9.8%	11.0%	
Targeted agents	4.7%	2.4%		5.4%	2.9%	
Cloretazine	4.7%	5.1%		5.9%	6.2%	
No treatment in MDACC	19.5%	17.3%		0.0%	0.0%	
Response						
Complete remission	46.9%	43.5%	.61	58.5%	52.9%	.519
Complete remission without complete platelet recovery	1.2%	1.2%		1.5%	1.4%	
Death before day 28	8.6%	7.8%		10.7%	9.5%	
Resistant	23.4%	29.8%		29.3%	36.2%	
NA	19.9%	17.6%		0.0%	0.0%	
Relapse						
Yes	61.8%	61.4%	.97	61.8%	61.4%	.966
Alive	19.9%	20.4%	.90	20.5%	21.0%	.907
Age, Years						
Range	16-86	17-87		17-86	18-86	
Median	65.22	66.06		63.76	64.93	
Mean	62.15	62.88	.57	60.65	61.35	.64
WBC						
Median	7.30	10.90		8.60	12.20	
Mean	26.21	26.41	.97	28.87	27.00	.666
Percentage of Bone Marrow Blasts						
Median	44.00	50.00		46.00	51.50	
Mean	49.60	50.92	.51	51.90	52.06	.97
Survival						
Weeks	44.57	38.43	.41	51.00	45.93	.65
CR Duration						
Weeks	45.29	46.79	.95	45.29	46.79	.95

The demographic and disease characteristics of patients that were treated are shown. The *P* values refer to comparisons of the training and test groups. Performance status uses the Zubrod scale. Abbreviations: AHD = antecedent hematologic disorder; AML = acute myeloid leukemia; AraC = cytosine arabinoside; DAC = decitabine; FAB = French-American-British classification; HDAC = high dose ara-C; IL = interleukin; ITD = internal tandem duplication; MDACC = M.D. Anderson Cancer Center; MYLO = myelotarg; RAEBT = refractory anemia with excess blasts in transformation; WBC = white blood cell; WHO = World Health Organization classification.

[a] Favorable = t(8:21) or inversion(16), intermediate = diploid or – y, unfavorable = all others including −5, −7, +8, t(6:9), miscellaneous changes, +21, 11q23.

Table 2

Multivariate Analysis, Final Model

Variable	β	Wald Statistic	Risk Ratio	Risk Ratio, 95% Lower CI	Risk Ratio, 95% Upper CI	*P*
All Patients						
Age at diagnosis	0.035286	62.74	1.035	1.026	1.045	<.001
Favorable cytogenetics	−0.847557	6.45	0.428	0.222	0.824	.011
Unfavorable cytogenetics	0.533663	20.34	1.705	1.352	2.150	<.001
FLT3 mutation	0.269996	3.94	1.309	1.003	1.710	.047
Platelets (categorical)	0.353812	10.09	1.424	1.145	1.771	.001
Albumin	−0.250617	10.10	0.778	0.666	0.908	.001
WBC (categorical)	0.185245	8.59	1.203	1.063	1.362	.003
TRIM62 (highest tertile)	−0.340555	6.91	0.711	0.551	0.916	.008
Patients With Intermediate Cytogenetics						
Age at diagnosis	0.032572	26.30	1.033	1.020	1.046	<.001
Performance status	0.435552	15.45	1.545	1.244	1.929	<.001
Creatinine	0.386230	10.10	1.471	1.159	1.866	.001
FLT3 mutation	0.637209	10.31	1.891	1.281	2.790	.001
NPM1 mutation	−0.695008	6.17	0.499	0.288	0.863	.012
Hemoglobin (categorical)	0.365083	11.02	1.440	1.161	1.786	.0001
TRIM62 (highest tertile)	−0.618031	10.17	0.539	0.368	0.787	.001

Abbreviation: WBC = white blood cell.