

# Early mortality in acute promyelocytic leukemia: Potential predictors (Review)

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**Abstract.** Acute promyelocytic leukemia (APL) is a rare leukemia characterized by the balanced reciprocal translocation between the promyelocytic leukemia gene on chromosome 15 and the retinoic acid receptor  $\alpha$  (RAR $\alpha$ ) gene on chromosome 17, and accounts for 10-15% of newly diagnosed acute myeloid leukemia each year. The combined use of all-trans retinoic acid and arsenic trioxide (ATO) as primary therapy has markedly improved the survival rate of patients with APL. Mortality in the first 30 days following therapy remains a major contribution to treatment failure. In the present study, published data was reviewed with a focus on the factors associated with early mortality. When treated with ATO as a primary treatment, the fms-like tyrosine kinase-internal tandem deletion has no impact on early mortality. Low lymphoid enhancer binding factor-1 expression may be a reliable marker for early mortality and the target of therapy if it could be proven by further studies. Cluster of differentiation (CD)56+ and CD34+/CD2+ may be candidates to select high-risk patients. The risk of early mortality in APL still cannot be predicted via the cell surface makers, despite multiple studies on their prognostic significance. Typically, a complex translocation did not alter the survival rate in patients with APL; however, if an abnormal karyotype [e.g., *Ide(17)*, *ZBTB16/RAR $\alpha$*  and

*STAT5B/RAR $\alpha$* ] appeared singularly or as part of a complex mutation, there is a high possibility of early mortality if clinicians are unable to identify or monitor it.

## Contents

1. Introduction
2. Fms-like tyrosine kinase 3 gene
3. Microgranular variant (M3V)
4. Immunophenotyping
5. Complex karyotype
6. Lymphoid enhancer binding factor-1 (LEF1)
7. Conclusion

## 1. Introduction

Acute promyelocytic leukemia (APL) is a rare leukemia characterized by the balanced reciprocal translocation between the promyelocytic leukemia (PML) gene on chromosome 15 and the retinoic acid receptor  $\alpha$  (RAR $\alpha$ ) gene on chromosome 17, and accounts for between 10 and 15% of newly diagnosed acute myeloid leukemia (AML) cases each year (1). Potentially life-threatening coagulopathy, and distinct morphologic and cytogenetic aberrations define APL as a unique subtype of AML (2). Early studies have demonstrated a median survival time of 1 week, ranging from 1 day to 1 month (3-6) in patients who were untreated or only received corticosteroids. The combined administration of all-trans retinoic acid receptor  $\alpha$  (ATRA) and arsenic trioxide (ATO) as primary therapy has notably improved the survival rate and decreased toxicity in patients. Early death (ED; mortality in the first 30 days following therapy) remains a major contribution to treatment failure (7). A previous study analyzed the data from surveillance, epidemiology and an end result program of 1,400 APL patients, revealing an ED rate (EDR) of 17.3% (8). Due to delayed diagnosis, delayed administration of ATRA and/or inadequate supportive care, the EDR of APL did not change substantially (9); if high-risk patients could be identified earlier and provided with better supportive care, such as the hemostatic targets protocol (e.g., platelets  $>30 \times 10^9/l$ , normal prothrombin time, normal activated partial thromboplastin time, fibrinogen  $>1.5$  g/l) (10), the EDR is expected to decrease.

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**Abbreviations:** APL, acute promyelocytic leukemia; ED, early death; EDR, early death rate; FLT3, Fms-like tyrosine kinase; PML, promyelocytic leukemia; RAR $\alpha$ , retinoic acid receptor  $\alpha$ ; ATRA, all-trans retinoic acid; ATO, arsenic trioxide; WBC, white blood cell count; M3V, microgranular variant; LEF1, lymphoid enhancer binding factor-1

**Key words:** acute promyelocytic leukemia, early mortality, Fms-like tyrosine kinase 3 gene, immunophenotyping, microgranular variant, lymphoid enhancer binding factor-1, complex karyotype

In the present study, published data is reviewed with a focus on the factors that may contribute to the ED of patients with APL, in order to improve the identification of high-risk patients.

## 2. *Fms*-like tyrosine kinase 3 (FLT3) gene

The FLT3 gene, a class III tyrosine kinase receptor, is located on chromosome 13q12 in humans (11). Somatic mutations in AML are common, including missense mutations in the activation loop domain (ALM) of the tyrosine kinase domain (FLT3/ALM) and internal tandem duplications of the juxta-membrane domain coding sequence [FLT3-internal tandem duplication (ITD)] (12-14). It is the most frequent genetic event in APL that may coincide with t(15;17) translocation. Several studies have demonstrated that 20-40% of APL patients possess the FLT3-ITD mutation and another 10-20% carry an FLT3/ALM mutation (15,16). Thus, Souza *et al* (17) suggested that FLT3-ITD positive APL patients may be classified as a new sub-type.

Previous studies have identified that FLT3-ITD is associated with a high white blood cell count (WBC) (17-28), the microgranular variant (M3V) type (17-20,23,25-27), short type PML/RAR $\alpha$  [break cluster region 3 (BCR3)] (17,19,20,23,24,26,27), sex (28), low-fibrinogen concentration (22), hemoglobin levels (17,26) and high lactate dehydrogenase (LDH) level (22). However, when discussed in the context of ED, the prognostic significance of this association remains unclear.

Wing *et al* (18) reported 82 patients who received a primary treatment with ATRA. FLT3 aberrations were detected in 35 cases (43%) at diagnosis, and were identified to be significantly associated with microgranular morphology and higher white blood cell count (WBC), but not the short type. A total of 7/19 patients succumbing to ED were carrying the ITD mutations, and an association was identified between the FLT3-ITD mutations and ED ( $P=0.06$ ), male, high WBC, and microgranular morphology (17). Gale *et al* (20) revealed results similar to the aforementioned Wing study, reporting an FLT3 mutation rate of 43% in 203 patients, with a number of them (183/203), who were diagnosed following the availability of ATRA for the treatment of APL, receiving ATRA as primary therapy. The authors identified a significantly high EDR in the FLT3-mutated group and the presence of FLT3-ITD reduced the additional cytogenetic abnormalities accompanying t(15;17) translocation. Furthermore, it was identified that the use of an FLT3 inhibitor CEP-701 had a greater effect on cell survival/proliferation in FLT3-ITD cells. However, it reduced the differentiation function of ATRA, which may have led to relapse (20). Kainz *et al* (29) identified that FLT3-ITD was associated with early mortality in 21 APL patients, while 2/5 ED patients did not accept ATRA. Barragán *et al* (30) analyzed the 739 patients assigned to the Pethema and Hovon groups between 1996 and 2005, which included the PETHEMA LPA96 and Pethema/Hovon LPA99 trials, identified a significantly higher EDR in the FLT3-ITD group ( $P=0.03$ ), which was associated with expression of CD2, CD34, human leukocyte antigen-DR, and CD11b surface antigens. This result was similar to Souza *et al* (17).

Chillon *et al* (21) observed that patients with an initial normalized copy number of PML-RARA transcripts less than the 25th percentile experienced increased incidence of ITDs ( $P=0.001$ ) and an adverse outcome of 5 year overall survival (OS) and relapse-free survival (RFS) but not EDR. Schnittger *et al* (25) identified that FLT3-ITD did not have a significant prognostic impact. Notably, when using a threshold mutation/wild-type ratio of 0.5, the ED rate was significantly higher for those with an FLT3-ITD/wt ratio  $>0.5$  ( $P=0.039$ ). The lack of association between FLT3-ITD and ED were also observed by Kiyoi *et al* (22), Callens *et al* (19), Noguera *et al* (23), Breccia *et al* (31), Mathews *et al* (24) and Lucena-Araujo *et al* (26), however, Breccia *et al* (31) observed a significant association between FLT3 and OS. Due to the controversy of the EDR and FLT3-ITD mutation, Beitinjaneh *et al* (32) conducted a systematic review to investigate the prognostic significance of FLT3 mutations for APL. A total of 11 observational studies were included and 10 of them received ATRA as primary therapy (the other received ATO). The authors identified a negative effect with ITD on OS and disease-free survival (DFS) for APL; however, there was no impact on complete remission (CR) rates with only 6 CR rates available in this study (32). Thus, the effect of FLT3 in APL remains difficult to determine.

In studies that used ATO as one of the introductory chemotherapeutic drugs for APL, the clinical outcomes presented were more favorable. For example, the study by Mathews *et al* (24) reported an ITD mutation rate of 33% in 98 APL patients and identified no impact on outcome. A study by the Shanghai Group (33) also suggested that the status of FLT3 did not associate with low EDR in 85 patients with APL receiving ATRA/ATO, and suggested that ATO may inhibit the negative effect of ITD. In a different study, 4/124 patients succumbed during primary therapy and no adverse outcomes influenced by FLT3 mutation status were identified (10). It has been demonstrated that inferior OS and DFS were significantly associated with FLT3-ITD (24). Therefore, any adverse effect of FLT3 mutations appears to be neutralized by the addition of ATO during primary therapy and consolidation. Furthermore, the authors suggest that FLT3 inhibitor therapy will serve no function in the future management of APL despite the occurrence of FLT3 mutations in APL. Poiré *et al* (27) analyzed 245 newly diagnosed adult patients with APL treated on intergroup trial C9710 and identified that FLT3 status had no association with EDR. The same result was also examined by Stock *et al* (16) in their study of 78 adults with newly diagnosed APL entered onto CALGB 9710, a North American Intergroup phase III trial. However, the authors suggested that targeted therapy with FLT3 inhibitors may improve relapse free survival for patients with FLT3 + APL.

The ITD mutation may have a relatively reduced function in the progression of APL and early mortality in patients that did not receive ATO as the initial chemotherapy. However, when ATO was used as a primary therapy, the inferior outcome was observed to be reversed. Continued study may resolve the mechanism of this phenomenon. Due to limitations in patient numbers and selection, more retrospective or prospective studies should focus on FLT3-ITD and early mortality in patients that received ATO as a first induction chemotherapy regimen.

### 3. Microgranular variant (M3V)

M3V is defined as leukemia promyelocytes with few Auer rods (34) and are devoid of or have only sparse granules (35). The incidence of this sub-type was 15-25% in APL. Several studies reported that M3v is associated with an increased white blood cell count (25,36,37), FLT3-ITD-mutations (17-20,23-28,37,38), the expression of CD2 (35-37,39-46) and CD34 (40,41), the relative incidence of the bcr-3 sub-type (37,39,47) and an increased platelet count (48). However, data regarding the prognostic significance of M3v of APL is rare, particularly in patients receiving ATRA and ATRA/ATO regimens.

Bassan *et al* (49) observed that M3v was associated with a higher incidence of ED in the conventional chemotherapy regimens, similar to the results of Cunningham *et al* (50). However, not all studies observed the same results. Davey *et al* (48) identified no impact of M3v on the early mortality of patients with APL in the chemotherapy regimens. The lack of significant impact of M3v on prognosis was also observed by Schnittger *et al*, however, their data did not identify the induction therapy of patients (25).

Utilizing ATRA as first therapy of APL, Tallman *et al* (7) identified no effect on ED in APL. Additionally, Kuchenbauer *et al* (51) suggested that the impact of poor prognosis of M3v in APL is associated with the frequency of FLT3-ITD mutation. Co-expression of FLT3-ITD yields an increased frequency of M3v with hypogranular blasts in lobulated nuclei (52). Thus Gale *et al* (20) suggest that the ITD may contribute to the generation of morphologic features of M3v. However, other studies (53-55) have published gene expression of a cohort of patients with APL and identified a gene group to distinguish M3 from M3v. The authors suggest that genes more suited to differentiate M3 from M3v are not markedly associated with FLT3-ITD (53,54) and morphology and FLT3 status partly affect gene expression independently (54). Other clinical data suggested that M3v is an independent factor in predicting a poor prognosis, and therefore not influenced by FLT3-ITD (18,19,23). A large study of 155 patients with APL focused on the outcome of patients with M3v (37), and identified no difference in morphology when treated with ATRA regimens when adjusting for WBC or relapse risk score ( $P=0.02$ , without adjustment). The authors also suggest that the poor outcome of M3v in previous studies may be due to the expression of CD2. Thus whether M3v is an independent predict mark of ED of APL remains unresolved.

### 4. Immunophenotyping

CD56, which is known as a neural cell adhesion molecule and associated with unfavorable clinical outcome in AML with t(8;21) (56), is expressed in 11-15% of patients with APL (57-59). It has been associated with CD2+ (57), CD34+ (57,59), CD7+ (57), HLA-DR+ (57), CD15+ (57), CD117+ (57), BCR3 isoform (57,60,61), fibrinogen range (60), and a trend toward M3v, CD11b+ and CD9+ has been demonstrated (57). Murray *et al* (60) first described a decreased CR rate associated with expression of CD56 and noticed the association between CD56 and natural killer and T cell markers. The association between CD56+ and immaturity-associated markers (CD117)

and natural killer and T-cell antigens, including CD2 and CD7 has been identified and it is hypothesized that these sub-groups of APL may have arisen in progenitors that have not undergone lineage restriction (57). The immature, undifferentiated and pluripotent leukemic stem cells are less sensitive to the primary therapy, which may explain why the CD56+ group experienced a decreased CR rate and an increased EDR (57). Ito *et al* (59) did not identify any impact of CD56+ on EDR in their study of 28 patients with APL. Similar results were also obtained by Ferrara *et al* (58), however, the authors observed that CD56 is an independent prognostic impact on survival that includes WBC count which indicated that the poor outcome of CD56+ APL is not associated with high WBC count. However, when drugs including ATO and gemtuzumab ozogamicin were administered as primary treatment, the predicted value of CD56 is waiting to be verified in the future studies (62).

The T-cell associated antigen CD2 was associated with PML/RAR $\alpha$  bcr3 (44,46,63), M3v (39,42,46,64) and increased leukocyte counts (46,63). In one study, CD2 predicted an improved CR rate and event-free survival (EFS) in APL (46). Xu *et al* (65) used a threshold value of 20% of positive cells to distinguish CD2+ to CD2-APL and identified that CD2+ APL had an increased EDR and is an independent risk factor of ED. The existing data of CD2 in APL is rare, therefore it cannot be confirmed whether this immunophenotyping will affect the prognosis.

The frequency of CD34 expression has been identified to range from 20 to 31% (28,35,45,46). The expression of CD34 in APL has been associated with leukocytosis (45,46,64,66,67), bcr3 isoform (45,46,64,63), M3V (45,46,64,63), increased frequency in females (45) and CD2 expression (45,46,64,63). It is often expressed at a significantly lower density on the surface of APL compared to AML (41,64). Breccia *et al* (63) and other studies (45,46) did not identify any association between early mortality and isolated CD34 expression, however, shorter OS was observed. A study of 40 *de novo* APL patients with a CD34+ rate of 32.5% confirmed a significantly increased EDR of CD34+ APL (66).

Grimwade *et al* (67) suggested that the CD2+ APL cell may derive from a different stem cell co-expressing CD2 and myeloid antigens. Thus, Breccia *et al* (63) continue to focus on the patients with CD34/CD2 double positive APL. The authors suggest that these patients may be identified as a subgroup with characteristic features associated with M3v, Bcr 3, FLT3-ITD, high incidence of differentiation syndrome and disseminated intravascular coagulation (DIC). This result was not consistent with the data of Albano *et al* (45) who identified no differences between the groups of complete remission, overall survival and disease-free survival, however, the authors did not evaluate the impact of CD34/CD2 on EDR. The poor clinical characteristics of this sub-type may predict a higher EDR and future studies may prove it.

A study (68) of 231 APL patients receiving various primary treatment suggested that carriers of a G>A polymorphism at position 1377 in the core promoter of the CD95 cell death receptor gene may predict a poor prognosis, particularly for ED ( $P=0.01$ ) with patients more likely to suffer infection-associated mortality. This genetic variation may be a reliable marker of ED in future studies. CD15 has been reported (60) to exhibit an unexpectedly high frequency

of relapses and a low OS. However the authors exclude the ED patients in this study, thus no data is available to reveal an association between ED and CD15.

### 5. Complex karyotype

The balanced reciprocal translocation t(15;17) (q22;q11-21) leading to PML gene and RARA gene fusion is the genetic characteristic of APL. The classical t(15;17) (q22;q12) is observed in between 70 and 90% of APL cases (69), and a number of patients exhibit complex translocations, involving chromosomes 15, 17 and other chromosomes (70,71). The most common abnormality is trisomy 8 (72,73). A number of the complex mutations were sensitive for the prediction of ED in APL. In a study by De Botton *et al* (73) and the Southwest Oncology Group (74), additional cytogenetic changes in patients with t(15;17) had no effect on the CR rate, EFS, relapse and overall survival at 2 years, which is in accordance with the findings of Grimwade *et al* (72). Mi *et al* (75) identified that a complex karyotype may contribute to an improved prognosis. Other reports have suggested the presence of complex karyotype changes adversely affects prognosis (76). A study of C9710 analyzed 245 newly diagnosed adult patients with APL (27). The authors identified a significantly lower CR rate in the complex karyotype [two or more additional chromosomal abnormalities (ACAs)] subgroup compared with one ACA or normal karyotype (P=0.001) independent of ATO treatment. This may be due to the reduced sensitivity to ATRA/ATO and the delay in administering ATO, which has been demonstrated by an Italian/German/Austrian cooperative group study that suggested earlier administration of ATO may overcome the negative effect of complex karyotypes (77). The natural resistance to primary therapy is also a factor contributing to ED in patients with APL.

Tetraploidy is rare and accounts for 0.75% in APL (78). Published data revealed a variety of clinical features of this mutation. CD2 was observed to be more frequent in this group (7 of 15) compared with the literature reported 23% of normal APL (39,42,44,45,64). It is more common in males with a median age of 49 (78). Since the majority of these cases did not acquire other complex mutations, the outcomes remain favorable despite the higher expression of CD2. The ATRA based primary therapy may be suitable for this complex mutation.

Co-expression of t(8;21) and t(15;17) is rare in APL, with only 6 patients reported at present (79-84). Neto *et al* (79) reported a case of APL-M3V which was sensitive to ATRA treatment, and detected a novel t(8;21) chromosomal aberration between 3 and 18 months after initial treatment. The authors noted the intermittent detection of t(8;21) during periods without ATRA suggested there was an antitumor effect of ATRA on M2 leukemic cells. Charrin *et al* (80) and Bonomi *et al* (81) reported two cases and identified that the t(15;17) may be acquired subsequent to t(8;21). A total of 5 patients received the ATRA-based treatment and achieved CR. No ED occurred during primary therapy, despite a high rate of relapse. A favorable response to chemotherapeutic induction indicated that the ATRA and idarubicin and Ara-C induction treatment was suitable for this complex karyotype (84). Another study also suggested that at the time of

diagnosis the rate of M2 leukemic cells could be tested using polymerase chain reaction detection and the alteration of bone marrow cell kinetics may trigger t(8;21) via complex mechanisms following chemotherapy (79).

Ider(17), which has been reported in only 72 APL cases globally, is a relatively rare variant cytogenetic abnormality among patients with APL (83,85-97). This isochromosomal abnormality may occur following initial reciprocal translocation of t(15;17), and is formed from the short arm and duplication of the long arm of ider(17)t(15;17) (85). Clinical outcome data were available for 36 patients with a CR rate of 77.8% and the response to ATRA and EDR were observed to be similar to that in normal APL. A total of 19 patients succumbed to the disease; however, the prognostic significance of this abnormal karyotype remains unclear due to the limited number of cases. The proportion of cells with the ider(17)(q10)t(15;17) is higher, in 9/12 cases. Since tumor protein p53 (TP53) is located on 17p, the ider(17)(q10)t(15;17) may provide a growth advantage to the relevant clone (85). The long type PMA/RAR $\alpha$  is prevalent in this type (57%). Bcr1 splicing PML exon 5-6 was associated with decreased sensitivity to ATRA (98), which may explain the ATRA resistance of ider(17) patients. Since the loss of TP53 and absence of PML exon 5 may occur in this rare subtype and present data have identified a trend of poor outcome, ider(17) is a candidate for further study.

The nuclear receptor binding SET domain protein 3, lysine acetyltransferase 6A and fibroblast growth factor receptor 1 which regulate cell transcription (99) and are associated with the stem cell myeloproliferative disorder are candidate genes involved with the loss of 8p (100). Otero *et al* (101) reported a patient with APL with dicentric t(8;13)(q10;q10) who succumbed due to a central nervous system hemorrhage on day 3 with ATRA based primary therapy. The authors hypothesized that the additional chromosomal changes were directly associated with the patient's prognosis, and that the novel chromosomal abnormalities may predict a poor outcome of APL.

Between 1 and 2% of APL cases are due to abnormal translocations including zinc finger and BTB domain containing 16 (ZBTB16)/RAR $\alpha$ , nucleophosmin (NPM)/RAR $\alpha$ , nuclear matrix associated/RAR $\alpha$ , signal transducer and activator of transcription 5B (STAT5B)/RAR $\alpha$ , protein kinase CAMP-dependent type 1 regulatory subunit  $\alpha$ /RAR $\alpha$ , BCL6 corepressor/RAR $\alpha$  and factor interacting with PAPOLA and SPSF1/RAR $\alpha$ , and all these translocations involve RARA (70). A review by Adams and Nassiri (102) discussed the various translocations of APL and identified certain features. t(5;17) NPM/RAR $\alpha$  has been diagnosed in patients younger than 10 years which is uncommon to normal APL (103). It responds well to ATRA but has higher risk of relapse. Diagnosis of ZBTB16/RAR $\alpha$  t(11;17) APL can be difficult. This translocation is more commonly associated with CD56 expression (104). Patients had an increased number of hypogranular pelgeroid neutrophils and a more regular nucleus compared with the bilobed nucleus typically found in APL (97). The majority of the translocations in APL can be successfully treated with ATRA/ATO, while patients with ZBTB16/RAR $\alpha$  and STAT5B/RAR $\alpha$  are resistant to ATRA and experienced a poor prognosis (105). There is currently limited data regarding the prognosis of patients with abnormal translocations. ZBTB16/RAR $\alpha$  and STAT5B/RAR $\alpha$  are

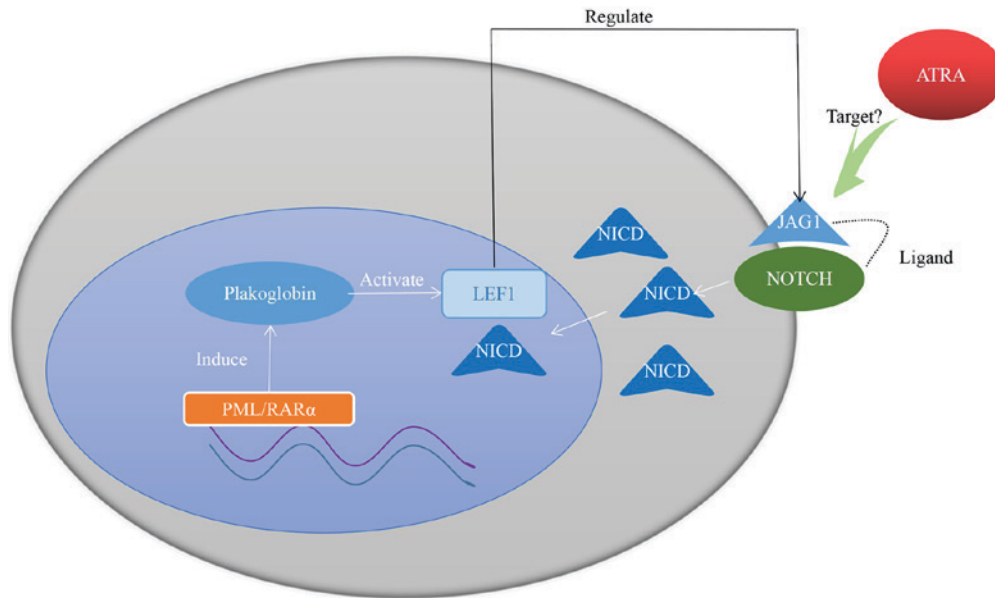


Figure 1. The hypothetical mechanism of LEF1 in the Notch signaling pathway. In the nucleus, PML/RAR $\alpha$  fusion gene may induce plakoglobin ( $\gamma$ -catenin) expression, leading to transcriptional activation of LEF1. LEF1 itself is a coactivator of NICD and may crosstalk with the Notch signaling pathway by regulating the expression of JAG1 on the cytomembrane. Furthermore, JAG1 is overexpressed in APL, and upon receiving ATRA therapy, it is downregulated in the NB4 cell line which indicates that JAG1 may be a therapeutic target of ATRA. PML, promyelocytic leukemia; RAR $\alpha$  retinoic acid receptor  $\alpha$ ; LEF1, lymphoid enhancer binding factor-1; NICD, Notch intracellular domain; APL, acute promyelocytic leukemia; ATRA, all-trans retinoic acid; JAG1, Jagged 1.

associated with ED, while other variant translocations may result in a similar outcome to cases with t(15;17) APL.

## 6. Lymphoid enhancer binding factor-1 (LEF1)

As an important member of the LEF/T-cell factor (TCF) family, LEF1 regulates cellular proliferation and cell cycle regulation (106). It is traditionally regarded as a central mediator of the wingless-type (Wnt) signaling pathway (106,107) and may serve a function in development and cancerogenesis, control self-renewal, cell proliferation and differentiation (108). Previous data reveal that it may serve a vital function in early hematopoiesis and leukemic transformation in murine models (109). However, certain functions independent of wnt signaling have also been reported (107,110). A study of 78 adult patients with APL (111) suggests a novel mechanism whereby LEF1 serves a specific function in the Notch signaling pathway and draws the conclusion that patients with APL overexpressing LEF1 are more likely to experience a favorable outcome.

In the nucleus, PML/RAR $\alpha$  fusion gene is able to induce plakoglobin ( $\gamma$ -catenin) expression in primary patient samples as well as in cell lines, leading to transcriptional activation of LEF1 (112). LEF1 itself is a coactivator of Notch intracellular domain (107). Jagged1 (JAG1) is a downstream target gene of LEF1 and is also the ligand of Notch (113). LEF1 is able to crosstalk with the Notch signaling pathway by regulating the expression of JAG1 on the cytomembrane (112). Furthermore, JAG1 is more frequently expressed in APL and, when receiving ATRA therapy, JAG1 is downregulated in the NB4 cell line (114,115). Taken together, these studies indicate that JAG1 may be a therapeutic target of ATRA, with high expression of LEF1 promoting the curative effect of ATRA. The hypothesized mechanism is presented in Fig. 1.

In trials, 103 newly diagnosed APL patients were observed and treated with the AIDA-0493 (116) and AIDA-2000 (117) protocols between January 1996 and December 2012. The median follow-up time was 5.7 years. Patients were divided in two groups according to the expression level of LEF1: A low LEF1 group with LEF1 values below the median value (<2.1 fold-change) and a high LEF1 group with LEF1 values above the median value (>2.1 fold-change). Fisher's exact test for categorical data and the nonparametric Mann-Whitney U test for continuous variables were used to identify the difference between two groups. Survival curves and influence factors of survival endpoints were measured by the Kaplan-Meier method and multivariate Cox proportional hazards models accordingly. They demonstrated that the LEF-high group exhibited lower WBC counts ( $P < 0.0001$ ), trended towards a younger age ( $P = 0.08$ ), and presented more frequent FLT3-ITD mutations ( $P = 0.02$ ). ED only occurred in the LEF-low group ( $n = 9$ ;  $P = 0.002$ ). This suggests that the expression of LEF may be studied as a novel marker of risk in APL if similar results can be confirmed by further studies.

## 7. Conclusion

In conclusion, published data has been reviewed with a focus on the factors associated with ED. When treated with ATO as primary treatment, the FLT3-ITD has no impact on ED. Low LEF expression may be a reliable marker of ED and a therapeutic target if it can be proven by further studies. CD56+ and CD34+/CD2+ may be candidates to select high-risk patients. High-risk patients still cannot be identified via the cell surface makers, despite a number of studies analyzing their prognostic significance. Complex translocations did not reduce the EDR in APL; however, if an abnormal karyotype [e.g., t(17), ZBTB16/RAR $\alpha$  and STAT5B/RAR $\alpha$ ] appeared singularly or

as part of a complex mutation, there is a high possibility of early mortality if clinicians are unable to identify or monitor it.

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