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The RTK–RAS signaling pathway is enriched in patients with rare acute myeloid leukemia harboring t(16;21)(p11;q22)/*FUS::ERG*

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Abstract

Acute myeloid leukemia (AML) with t(16;21)(p11;q22)/*FUS::ERG* is a rare AML subtype associated with poor prognosis. However, its clinical and molecular features remain poorly defined. We determined the clinicopathological, genomic, and transcriptomic characteristics and outcomes of patients with AML harboring *FUS::ERG* at our center. Thirty-six AML patients harboring *FUS::ERG* were identified, with an incidence rate of 0.3%. These patients were characterized by high lactate dehydrogenase levels (median: 838.5 U/L), elevated bone marrow blast counts (median: 71.5%), and a CD56-positive immunophenotype (94.3%). Notably, we found that RTK–RAS GTPase (RAS) pathway genes, including *NRAS* (33%) and *PTPN11* (24%), were frequently mutated in this subtype. Transcriptome analysis revealed enrichment of the phosphatidylinositol-3-kinase-Akt (PI3K-Akt), mitogen-activated protein kinase (MAPK), and RAS signaling pathways and upregulation of *BCL2*, the target of venetoclax, in *FUS::ERG* AML compared to *RUNX1::RUNX1T1* AML, a more common AML subtype with good prognosis. The median event-free survival in patients with *FUS::ERG* AML was 11.9 (95% confidence interval [CI]: 9.0–not available [NA]) months and the median overall survival was 18.2 (95% CI: 12.4–NA) months. Allogeneic hematopoietic stem cell transplantation failed to improve outcomes. Overall, the high incidence of RTK–RAS pathway mutations and high expression of *BCL2* may indicate promising therapeutic targets in this high-risk AML subset.

Key Words: Acute myeloid leukemia; BCL2; t(16; 21)(p11; q22)/*FUS::ERG*; RTK-RAS signaling pathway

1. INTRODUCTION

t(16;21)(p11;q22) is a rare chromosome translocation in acute myeloid leukemia (AML) that results in the fusion gene *FUS::ERG*,[1] which has been listed as an AML class-defining genetic abnormality in the 2022 European Leukemia Net (ELN) AML classification.[2] Although *FUS::ERG* AML was not included in the 2022 ELN risk stratification, patients with the fusion gene were consistently reported to have a poor outcome with a median overall survival (OS) of 11 to 22 months.[1–3] However, published results regarding the role of allogeneic hematopoietic stem cell transplantation (HSCT) in these patients are discordant.[1,4]

AML is characterized by concurrent mutations that cooperate in driving leukemogenesis and refractory/relapse mechanisms.[5,6] However, few studies have described the mutational landscape of *FUS::ERG* AML. Zerkalenkova et al[7] discovered concomitant *DNMT3A*, *ASXL1*, *RUNX1*, and *BCOR* mutations in patients with *FUS::ERG* AML; however, the study was limited by a small case number. Alternatively, mutations in the RTK–RAS GTPase (RAS) pathway are common in AML, causing the proliferative and survival advantage of leukemic cells.[8] Targeting mutations involved in the RTK–RAS pathway (eg, *FLT3*) combined with chemotherapy yielded significant clinical effect on improving the poor survival of certain patients with AML, suggesting the potential promise of this approach for the treatment of *FUS::ERG* AML with concurrent RTK–RAS mutations.[9] The results of transcriptome analysis in pediatric *FUS::ERG* AML have revealed the upregulation of *EZH2* and downregulation of

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The detailed clinical and sequencing data of the current study are provided upon rational request.

Written informed consent was attained from all patients.

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antigen processing and presentation-associated genes compared to that in other types of primary AML.[3] In addition, a similar expression pattern between *FUS::ERG* AML and Ewing sarcoma was reported in a pediatric cohort.[10] Nevertheless, transcriptome analysis of adult patients with *FUS::ERG* AML is still lacking.

Therefore, we aimed to assess the clinical outcome of patients with *FUS::ERG* AML and the effect of HSCT using a relatively large cohort. Furthermore, the genomic and transcriptomic alterations were analyzed, providing new insights regarding potential targeted therapies for this specific aggressive leukemia.

2. MATERIALS AND METHODS

2.1. Patients

We reviewed the karyotypes and/or reverse transcription-polymerase chain reaction (PCR) analysis results of 12,948 patients diagnosed with AML according to the 2016 World Health Organization classification[11] between December 2012 and August 2022 at the Blood Disease Hospital, Chinese Academy of Medical Science. Patients with t(16;21)(p11;q22) or *FUS::ERG* fusion genes detected at the time of diagnosis were included in this study, whereas patients with these abnormalities detected at disease progression were excluded. All adult patients received anthracycline- and cytarabine-based induction therapy; etoposide was also included in the pediatric regimen. Patients received 2 or 3 courses of intermediate/high-dose cytarabine-based consolidation therapy or HSCT according to their risk stratification.[2,12] Written informed consent was obtained from all patients, and the study was conducted in accordance with the Declaration of Helsinki and approved by the Hospital Ethics Committee (NKRDP2021005-EC-2).

2.2. High throughput sequencing and data processing

Targeted sequencing was conducted for 21 patients; the sequenced genes are listed in Table S1, http://links.lww.com/BS/A94. Additionally, whole exome sequencing (WES) was performed for 4 of the 21 patients. Of the 21 patients, 6 adult patients with *FUS::ERG* AML had available RNA sequencing data. Healthy bone marrow mononuclear cells (BMMCs) from a published dataset (GSE120444)[13] and patients with *RUNX1::RUNX1T1* and *CBFB::MYH11* AML—2 more common AML subtypes with favorable prognosis, treated at our hospital, for which transcriptome results were available, were designated as controls. Details of the library preparation and data analysis are described in the Supplementary Material, http://links.lww.com/BS/A94.

2.3. Statistical analysis

OS was calculated from the date of diagnosis to the date of death or censoring. Event-free survival (EFS) was calculated from the date of diagnosis to the date of refractory disease, relapse, death, or censoring, whichever came first. Patients with a follow-up duration longer than 30 days were included in the survival analysis. OS and EFS were analyzed using the Kaplan–Meier method. Additionally, the survival data of patients with *RUNX1::RUNX1T1* and *CBFB::MYH11* AML treated at our center were analyzed as a reference. The imbalance in age and sex between the *FUS::ERG* and reference groups was corrected using propensity score matching analysis with 1:2 and 1:1 matching ratios for *RUNX1::RUNX1T1* and *CBFB::MYH11* subgroups, respectively. The effect of HSCT as a time-dependent variable was assessed using the Mantel–Byar test and depicted using Simon–Makuch plots,[14,15] in which only patients who achieved complete remission (CR) were analyzed; OS and EFS were calculated from the date of first CR. Significance was set at $P < .05$. Statistical analyses were performed using R software (version 4.3.1).

3. RESULTS

3.1. Baseline characteristics

A total of 36 consecutive patients with AML harboring t(16;21)(p11;q22)/*FUS::ERG* were included in this study. The median age of our cohort was 27 years (range: 1–64). Patients with t(16;21)(p11;q22)/*FUS::ERG* AML appeared to have a high tumor burden at diagnosis, as reflected by high lactate dehydrogenase levels (median: 838.5 U/L) and bone marrow blast counts (median: 71.5%). Seven patients (19.4%, 7/36) had central nervous system involvement at the time of the first lumbar puncture or relapse. M5 was the predominant French–American–British subtype. In terms of immunophenotype, the NK cell marker CD56 (94.3%, 33/35) and leukemia stem cell (LSC) marker CD123 (85.7%, 30/35) were expressed in most patients, similar to the results of previous reports.[1] Notably, the B cell marker cCD79a was partially or weakly expressed in 2 separate patients. According to the refined Medical Research Council risk stratification,[12] the majority (86.1%, 31/36) of patients with *FUS::ERG* AML had intermediate cytogenetic risk and more than half (54.5%, 12/22) of the patients with evaluable information were classified in the intermediate-risk group based on the 2022 ELN risk stratification (Table 1).[2]

3.2. Mutational landscape

In our cohort, 21 patients had targeted or WES data available. A total of 23 non-silent somatic mutations involving nine genes were identified by targeted sequencing, whereas 46 mutations involving 42 genes were identified by WES (**Fig. 1A**, Table S2,

Table 1

Characteristics of patients with AML harboring t(16;21)(p11;q22)/*FUS::ERG*.

	Overall
N	36
Male (%)	22/36 (61.1)
Age (median [range])	27 [1, 64]
WBC (×10^9/L, median [range])	13.5 [0.8, 157.5]
HGB (g/L, median [range])	85.8 [49.0, 128.0]
PLT (×10^9/L, median [range])	41.5 [2.0, 274.0]
LDH (U/L, median [range])	838.5 [211.5, 4574.0]
Bone marrow blast% (median [range])	71.5 [14.0, 98.0]
FAB subtype (%)	
M2	2/35 (5.7)
M4	1/35 (2.9)
M5	32/35 (91.4)
CD34 (%)	35/35 (100)
CD56 (%)	33/35 (94.3)
CD123 (%)	30/35 (85.7)
cCD79a (%)	2/35 (5.7)
Refined MRC cytogenetic risk stratification (%)	
Intermediate	31/36 (86.1)
Adverse	5/36 (13.9)
2022 ELN risk stratification (%)	
Intermediate	12/22 (54.5)
Adverse	10/22 (45.5)
Complex karyotype (%)	8/36 (22.2)
+8	7/36 (19.4)
CNS involvement	7/36 (19.4)
CR after 1st course (%)	13/29 (44.8)
CR after 2nd course (%)	26/29 (89.7)
Allo-HSCT (%)	15/36 (41.7)

allo-HSCT = allogeneic hematopoietic stem cell transplantation, AML = acute myeloid leukemia, CNS = central nervous system, CR = complete remission, ELN = European Leukemia Net, FAB = French–American–British classification system, HGB = hemoglobin, LDH = lactate dehydrogenase, MRC = Medical Research Council (criteria-based), PLT = platelet count, WBC = white blood cell count.

http://links.lww.com/BS/A94). Six patients had no mutations, as detected by targeted sequencing (Table S2, http://links.lww.com/BS/A94). By combining targeted sequencing and WES data, we identified a unique mutational profile of patients with *FUS::ERG* AML. The most prevalent somatic variants in our cohort were in the *NRAS* (33%, 7/21), *PTPN11* (24%, 5/21), *ARID1A* (10%, 2/21), *RUNX1* (10%, 2/21), and *WT1* (10%, 2/21) genes. Notably, the RTK–RAS signaling pathway was identified as being the pathway most involved after assigning functional categories to the mutated genes (*NRAS* and *PTPN11*), indicative of constitutive activation of RAS signaling leading to the promotion of AML cell survival and proliferation (**Fig. 1B**).[8] In particular, all but one *PTPN11* mutation affected the amino terminal (N)-SH2 domain. *NRAS* mutations occurred at amino acids G12, G13, Q61, and R102 (Table S2, http://links.lww.com/BS/A94).

3.3. Transcriptome analysis

We used healthy BMMCs as a control group (n = 8) to explore the expression profile of *FUS::ERG* AML. As expected, patients with *FUS::ERG* AML clustered separately from healthy controls following principal component and unsupervised hierarchical clustering analysis (**Fig. 2A, B**). A total of 3381 genes were found to be differentially expressed with a log2 fold change >2 and an adjusted *P* value <.05, among which 1543 genes were upregulated and 1838 genes were downregulated in *FUS::ERG* AML compared to the control group (Tables S3 and S4, http://links.lww.com/BS/A94). Notably, the leukemia stem/progenitor cell (LSPC) marker *CD34* and LSC marker *CD123* (*IL3RA*) were highly expressed, consistent with the immunophenotype of *FUS::ERG* AML (**Fig. 2C**, Table 1). Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of the differentially expressed genes (DEGs) revealed the upregulation of oncogenic pathways, including the phosphatidylinositol-3-kinase-Akt (PI3K–Akt), mitogen-activated protein kinase (MAPK), and RAS signaling pathways (**Fig. 2D**). The results of gene set enrichment analysis revealed upregulation of the gene set associated with LSCs (**Fig. 2E**). The application of deconvolution analysis to inspect the cell composition of *FUS::ERG* AML revealed the presence of hematopoietic stem cells, progenitor cells, monocytes, and neutrophils in the tumor microenvironment (Figure S1, http://links.lww.com/BS/A94).

To gain further insight regarding the expression profile characterizing *FUS::ERG* AML, we included available transcriptome data from patients with *RUNX1::RUNX1T1* (n = 13) and *CBFB::MYH11* (n = 7) AML, which are common AML subtypes that predict favorable outcome, for comparison. Principal component analysis and unsupervised hierarchical clustering showed that *FUS::ERG* clustered separately from *RUNX1::RUNX1T1* AML (**Fig. 3A, B**). Comparison of *FUS::ERG* with *RUNX1::RUNX1T1* AML identified 1778 DEGs, among which 947 were upregulated and 831 were downregulated (Tables S5 and S6, http://links.lww.com/BS/A94). DEGs were further subjected to KEGG analysis, which revealed upregulation of the PI3K–Akt, MAPK, and RAS signaling pathways (**Fig. 3C**). Similar results were observed when comparing *FUS::ERG* to *CBFB::MYH11* AML (Figure S2, http://links.lww.com/BS/A94 and Tables S7 and S8, http://links.lww.com/BS/A94). The application of consensus non-negative matrix factorization to the transcriptomes of patients with *FUS::ERG* and *RUNX1::RUNX1T1* AML, with $k = 2$ chosen to obtain better stability and interpretation, resulted in the identification of 2 clusters. In particular, *FUS::ERG* and *RUNX1::RUNX1T1* clustered exclusively into subgroups C1 and C2, respectively (**Fig. 3D**). The genes most associated with C1, namely the *FUS::ERG* subgroup, included the progenitor cell marker *CD44* and the anti-apoptotic gene *BCL2*, which was more highly expressed in the *FUS::ERG* than in the *RUNX1::RUNX1T1* subgroup (**Fig. 3E**). To identify factors that potentially contribute to *FUS::ERG*-mediated regulation of *BCL2*, we used the STRING database (v12.0) and found that *FUS::ERG* might modulate the expression of *BCL2* through the upregulation of *EZH2*, *KDR*, *RUNX3*, *H4C6*, *NTRK1*, *TNF*, and *CTSB* (Figure S3, http://links.lww.com/BS/A94).[16]

3.4. Clinical outcome

After 2 courses of chemotherapy, 89.7% (26/29) of patients achieved CR (Table 1). The remaining 3 patients attained CR

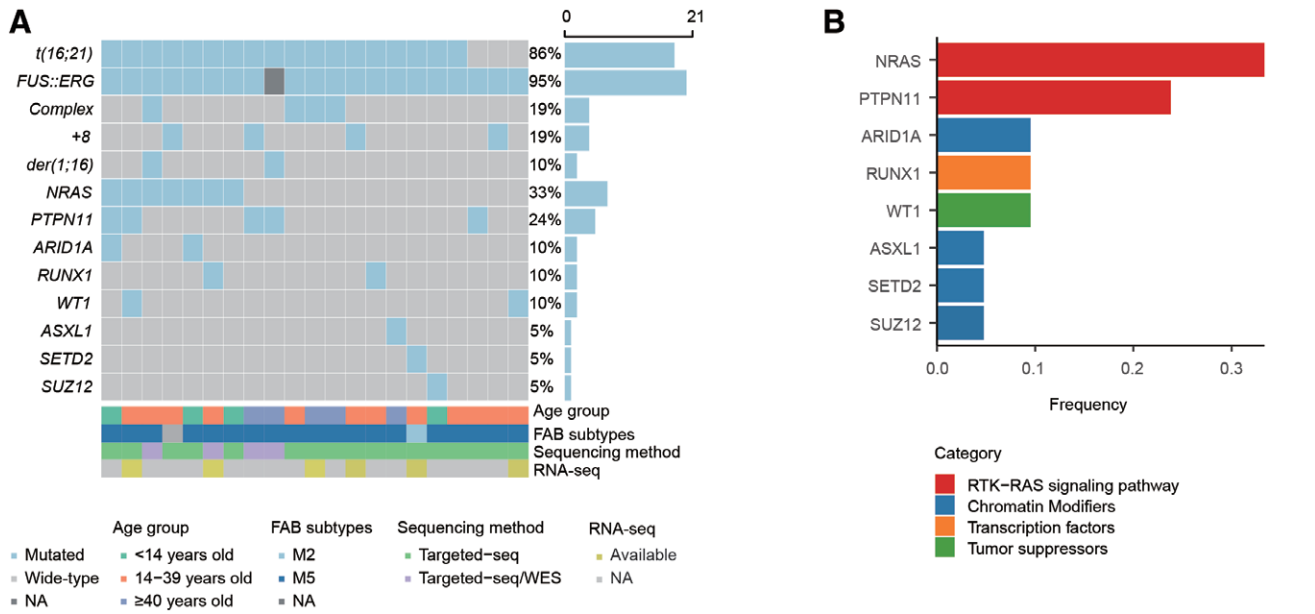

Figure 1. Genomic landscape of patients with t(16;21)(p11;q22)/*FUS::ERG* AML. (A) Oncoplot displaying recurrent cytogenetic abnormalities and hotspot mutations. (B) Functional categories of the mutated genes. AML = acute myeloid leukemia, NA = not available, RNA-seq = RNA sequencing, Targeted-seq = targeted sequencing, WES = whole exome sequencing.

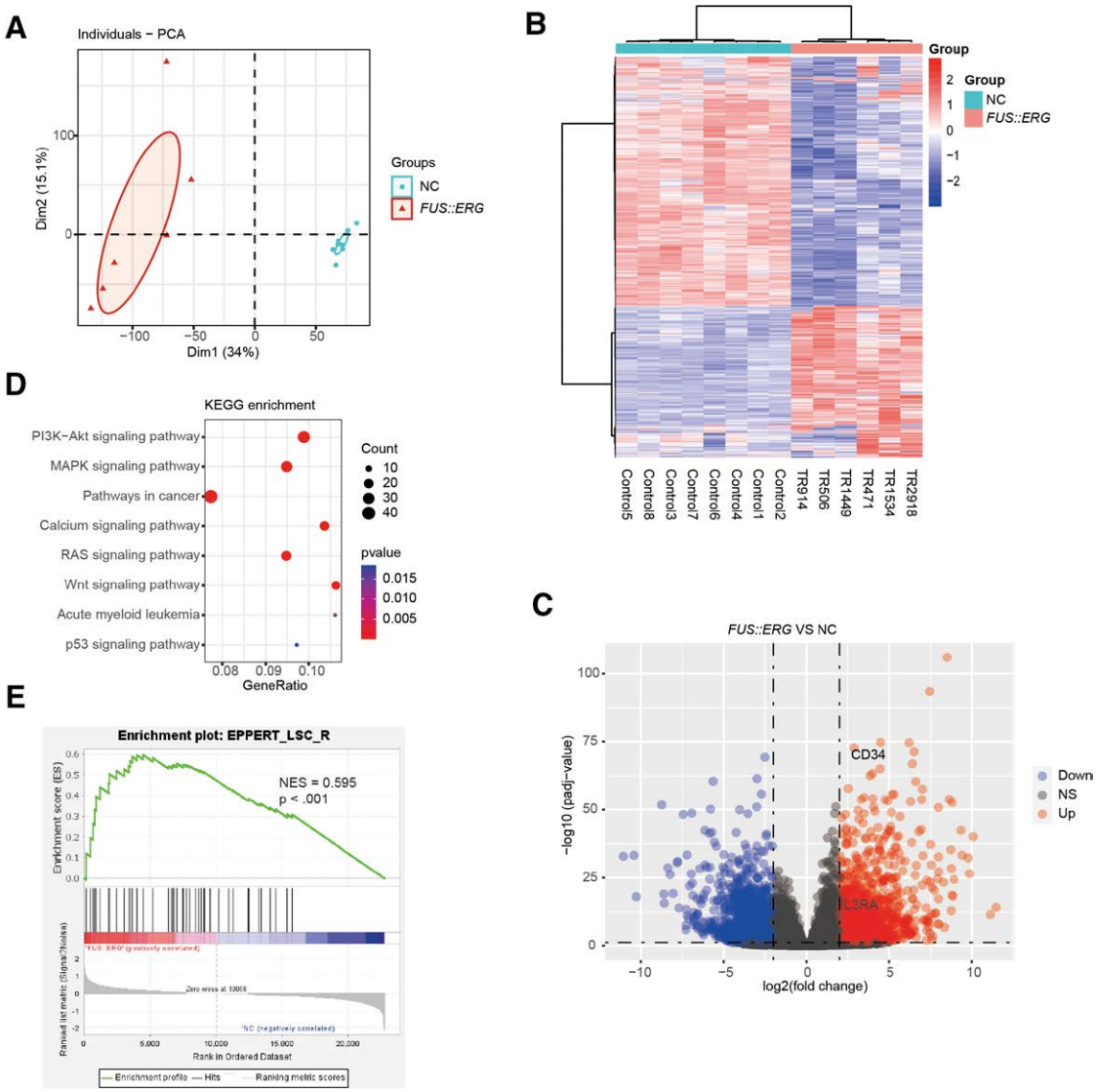

Figure 2. Transcriptome characteristics of patients with *FUS::ERG* AML compared with the normal control group. (A) PCA results of all expressing genes. (B) Unsupervised hierarchical clustering of top 2000 variable genes. (C) KEGG enrichment analysis depicting biologic terms associated with upregulated genes in patients with *FUS::ERG* AML. (D) Volcano plot indicating the upregulated and downregulated genes. (E) Gene set enrichment analysis plot showing the gene set associated with leukemia stem cells. AML = acute myeloid leukemia, KEGG = Kyoto Encyclopedia of Genes and Genomes, MAPK = mitogen-activated protein kinase, NC = normal control, NES = normalized enrichment score, *p*adj-value = adjusted *P* value, PCA = principal component analysis, PI3K-Akt = phosphatidylinositol-3-kinase-Akt, RAS = RAS GTPase.

after 3 courses of induction therapy. With a median follow-up of 14.9 months (95% confidence interval [CI]: 11.3–23.7), the median EFS of the patients with *FUS::ERG* AML was 11.9 months (95% CI: 9.0–not available [NA]) and the median OS was 18.2 months (95% CI: 12.4–NA), whereas the median EFS and OS of the reference *RUNX1::RUNX1T1* and *CBFB::MYH11* groups were not reached (EFS: $P < .001$; OS: $P < .001$) (**Fig. 4A, B**, Tables S9 and S10, http://links.lww.com/BS/A94).

Owing to the poor outcome of this AML subtype, allogeneic HSCT is highly recommended. Fifteen patients underwent allogeneic HSCT. Notably, 7 of 11 patients were positive for minimal residual disease (MRD) as assessed by flow cytometry (FCM) at the time of transplantation, whereas 1 of 4 FCM-negative patients still had detectable *FUS::ERG* by PCR. However, we did not observe a statistically significant effect of HSCT on improving the EFS (hazard ratio [HR] = 0.7, 95% CI: 0.2–2.5; $P = .650$) or OS (HR = 0.8, 95% CI: 0.2–3.0; $P = .765$) of our patients mainly owing to the limited sample size (**Fig. 4C, D**).

4. DISCUSSION

We retrospectively analyzed the clinical and molecular features of patients with t(16;21)(p11;q22)/*FUS::ERG* AML, identifying potential therapeutic targets for this subgroup. The survival outcome of patients with *FUS::ERG* AML was poor, and the beneficial effect of HSCT could not be determined owing in part to the limited sample size. In comparison, Pan et al[1] conducted a retrospective study of patients with *FUS::ERG* AML and found that HSCT could improve OS but not leukemia-free survival. Alternatively, Qin et al[4] demonstrated that 5 of 11 patients with AML harboring *FUS::ERG* who underwent haploidentical transplantation were alive without relapse. The heterogeneity of the chemotherapy regimen and supportive care, as well as the small sample size in each study, might account for such inconsistent results. Furthermore, most patients in the HSCT group in our cohort had MRD as detected by either FCM or PCR prior to transplantation. This suggested that traditional chemotherapy failed to eradicate leukemic cells in these patients, which might partially explain the limited effect of HSCT in our study.[4,17]

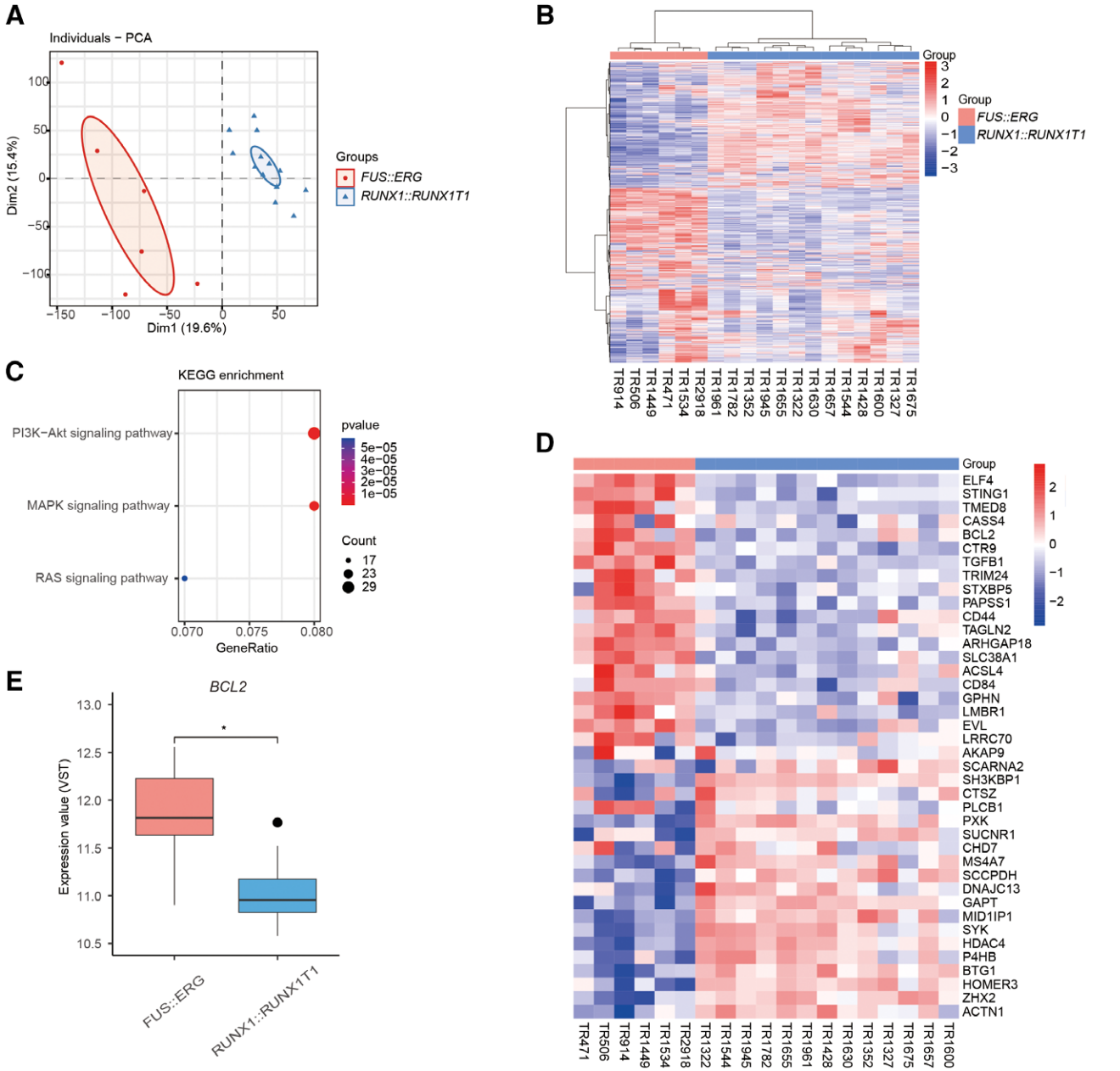

Figure 3. Transcriptome characteristics of patients with *FUS::ERG* compared with *RUNX1::RUNX1T1* AML (A) PCA results of all expressing genes. (B) Unsupervised hierarchical clustering of top 2000 variable genes. (C) KEGG enrichment analysis depicting biologic terms associated with upregulated genes in patients with *FUS::ERG* versus *RUNX1::RUNX1T1* AML. (D) Heatmap depicting the relative expression value of the top 20 genes most associated with cNMF C1 and C2 subgroups. (E) Boxplot showing the expression value of *BCL2* in the 2 fusion-gene groups, **P* < .05, ● outlier. AML = acute myeloid leukemia, cNMF = consensus non-negative matrix factorization, KEGG = Kyoto Encyclopedia of Genes and Genomes, MAPK = mitogen-activated protein kinase, PCA = principal component analysis, PI3K-Akt = phosphatidylinositol-3-kinase-Akt, RAS = RAS GTPase, VST = variant stabilizing transformation using DESeq2.

As an alternative, we performed genomic and transcriptomic sequencing to identify novel therapeutic targets for this AML subtype. Genomic analysis demonstrated that mutations involved in the RTK–RAS signaling pathway, including in the *NRAS* and *PTPN11* genes, were enriched in patients with *FUS::ERG* AML. Larger cohort studies suggested that *PTPN11* mutation could lead to worse OS of patients with AML.[18] Additionally, patients harboring mutations in the N-terminal SH2 domain of *PTPN11* had an increased rate of early death.[19] The functional cooperation of mutated *PTPN11* and *NRAS* with an oncogenic fusion protein has been demonstrated in a preclinical AML model.[5,6,20] Specifically, accelerated leukemogenesis was observed with an increase of LSCs and activation of anti-apoptotic pathway.[5] However, direct targeting of the mutated RAS protein had limited clinical effect in solid tumors.[21] Targeting downstream proteins including RAF using the pan-RAF inhibitor LY30098120 and shp2 (*PTPN11*) using the allosteric inhibitor RMC4550 or SHP099 might thus be an alternative strategy for the treatment of *FUS::ERG* AML with RTK–RAS signaling pathway mutations.[22–24]

Transcriptome analysis revealed a distinct expression profile in patients with *FUS::ERG* AML compared to that in normal BMMCs and patients with AML harboring the fusion genes *RUNX1::RUNX1T1* or *CBFB::MYH11*. The LSPC marker *CD34*, LSC marker *CD123*, and LSC-related gene set were expressed at higher levels in *FUS::ERG* AML than in the normal control, suggesting an oncogenic role of *FUS::ERG*.[10,25] Although *NRAS* mutation was also frequently

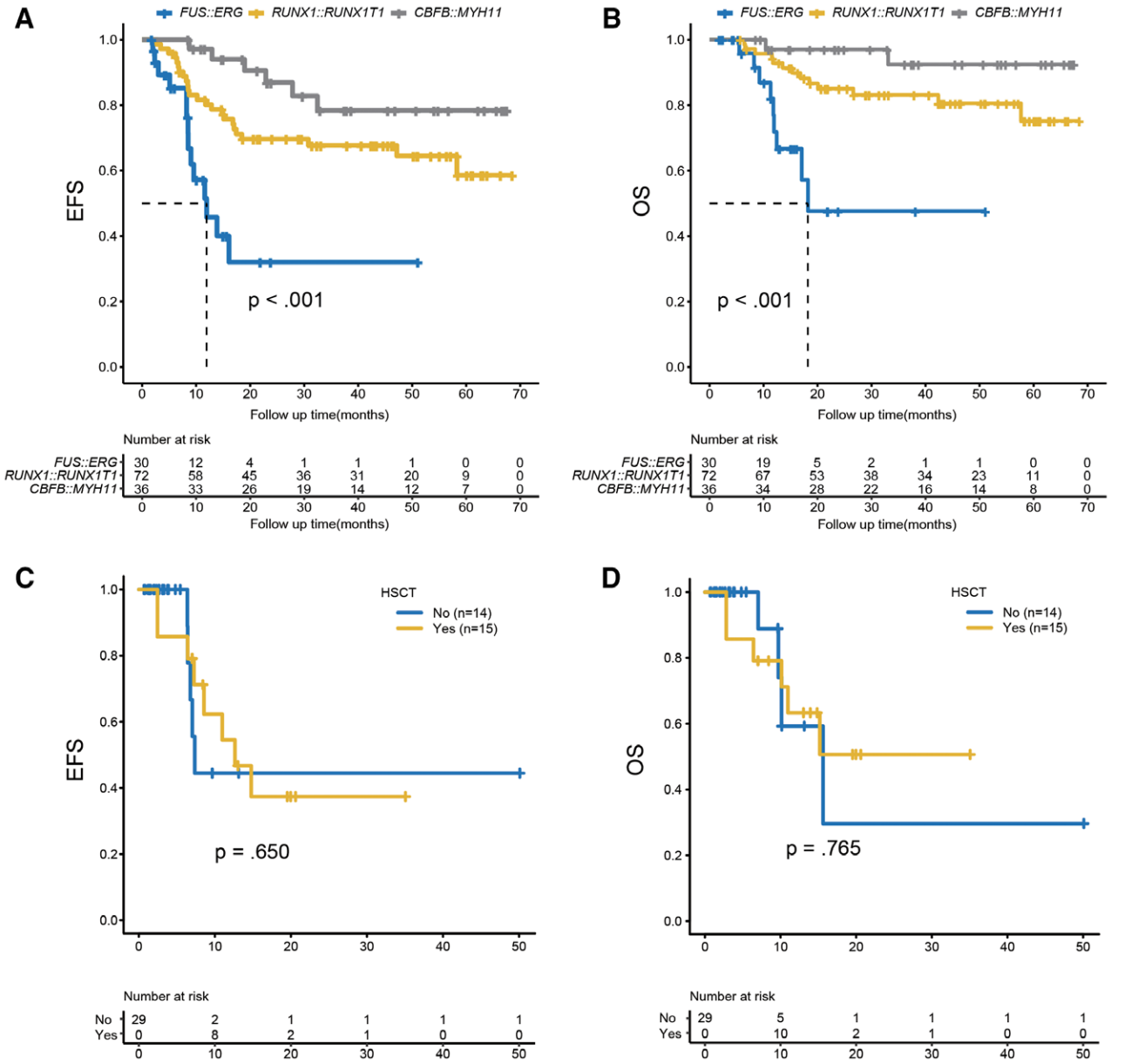

Figure 4. Clinical outcome of patients with *FUS::ERG* AML. (A–B) Kaplan–Meier curves showing the EFS and OS of patients with *FUS::ERG* AML and the reference groups; (C–D) Simon–Makuch plot depicting the effect of HSCT in patients with *FUS::ERG* AML who finally achieved CR. AML = acute myeloid leukemia, CR = complete remission, EFS = event-free survival, HSCT = hematopoietic stem cell transplantation, OS = overall survival.

present in patients with *RUNX1::RUNX1T1* (21.8%, 42/193) or *CBFB::MYH11* (61.2%, 52/85) AML, KEGG pathway analysis revealed that the PI3K–Akt, MAPK, and RAS signaling pathways were upregulated in *FUS::ERG* AML compared to normal control and the *RUNX1::RUNX1T1* and *CBFB::MYH11* fusion-gene groups, consistent with the aggressive phenotype of *FUS::ERG* AML.[21] Moreover, the anti-apoptosis gene *BCL2* was upregulated in *FUS::ERG* versus *RUNX1::RUNX1T1* AML, corresponding to prior findings that *FUS::ERG* knockdown resulted in the downregulation of *BCL2* and induced cell death.[26] Further studies are needed to elucidate the mechanism by which the fusion protein regulates the expression of *BCL2*. Nevertheless, combining venetoclax inhibition of BCL2 and intensive chemotherapy is an effective treatment in newly diagnosed AML, inducing high MRD-negative CR rates, which could translate into improved survival.[27,28] Therefore, combined regimens, including venetoclax plus intensive chemotherapy to eradicate the frequently presenting MRD, followed by HSCT, might improve the outcome of *FUS::ERG* AML.

In conclusion, we identified *NRAS* and *PTPN11* as the most frequent mutations and the RTK–RAS signaling pathway as the most involved pathway in patients with the rare AML harboring t(16;21)(p11;q22)/*FUS::ERG* subtype. The addition of signaling pathway inhibitors and/or venetoclax, followed by HSCT, may be an effective strategy to overcome the poor outcomes associated with this subtype. Our study is limited by the small number of *FUS::ERG* cases, as the incidence of this fusion gene in AML is low. Larger cohort studies are warranted to further investigate the molecular characteristics and evaluate the clinical activity of small-molecule inhibitors in patients with *FUS::ERG* AML.

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ETHICAL APPROVAL

The study was in accordance with the Declaration of Helsinki and approved by the Hospital Ethics Committee (NKRDP2021005-EC-2).

AUTHOR CONTRIBUTIONS

S.Q. and J.W. were responsible for designing the study. S.Q., W.L., and A.L. were responsible for analyzing and interpreting the data and writing the report. All authors contributed to the collection and assembly of clinical and mutational data. H.W., Y.W., D.L., C.Z., B.L., S.Q., R.G., Y.L., S.W., B.G., K.L., X.G., Y.L, G.Z., and J.Z. provided the patients and materials. All authors reviewed the article and approved the final version.

REFERENCES

[1] Pan J, Zhang Y, Zhao YL, et al. Impact of clinical factors on outcome of leukemia patients with TLS-ERG fusion gene. *Leuk Lymphoma* 2017;58(7):1655–1663. doi:10.1080/10428194.2016.1260124.

[2] Döhner H, Wei AH, Appelbaum FR, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood* 2022;140(12):1345–1377. doi:10.1182/blood.2022016867.

[3] Buteyn NJ, Burke CG, Smith JL, et al. EZH2-mediated MHC class II silencing drives immune evasion in AML with t(16;21) (FUS-ERG). *Blood* 2021;138(Suppl 1):374–374. doi:10.1182/blood-2021-153256.

[4] Qin YZ, Chen Y, Xu LP, et al. Outcome and minimal residual disease monitoring in patients with t(16;21) acute myelogenous leukemia undergoing allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2018;24(1):163–168. doi:10.1016/j.bbmt.2017.09.002.

[5] Chen L, Chen W, Mysliwski M, et al. Mutated Ptpn11 alters leukemic stem cell frequency and reduces the sensitivity of acute myeloid leukemia cells to Mcl1 inhibition. *Leukemia* 2015;29(6):1290–1300. doi:10.1038/leu.2015.18.

[6] Xue L, Pulikkan JA, Valk PJ, Castilla LH. NrasG12D oncoprotein inhibits apoptosis of preleukemic cells expressing Cbfβ-SMMHC via activation of MEK/ERK axis. *Blood* 2014;124(3):426–436. doi:10.1182/blood-2013-12-541730.

[7] Zerkalenkova E, Panfyorova A, Kazakova A, et al. Molecular characteristic of acute leukemias with t(16;21)/FUS-ERG. *Ann Hematol* 2018;97(6):977–988. doi:10.1007/s00277-018-3267-z.

[8] Doepfner KT, Boller D, Arcaro A. Targeting receptor tyrosine kinase signaling in acute myeloid leukemia. *Crit Rev Oncol Hematol* 2007;63(3):215–230. doi:10.1016/j.critrevonc.2007.05.005.

[9] Stone RM, Mandrekar SJ, Sanford BL, et al. Midostaurin plus chemotherapy for acute myeloid leukemia with a FLT3 mutation. *N Engl J Med* 2017;377(5):454–464. doi:10.1056/NEJMoa1614359.

[10] Noort S, Oosterwijk JV, Ma J, et al. Analysis of rare driving events in pediatric acute myeloid leukemia. *Haematologica* 2023;108(1):48–60. doi:10.3324/haematol.2021.280250.

[11] Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood* 2016;127(20):2391–2405. doi:10.1182/blood-2016-03-643544.

[12] Grimwade D, Hills RK, Moorman AV, et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood* 2010;116(3):354–365. doi:10.1182/blood-2009-11-254441.

[13] Oetjen KA, Lindblad KE, Goswami M, et al. Human bone marrow assessment by single-cell RNA sequencing, mass cytometry, and flow cytometry. *JCI Insight* 2018;3(23):e124928. doi:10.1172/jci.insight.124928.

[14] Mantel N, Byar DP. Evaluation of response-time data involving transient states: an illustration using heart-transplant data. *J Am Stat Assoc* 1974;69(345):81–86. doi:10.2307/2285503.

[15] Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* 2013;48(3):452–458. doi:10.1038/bmt.2012.244.

[16] Szklarczyk D, Gable AL, Nastou KC, et al. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res* 2021;49(D1):D605–D612. doi:10.1093/nar/gkaa1074.

[17] Buckley SA, Wood BL, Othus M, et al. Minimal residual disease prior to allogeneic hematopoietic cell transplantation in acute myeloid leukemia: a meta-analysis. *Haematologica* 2017;102(5):865–873. doi:10.3324/haematol.2016.159343.

[18] Alfayez M, Issa GC, Patel KP, et al. The Clinical impact of PTPN11 mutations in adults with acute myeloid leukemia. *Leukemia* 2021;35(3):691–700. doi:10.1038/s41375-020-0920-z.

[19] Fobare S, Kohlschmidt J, Ozer HG, et al. Molecular, clinical, and prognostic implications of PTPN11 mutations in acute myeloid leukemia. *Blood Adv* 2022;6(5):1371–1380. doi:10.1182/bloodadvances.2021006242.

[20] Mohanty S, Jyotsana N, Sharma A, et al. Targeted inhibition of the NUP98-NSD1 fusion oncogene in acute myeloid leukemia. *Cancers (Basel)* 2020;12(10):2766. doi:10.3390/cancers12102766.

[21] Yuan J, Dong X, Yap J, Hu J. The MAPK and AMPK signalings: interplay and implication in targeted cancer therapy. *J Hematol Oncol* 2020;13(1):113. doi:10.1186/s13045-020-00949-4.

[22] Khoury JD, Tashakori M, Yang H, et al. Pan-RAF inhibition shows anti-leukemic activity in RAS-mutant acute myeloid leukemia cells and potentiates the effect of sorafenib in cells with FLT3 mutation. *Cancers (Basel)* 2020;12(12):1–13. doi:10.3390/cancers12123511.

[23] Popescu B, Stahlhut C, Tarver TC, III, et al. Allosteric SHP2 inhibitor RMC4550 synergizes with venetoclax in FLT3 and KIT mutant acute myeloid leukemia. *Blood* 2021;138(Suppl 1):2231–2231. doi:10.1182/blood-2021-145964.

[24] Wu J, Zhang H, Zhao G, Wang R. Allosteric inhibitors of SHP2: an updated patent review (2015-2020). *Curr Med Chem* 2021;28(19):3825–3842. doi:10.2174/1568011817666200928114851.

[25] Yuen KC, Tran B, Anton A, et al. Molecular classification of hormone-sensitive and castration-resistant prostate cancer, using non-negative matrix factorization molecular subtyping of primary and metastatic specimens. *Prostate* 2022;82(9):993–1002. doi:10.1002/pros.24346.

[26] Sotoca AM, Prange KH, Reijnders B, et al. The oncofusion protein FUS-ERG targets key hematopoietic regulators and modulates the all-trans retinoic acid signaling pathway in t(16;21) acute myeloid leukemia. *Oncogene* 2016;35(15):1965–1976. doi:10.1038/onc.2015.261.

[27] DiNardo CD, Lachowiez CA, Takahashi K, et al. Venetoclax combined with FLAG-IDA induction and consolidation in newly diagnosed and relapsed or refractory acute myeloid leukemia. *J Clin Oncol* 2021;39(25):2768–2778. doi:10.1200/jco.20.03736.

[28] Walter RB, Buckley SA, Pagel JM, et al. Significance of minimal residual disease before myeloablative allogeneic hematopoietic cell transplantation for AML in first and second complete remission. *Blood* 2013;122(10):1813–1821. doi:10.1182/blood-2013-06-506725.