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CASE REPORT

RUNX1::CBFA2T2 rearranged acute myeloid leukemia transformed from *JAK2* V617F mutated primary myelofibrosis

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Abstract

Acute myeloid leukemia (AML) with *RUNX1::CBFA2T2* fusion is rare with largely unknown clinicopathological features and genomic characterization. We present one such case of AML transformed from *JAK2* V617F mutated primary myelofibrosis and review the literature on this topic. The immunophenotype and the landscape of cooperative gene alterations in AML with *RUNX1::CBFA2T2* resemble those of AML with *RUNX1::RUNX1T1*, including expression of CD19, cooperative gene alterations in signaling pathway (*JAK2*), epigenetic/chromatin and cell cycle regulation (*TET2*, *SMC3*, and *CDKN2A/B*), and additional chromosomal abnormalities (trisomies 8 and 15). This case study provides insights into the pathogenesis of this rare subtype of AML.

KEYWORDS
acute myeloid leukemia, *JAK2*, primary myelofibrosis, *RUNX1::CBFA2T2*, *RUNX1::RUNX1T1*

1 | INTRODUCTION

Acute myeloid leukemia (AML) with *RUNX1::RUNX1T1* fusion, resulting from chromosomal translocation t(8;21)(q22;q22.1), is one of the core-binding factor (CBF) leukemias. This subtype constitutes 1%–5% of AML, characterized by blasts expressing CD19/CD34, presence of additional cytogenetic abnormalities, and a favorable clinical outcome [1–3]. The *RUNX1::RUNX1T1* fusion forms a co-repressor complex, blocking the *RUNX1*-mediated genes involved in regulation of myeloid differentiation [4]. Cooperative gene mutations, such as *KIT*, *NRAS*, *FLT3*-ITD, *ASXL1*, and *SMC3*, are thought to be required in the *RUNX1::RUNX1T1* fusion-induced leukemogenesis [5, 6].

RUNX1T1 is one of the myeloid translocation gene (MTG) family members, which consists of *RUNX1T1* (also known as *MTG8*, *CBFA2T1*, or *ETO*), *CBFA2T2* (*MTGR1*), and *CBFA2T3* (*MTG16* or *RUNX1T3*) [7]. The high homology between *CBFA2T2*, *RUNX1T1*, and *CBFA2T3* suggests that when fused with *RUNX1*, all three chimeric genes may have similar effects on leukemogenesis [8]. Several reports have shown similarity of AML with t(16;21)(q24;q22)/*RUNX1::CBFA2T3* to AML with *RUNX1::RUNX1T1* with regard to morphology, immunophenotype, gene expression profiling, and response to therapy [9, 10].

RUNX1::CBFA2T2 is a rare gene rearrangement in AML, with only four patients having been previously reported [8, 11–13]. The clinicopathological and molecular features of AML harboring *RUNX1::CBFA2T2* are largely unknown. This case report is the first study to characterize the morphology, immunophenotype, cooperative gene alterations, and clonal evolution of this rare subtype of AML.

2 | METHOD

Clinical data were retrieved from the electronic medical records. Flow cytometric immunophenotyping, morphologic evaluation, genetic karyotyping, and comprehensive pan-cancer next-generation sequencing (NGS) were performed, as described previously [14].

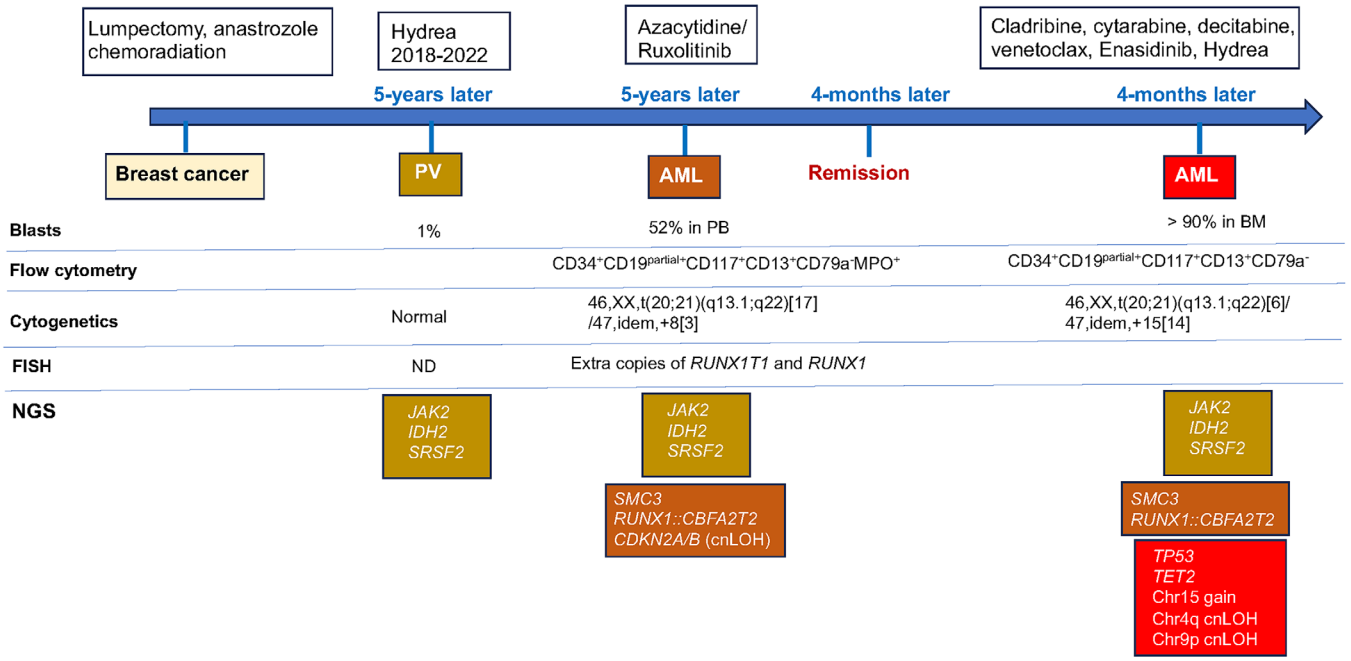

FIGURE 1 Timeline of the patient's clinical course including disease progression, treatment, cytogenetic/FISH, immunophenotype by flow cytometry, and gene alterations by NGS (cnLOH, copy neutral loss of heterozygosity).

3 | CASE DESCRIPTIONS

A 62-year-old female with a history of breast cancer (treated with lumpectomy, anastrozole, radiation, and adjuvant chemotherapy) was diagnosed with primary myelofibrosis (PMF, MF1-2, prefibrotic, 1% blasts) 5 years later, with mutations in *JAK2* (p.V617F, variant allele frequency [VAF%] 51%), *IDH2* (p.R140, 48%), and *SRSF2* (p.P95R, 47%), and normal cytogenetics (Figure 1). Four years after the PMF diagnosis, she presented with petechiae and generalized weakness. Laboratory studies showed leukocytosis (WBC 15.7 × 10^9/L), anemia (Hb 10.4 g/dL), thrombocytopenia (platelet 38 × 10^9/L), and 44% circulating myeloblasts in peripheral blood (PB). A bone marrow (BM) evaluation was limited by a dry tap. Flow cytometric analysis on a PB sample revealed myeloblasts with the following immunophenotype: $CD34^{bright+}$/$CD19^{partial+}$/$CD38^+$/$CD13^+$/$CD15^+$/$CD33^+$/$CD117^+$ /HLA-DR^+/MPO^+/$CD79^-$/$CD20^-$/$CD22^-$/$CD3^-$ (Figure 2A, Table 1). Cytogenetic analysis revealed an abnormal karyotype with two related clones harboring t(20;21):46,XX,t(20;21)(q13.1;q22)[17]/47, idem,+8[3] (Figure 2B). An AML FISH panel analysis showed extra copies of *RUNX1* and *RUNX1T1* in 93% and 7% of examined cells, respectively. An NGS study demonstrated persistent mutations in *JAK2*, *IDH2*, and *SRSF2* (at similar VAF to the previously diagnosed PMF except for an increased *JAK2* VAF to 97.5%), with additional gene alterations in *SMC3* (p.R381Q), *CDKN2A/2B* (deletion), and *RUNX1::CBFA2T2* (resulting from t(20;21)(q13.1;q22)) (Figure 1). These results demonstrate clonal evolution and transformation of PMF to AML.

The patient was treated with azacytidine/ruxolitinib and achieved remission after 4 months. Her AML relapsed 5 months later at which time she was treated with cytarabine, venetoclax, and enasidenib. Repeat BM evaluation showed persistent AML with 90% blasts (Figure 2D). Flow cytometric analysis revealed 69% neoplastic myeloblasts with a similar immunophenotype to prior analysis. Cytogenetic studies identified recurrent abnormal karyotype with an additional clone with trisomy 15 (Figure 2C). NGS detected recurrent gene alterations with additional alterations in *TP53* (p.P152R, 40.2%) and *TET2* (p.M906V) (Figure 2E). These results indicate continued clonal evolution at relapse. The patient expired 13 months after initial diagnosis of AML.

4 | DISCUSSION

AML with *RUNX1::CBFA2T2* fusion is extremely rare, with only four patients reported in the literature (Table 1) and with largely unknown clinicopathological and genomic features [8, 11–13]. A summarized analysis, including our patient, suggests that *RUNX1::CBFA2T2* rearranged AML is characterized by a male predominance and variable age of onset ranging from infancy to elderly. Three patients were diagnosed as de novo AML and two patients as secondary AML. Patient 4 had an extramedullary AML relapse post-transplant of *FIP1L1::PDGFRA* rearranged AML. Our patient (Patient 5) had an AML transformed from *JAK2* mutated primary myelofibrosis. The prognosis of *RUNX1::CBFA2T2* rearranged AML is largely unknown; two patients were deceased, and our patient died 13 months after AML diagnosis.

The immunophenotypic features of this subtype of AML were only available in two patients, which showed distinctly different profiles. Patient 4 had monocytic differentiation

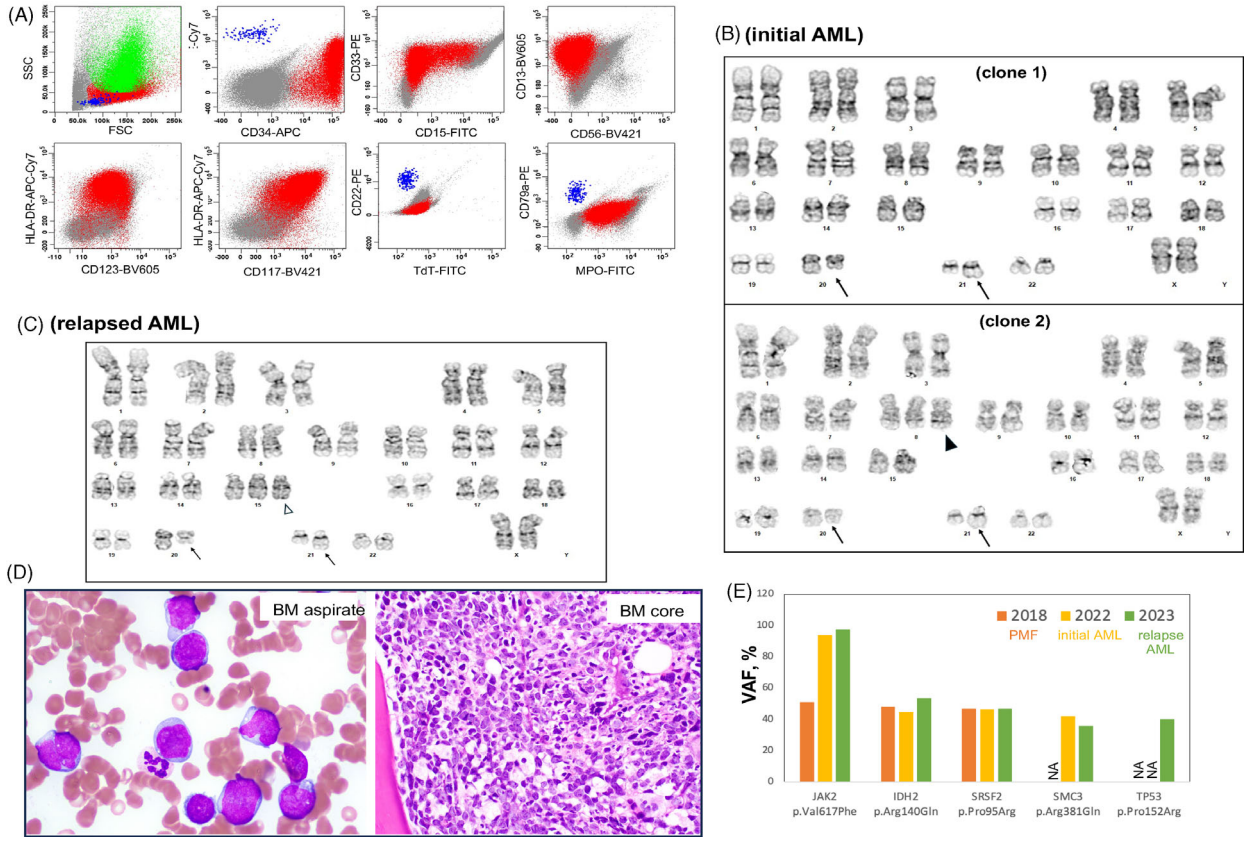

FIGURE 2 Clinicopathological and molecular characteristics of *RUNX1::CBFA2T2* rearranged AML transformed from *JAK2* mutated primary myelofibrosis. (A) Flow cytometric immunophenotype of bone marrow aspirate. Myeloblasts (in red) are medium sized to large with low side scatter, positive for CD34 (bright), CD33, CD13, HLA-DR, CD117, and MPO, partially positive for CD19 and CD15, and negative for CD22, CD79a, or TdT. Color code: green, granulocytes; blue, normal B cells. (B) and (C) The abnormal karyotype at initial AML (C): clone 1 with t(20;21)(q13.1;q22) (arrow), and clone 2 with additional trisomy 8 (arrowhead); and at relapsed AML (D): second clone with additional trisomy 15 (arrowhead). (D) The morphology of myeloblasts in relapsed AML: medium-sized to large blasts in BM aspirate (Wright–Giemsa stain, 1000×) and core biopsy stain (hematoxylin and eosin stain, 200×). (E) The variant allele frequency (VAF%) of gene mutations detected by NGS. ND, not detected.

TABLE 1 Literature review on *RUNX1::CBFA2T2* rearranged AML.

Cases	Diagnosis	Age/sex	Immunophenotype	Cytogenetics	Cooperative gene alterations	F/U	Survival
Patient 1[11]	de novo AML	1.5/M	NA	47,XY,+8,t(14;20)(q11.2;q11.2),del(21)(q21q2)	No *FLT3*-ITD (NA on other genes)	NA	Alive
Patient 2[12]	de novo AML	Infant/NA	NA	NA	NA	NA	NA
Patient 3[8]	de novo AML	69/M	NA	46,XY,ins(21;20)(q22.12;q13.33)[5]/46,indem,del(7)(q22)[7]/46,XY[4]	NA	NA	Dead
Patient 4[13]	Extramedullary relapse (post-transplant for AML *FIP1L1::PDGFRA)*	25/M	Monocytic diff: $CD45^{-}CD34^{-}CD117^{-}$ $CD19^{-}CD33^{+}CD64^{+}$ $HLADR^{+}$ $CD56^{+}CD15^{+}$ $CD13^{+}$ $CD11c^{+}MPO^{+}$	NA	*FIP1L1::PDGFRA* (no other gene alterations reported by NGS)	280 days[a]	NA
Patient 5 (our patient)	AML from PMF (breast cancer)	64/F	Myeloblast diff: $CD45^{+}CD34^{bgt+}CD19^{partial+}$ $CD15^{partial+}CD13^{+}CD117^{+}$ $CD33^{+}HLA\text{-}DR^{+}/MPO^{+}$	46,XX,t(20;21)(q13.1;q22)[17]/47,dem,+8[3]	*JAK2*, *IDH2*, *SRSF2*, *SMC3*, *TP53*, *TET2*	4 years[b]	Dead

Abbreviations: AML, acute myeloid leukemia; bgt, bright; diff, differentiation; F, female; F/U, follow-up; M, male; NA, not available; PMF, primary myelofibrosis.
[a]Interval from transplant to extramedullary AML relapse.
[b]Interval from PMF to AML.

(CD34^{-}/CD117^{-}/CD19^{-}/monocytic markers^{+}) and presented with extramedullary relapsed AML harboring both *FIP1L1::PDGFRA* and *RUNX1::CBFA2T2* fusions (without other gene alterations) [13]. Our patient had myeloblast differentiation (CD34$^{bright+}$/CD117^{+}/CD19$^{partial+}$/MPO^{+}) and presented as transformed AML (from *JAK2* mutated PMF) harboring *RUNX1::CBFA2T2* fusion with other cooperative gene alterations. It is unknown whether these different immunophenotypes are associated with extramedullary infiltration and/or cooperative gene alterations. Nonetheless, our case presented with an immunophenotype resembling AML with *RUNX1::RUNX1T1* and with *RUNX1::CBFA2T3*, such as bright CD34 expression and aberrant expression of CD19 on myeloblasts [1–3, 9]. Differentiating between these CD19(+) *RUNX1* rearranged AML requires cytogenetic and molecular studies. Additionally, CD19 expression raises the differential diagnosis of mixed phenotype acute leukemia (MPAL). Evaluation of other B lineage markers, such as CD20, CD22, and CD79a, is important to distinguish AML from MPAL.

Our case study is the first to reveal the landscape of cooperative gene alterations in *RUNX1::CBFA2T2* rearranged AML. A recent case of *RUNX1::CBFA2T2* and *FIP1L1::PDGFRA* rearranged AML did not report any additional gene alterations [13]. Our case of *RUNX1::CBFA2T2* rearranged AML transformed from *JAK2/IDH2/SRSF2* mutated PMF demonstrates cooperative gene alterations in signaling pathways (*JAK2*), epigenetic/chromatin and cell cycle regulations (*SMC3*, *TET2*, and *CDKN2A/B*), and tumor suppressor genes (*TP53*), along with chromosomal abnormalities during development and progression of AML. This genomic alteration landscape is similar to that of AML with *RUNX1::RUNX1T1* [5, 6], and provides insight into the pathogenesis of this rare AML subtype.

In conclusion, this is the first case study to describe the clinicopathological and genomic features of *RUNX1::CBFA2T2* rearranged AML transformed from *JAK2* mutated PMF and demonstrates that the immunophenotype and the landscape of cooperative gene alterations resemble those in *RUNX1::RUNX1T1* rearranged AML. Future studies on larger cohorts of patients are needed to further characterize the clinicopathologic and molecular landscape of this disease, which in turn may guide evidence-based treatment rationales for improved clinical management.

AUTHOR CONTRIBUTIONS

Lina Han and Weina Chen conceptualized the case study and wrote the original draft. All authors critically revised the manuscript and approved the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest.

FUNDING INFORMATION

The authors received no specific funding for this work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

All procedures performed in this case study were part of the clinical management in accordance with the ethical standards.

CLINICAL TRIAL REGISTRATION

The authors have confirmed clinical trial registration is not needed for this submission.

PATIENT CONSENT STATEMENT

Patient consent statement was waived per approved protocol (IRB STU 122013-023).

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REFERENCES

1. Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka HM, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Blood. 2022;140(11):1200–1228.
2. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization classification of haematolymphoid tumours: myeloid and histiocytic/dendritic neoplasms. Leukemia. 2022;36(7):1703–19.
3. Han SY, Mrozek K, Voutsinas J, Wu Q, Morgan EA, Vestergaard H, et al. Secondary cytogenetic abnormalities in core-binding factor AML harboring inv(16) vs t(8;21). Blood Adv. 2021;5(10):2481–89.
4. Al-Harbi S, Aljurf M, Mohty M, Almohareb F, Ahmed SOA. An update on the molecular pathogenesis and potential therapeutic targeting of AML with t(8;21)(q22;q22.1);RUNX1-RUNX1T1. Blood Adv. 2020;4(1):229–38.
5. Krauth MT, Eder C, Alpermann T, Bacher U, Nadarajah N, Kern W, et al. High number of additional genetic lesions in acute myeloid leukemia with t(8;21)/RUNX1-RUNX1T1: frequency and impact on clinical outcome. Leukemia. 2014;28(7):1449–58.
6. Faber ZJ, Chen X, Gedman AL, Boggs K, Cheng J, Ma J, et al. The genomic landscape of core-binding factor acute myeloid leukemias. Nat Genet. 2016;48(12):1551–56.
7. Davis JN, McGhee L, Meyers S. The ETO (MTG8) gene family. Gene. 2003;303:1–10.
8. Guastadisegni MC, Lonoce A, Impera L, Di Terlizzi F, Fugazza G, Aliano S, et al. CBFA2T2 and C20orf112: two novel fusion partners of RUNX1 in acute myeloid leukemia. Leukemia. 2010;24(8):1516–19.
9. Liu H, Wang SA, Schlette EJ, Xu J, Jorgensen JL, Cameron Yin C, et al. Myeloid neoplasms with t(16;21)(q24;q22)/RUNX1-RUNX1T3 mimics acute myeloid leukemia with RUNX1-RUNX1T1. Ann Hematol. 2018;97(10):1775–83.
10. Lavallee VP, Lemieux S, Boucher G, Gendron P, Boivin I, Armstrong RN, et al. RNA-sequencing analysis of core binding factor AML identifies recurrent ZBTB7A mutations and defines RUNX1-CBFA2T3 fusion signature. Blood. 2016;127(20):2498–501.
11. Shiba N, Yoshida K, Hara Y, Yamato G, Shiraishi Y, Matsuo H, et al. Transcriptome analysis offers a comprehensive illustration of the genetic background of pediatric acute myeloid leukemia. Blood Adv. 2019;3(20):3157–69.
12. Bolouri H, Farrar JE, Triche T Jr, Ries RE, Lim EL, Alonzo TA, et al. The molecular landscape of pediatric acute myeloid leukemia reveals recurrent structural alterations and age-specific mutational interactions. Nat Med. 2018;24(1):103–12.

13. Panda D, Pathak A, Tejwani N, Pandey P, Mehta A. Acquired RUNX1::CBFA2T2 fusion at extramedullary relapse in a patient of PDGFRA rearranged acute myeloid leukemia post allogenic HSCT. Cytometry B Clin Cytom. 2023;104(5):404–6.
14. Zheng R, Fuda F, Gagan JR, Weinberg OK, Koduru P, Cantu M, et al. Genomic heterogeneity within B/T mixed phenotype acute leukemia in a context of an immunophenotype. Leuk Res Rep. 2024;21:100410.

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