LETTERS TO THE EDITOR
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Identification of a Novel *MAPK1::BCR* Fusion Gene/t(9;22)(q34;q11) in a Patient with Acute Promyelocytic Leukemia

Wang Q. et al.: A Novel *MAPK1::BCR* in a Patient with APL

Qian Wang[1,2]*, Ling-ji Zeng[3]*, Man Wang[1,2], Jian-yu Weng[3], Jin-lan Pan[1,2]
[1]National Clinical Research Center for Hematologic Diseases, Jiangsu Institute of Hematology, the First Affiliated Hospital of Soochow University, Suzhou, China
[2]Institute of Blood and Marrow Transplantation, Collaborative Innovation Center of Hematology, Soochow University, Suzhou, China
[3]Department of Haematology, Guangdong Provincial People's Hospital, Guangzhou, China

*These authors have contributed equally to this work.

Jin-lan Pan, Ph.D., National Clinical Research Center for Hematologic Diseases, Jiangsu Institute of Hematology, the First Affiliated Hospital of Soochow University; Institute of Blood and Marrow Transplantation, Collaborative Innovation Center of Hematology, Soochow University, Suzhou, China
jinlanpan@aliyun.com

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To the Editor,

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The t(15;17)(q24;q21) is a highly specific cytogenetic change in acute promyelocytic leukemia (APL), leading to the fusion of *RARA* with *PML* gene. The t(9;22)(q34;q11), which results in the *BCR::ABL1* fusion gene, has been found in various hematologic neoplasms, including chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). The occurrence of both t(15;17)(q24;q21) and t(9;22)(q34;q11) in APL patients is extremely rare. Herein, we report the identification and treatment of the APL patient with coexistence of both t(15;17) and t(9;22) with a novel *MAPK1::BCR* fusion gene.

A 64-year-old woman was admitted to hospital on June 4, 2022, because of scattered petechial hemorrhages and ecchymosis on the abdomen skin for three days. Complete peripheral blood counts showed a white blood cell count of 6.86×10^9/L, a hemoglobin level of 90 g/L, and a platelet count of 70×10^9/L. Fibrinogen and D-dimer levels were 1.99 g/L (reference, 1.90 - 4.00 g/L) and 20 μg/mL (reference, 0.00 - 0.50 μg/mL), respectively. A bone-marrow (BM) smear revealed hypercellular marrow with 94.5% hypergranular promyelocytes with Auer rods. Multiparameter flow cytometry showed that the blasts were positive for CD13, CD33, CD117 and MPO, weakly expressed CD4, CD15 and CD38, but were negative for CD19, CD34, cCD3, cCD79a, TDT and HLA-DR. Karyotype analysis at first diagnosis revealed t(9;22)(q34;q11) in addition to t(15;17)(q24;q21) in 15 of 20 metaphase cells (Figure 1A). Fluorescence in situ hybridization (FISH) analysis revealed evidence of *PML::RARA* rearrangement (Figure 1B). FISH analysis using the *BCR::ABL1* dual-color dual-fusion probe revealed two normal size red (*ABL1*) signals and two reduced size and one normal size green (*BCR*) signals in 95% of the cells analyzed (Figure 1C). It is noteworthy that among five separate signals (3G2R), one small green signal was always adjacent to one large red signal in addition to other three separate signals (2G1R), which suggested that the translocation breakpoint was in the *BCR* gene and ruled out the *BCR::ABL1* rearrangement.

To characterize the rearrangement involving *BCR* gene, we performed RNA sequencing of the BM samples with Next-seq550 (Illumina Inc., San Diego, CA, USA) and found *PML::RARA* fusion and a novel *MAPK1::BCR* fusion (Figure 1D-E). For validation of this novel fusion, we performed reverse transcription-polymerase chain reaction (RT-PCR) and Sanger sequencing and confirmed *MAPK1::BCR* transcript, but reciprocal *BCR::MAPK1* transcript was not detected (Figure 1F). Exon 1 of *MAPK1* was fused to exon 2 of *BCR* out-of-frame, which led to a premature stop codon near the fusion breakpoint (Figure 1G). This fusion transcript encodes a protein of 40 amino acids, which does not lead to a fusion protein. We performed targeted next-generation sequencing of the entire coding sequences of 136 known or putative mutational gene targets in hematologic malignancies (Supplementary table 1). *ASXL1*-p.Gly643Val, *CALR*-p.Gly65fs, *KRAS*-p.Gly12Arg, and *NOTCH1*-p.Arg1672His variations were identified in this patient (Supplementary table 2).

The patient was diagnosed with APL and treated using all-trans retinoic acid (ATRA). She achieved complete hematologic remission 6 weeks after induction treatment. BM smear showed normocellular with 1.0% promyelocytes. The fusion transcript of *PML::RARA* was detected by quantitative RT-PCR at a ratio of 0.084%. After consolidation therapies, molecular remission of *PML::RARA* was also achieved. The normal *BCR* and *ABL1* signals (two green and two red signals) were also observed by FISH using *BCR::ABL1* probe. The patient has been tolerating maintenance therapy well in molecular remission more than 12 months from diagnosis.

APL is characterized by a balanced reciprocal translocation between chromosomes 15 and 17. However, additional chromosomal abnormalities (ACA) in addition to the t(15;17) can be detected and have also been reported in about 40% of cases. Presence of t(15;17) along with other recurrent genetic rearrangements such as t(9;22) is rare in

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APL. To date, the chromosomal abnormality of t(9;22) has been reported in several APL cases, and both *BCR::ABL1* and *PML::RARA* can be detected in these cases (Supplementary table 3) [1-7]. Interestingly, we describe a case of APL that t(15;17)(q22;q21) and t(9;22)(q34;q11) were detected in the same clone at the time of diagnosis by R bands. However, *BCR::ABL1* fusion gene was not detected, while a novel fusion gene *MAPK1::BCR* was detected by RNA sequencing. To the best of our knowledge, this is the first reported APL case with both *PML::RARA* and *MAPK1::BCR*. Both *MAPK1* and *BCR* genes located on chromosome 22q11. Based on the results of karyotype, FISH and RNA sequencing, we infer that the occurrence of t(9;22)/*MAPK1::BCR* occurs in a two-step mechanism shown in Supplementary Figure 1. The first step is an inversion between exon 1 of *MAPK1* and exon 1 of *BCR* within der(22), theoretically resulting in the *MAPK1* exon 1::*BCR* exon 2 and *BCR* exon 1::*MAPK1* exon 2 fusion genes. A fragment of 281bp (*MAPK1*::*BCR*) was detected by RT-PCR, whereas *BCR*::*MAPK1* was not detected. The second step is a translocation between chromosome 9 with breakpoint of 9q34 mapped upstream of *ABL1* gene and der(22) with breakpoint located between *BCR* exon 1 and *MAPK1* exon 1. The abnormality of t(9;22)(q34;q11) was very similar to standard t(9;22)/*BCR::ABL1* which masked complex rearrangements. Therefore, *BCR::ABL1* fusion gene should be detected by FISH, RT-PCR, or RNA sequencing when R/G banding karyotypes indicated concurrent t(9;22)(q34;q11) and t(15;17)(q24;q21) in APL.

The most common additional somatic mutations in APL mainly involve *FLT3*, *WT1*, *NRAS* genes, whereas variations detected in our case including *ASXL1*, *CALR*, *KRAS* and *NOTCH1* were rare [8]. The clinical significance of these variations is unclear and needs to be confirmed in larger samples. There are conflicting data in the literature concerning the prognostic relevance of ACA presented at diagnosis in APL. Epstein-Peterson ZD et al showed no influence of ACA (≥1 cytogenetic abnormality besides t(15;17)) on event-free survival (EFS), but complex karyotype (≥2 cytogenetic abnormalities besides t(15;17)) conferred inferior EFS [9]. Zeng H et al reported that ACA was an independent adverse factor for EFS [10]. The majority of APL patients with t(9;22)/*BCR::ABL1* achieved complete remission after ATRA-based induction therapy regardless of whether combined with tyrosine kinase inhibitors (TKIs) [4, 7]. Our patient was treated with ATRA for the induction chemotherapy, followed by the consolidation therapy including ATRA. Up to date, the patient remains in CR for 12 months. This report suggested that the presence of additional abnormality of t(9;22)/*MAPK1::BCR* might not reduce the sensitivity to ATRA and did not affect the clinical outcome of treatment.

In summary, we report the first case of *MAPK1::BCR* in an adult APL patient with concurrent of t(9;22)(q34;q11) and t(15;17)(q24;q21). It is worth noting that *BCR::ABL1* fusion gene should be detected by FISH, RT-PCR or RNA sequencing when t(9;22)(q34;q11) was detected by conventional karyotyping in APL with t(15;17)(q24;q21). *MAPK1::BCR* fusion gene appears to have no influence on the clinical outcome of the APL patient treated with ATRA. Further molecular studies involved larger series of cases are needed to understand the mechanism of *MAPK1::BCR* in leukemogenesis.

Keywords: acute promyelocytic leukemia, *PML::RARA*, *MAPK1::BCR*, t(9;22)(q34;q11), RNA sequencing

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Abbreviations： APL, acute promyelocytic leukemia; CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; BM, bone-marrow; RT-PCR, reverse transcription-polymerase chain reaction; ATRA, all-trans retinoic acid; ACA, additional chromosomal abnormalities; EFS, event-free survival; TKIs, tyrosine kinase inhibitors.

Ethics

Informed Consent: Informed consent was obtained from the patient.

Author Contributions

Concept: J.L.P.; Design: J.L.P., J.Y.W.; Data Collection or Processing: Q.W., L.J.Z.; Analysis or Interpretation: M.W.; Literature Search: Q.W., L.J.Z.; Writing: Q.W., J.L.P.

Conflict of Interest: No conflict of interest was declared by the authors.

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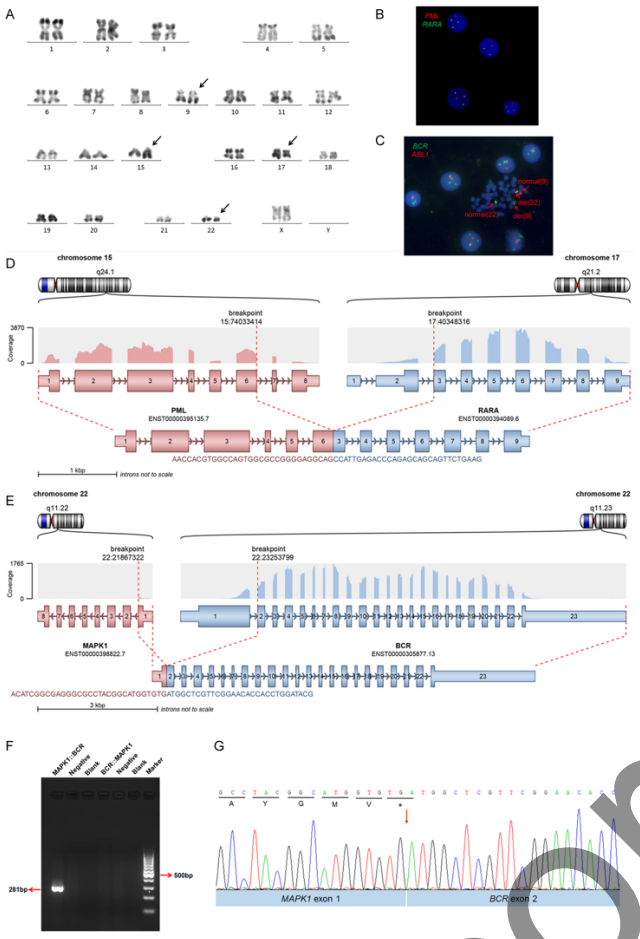

Figure 1. Laboratory characteristics of the adult patient with APL with the *MAPK1::BCR* fusion. **(A)** Karyotype analysis at diagnosis. R-banding analysis using the bone marrow sample revealed a karyotype of 46,XX,t(9;22)(q34;q11),t(15;17)(q24;q21)[15]/46,XX[5]. **(B)** FISH analysis using the *PML::RARA* dual-color dual-fusion probe revealed evidence of *PML::RARA* rearrangement (two yellow fusion signals, one red signal and one green signal). **(C)** FISH analysis using the *BCR::ABL1* dual-color dual-

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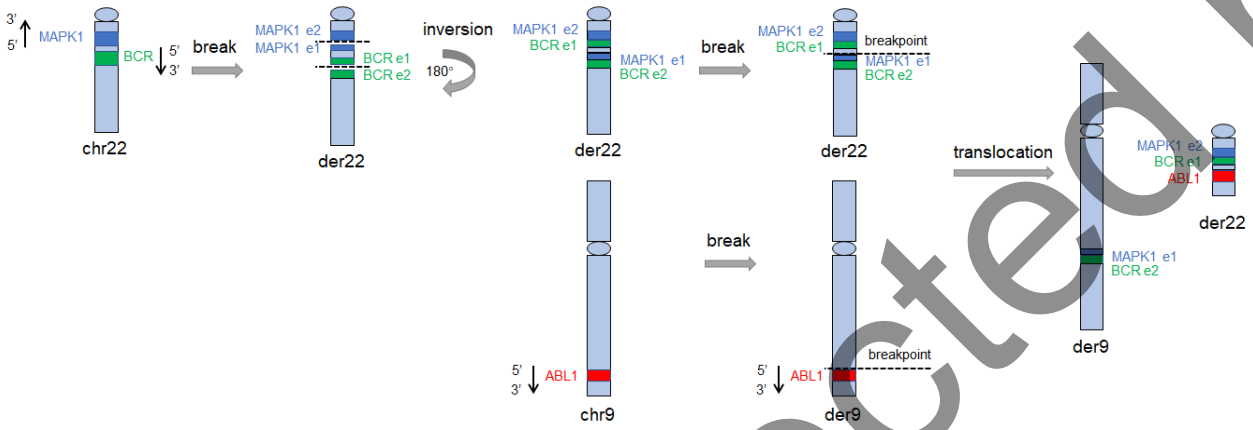
fusion probe revealed two red (*ABL1*) signals and three green (*BCR*) signals in 95% of the cells analyzed. Metaphase showed a yellow fusion signal on one chromosome 22. **(D)** RNA sequencing analysis revealed that exon 6 of the *PML* gene fused with exon 3 of the *RARA* gene. **(E)** RNA sequencing analysis revealed that exon 1 of the *MAPK1* gene fused with exon 2 of the *BCR* gene. **(F)** A product of 281 bp was detected by RT-PCR in the sample at diagnosis. Marker: GeneRuler 100 bp DNA ladder. **(G)** Sequence alignment of the amplified product revealed breakpoints between exon 1 of *MAPK1* and exon 2 of *BCR*.



Supplementary figure 1. Schematic illustration of the occurrence of t(9;22)(q34;q11)/*MAPK1::BCR*.

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Supplementary table 1. A panel of 136 genes detected by next-generation sequencing

ABL1	*ANKRD26*	*ARID1A*	*ARID1B*	*ASXL1*	*ASXL2*	*ASXL3*	*ATG2B*
ATM	*ATRX*	*BCOR*	*BCORL1*	*BLM*	*BRAF*	*BRCA1*	*BRCA2*
BRINP3	*CALR*	*CARD11*	*CBL*	*CCND1*	*CCND2*	*CD28*	*CDKN2A*
CEBPA	*CELA2A*	*CHD1*	*CHD4*	*COL12A1*	*CREBBP*	*CRLF2*	*CROCC*
CSF3R	*CSMD1*	*CUX1*	*DDX11*	*DDX41*	*DIS3*	*DKC1*	*DNMT3A*
DOT1L	*EP300*	*EPPK1*	*ETNK1*	*ETV6*	*EZH2*	*FAT1*	*FBXW7*
FLT3	*GATA1*	*GATA2*	*GFI1*	*GNAS*	*GSKIP*	*HAX1*	*HRAS*
IDH1	*IDH2*	*IKZF1*	*IL7R*	*JAK1*	*JAK2*	*JAK3*	*KDM5A*
KDM6A	*KIT*	*KMT2A*	*KMT2C*	*KMT2D*	*KMT2E*	*KMT6A*	*KRAS*
MECOM	*MPL*	*MUC16*	*MYC*	*MYD88*	*NCOR1*	*NCOR2*	*NF1*
NFE2	*NOTCH1*	*NOTCH2*	*NOTCH3*	*NPM1*	*NRAS*	*NT5C2*	*PAX5*
PBRM1	*PCLO*	*PDGFRA*	*PHF6*	*PIGA*	*PML*	*PPM1D*	*PRPF40B*
PRPF8	*PTEN*	*PTPN11*	*PTPRT*	*RAD21*	*RB1*	*ROBO1*	*ROBO2*
RUNX1	*RUNX2*	*SBDS*	*SETBP1*	*SF1*	*SF3B1*	*SH2B3*	*SMARCA2*
SMC1A	*SMC3*	*SMN1*	*SPN*	*SRP72*	*SRSF2*	*STAG1*	*STAG2*
STAT3	*STAT5B*	*SUZ12*	*TERC*	*TERT*	*TET2*	*TP53*	*TTN*
TYK2	*U2AF1*	*U2AF2*	*WAC*	*WT1*	*ZMYM3*	*ZNF608*	*ZRSR2*

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Supplementary table 2. Gene variations detected by next-generation sequencing.

Gene	Transcript ID	Chromosome	Exon	DNA change	AA change	VAF	Type of variation
ASXL1	NM_015338.5	chr20	12	c.1928G>T	p.Gly643Val	0,5344	missense
CALR	NM_004343.3	chr19	2	c.191_192dupAA	p.Gly65fs	0,3466	frameshift
KRAS	NM_004985.4	chr12	2	c.34G>C	p.Gly12Arg	0,3849	missense
NOTCH1	NM_017617.4	chr9	26	c.5015G>A	p.Arg1672His	0,4339	missense

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Supplementary table 3. Summary of published cases of co-expressing t(15;17)/*PML::RARA* and t(9;22)/*BCR::ABL1*, and this case.

No.	Age (years)	Sex	WBC ($\times 10^9$/L)	Hb (g/L)	PLT ($\times 10^9$/L)	Diagnosis	BM morphology	Cytogenetics	Immunophenotype	PCR	Treatment	CR	Outcome, months	Reference
1	39	Female	242,2	88	20	APL	promyelocytic cell population	46,XX,t(9;22)	positive for CD33 (72%), CD13 (88%), CD2 (4%) negative for HLA-DR and CD34	*PML::RARA* *BCR::ABL1*	idarubicin + Ara-C	Yes	Dead, 5	1995 Emilia *et al.*
2	38	Female	1,8	61	12	APL	73% promyelocytes	46,XX,t(9;22)(q34;q11),t(15;17)(q22;q21)[4]/46,XX[16]	positive for CD33 (98.69%), CD117 (35.65%), MPO (31.27%), CD13 (23.64%) negative for CD7, CD10, CD19, HLA-DR, CD34, CD61, CD64, CD14, CD35, CD56, CD15, CD11b and CD65s	*PML::RARA* *BCR::ABL1*	ATRA + Arsenic trioxide	No	Dead, <1	2009 Mao *et al.*

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3	50	Male	0,45	73	3	APL	38% blasts and 20% promyelocytes	46,XY,t(15;17)(q22;q12)[9]/46,XY,del(6)(q?),t(9;22)(q34;q11.2)[1]/46,XY[10]	not available	*PML::RARA* *BCR::ABL1*	ATRA	Yes	Alive, >2	2011 Takahashi *et al*.
4	48	Male	1,15	86	15	APL	1% myeloblasts and 91.5% promyelocytes	46,XY,t(9;22)(q34;q11),t(15;17)(q22;q21)[10]/47,idem,+8[4]/46,idem,der(14)t(9;14)(q10;q10)[6]	positive for CD13, CD33, CD38, CD64, MPO, CD4, CD11b, CD117 negative for CD34 and HLA-DR	*PML::RARA* *BCR::ABL1*	ATRA + Arsenic trioxide + imatinib	Yes	Alive, 18	2014 Sun *et al*.
5	51	Female	287,83	74	116	APL	8.5% myeloblasts and 43.5% promyelocytes	46,XX,t(9;22)(q34;q11),t(15;17)(q22;q21)	positive for MPO, CD13, CD33, CD34 negative for HLA–DR	*PML::RARA* *BCR::ABL1*	ATRA + DA	Yes	Alive, 11	2015 Zhang *et al*.
6	69	Female	1,17	118	79	APL	5.2% blasts and 48% abnormal promyelocytes	46,XX,t(15;17)(q24.1;q21.1)[8]/46,XX[16]	positive for CD13, CD33, cytoplasmic MPO, CD11c, CD10 negative for HLA-DR	*PML::RARA* *BCR::ABL1*	ATRA + idarubicin	Yes	Dead, 6	2017 An *et al*.
7	49	Female	6,48	69	27	APL	10% blasts and 80% abnormal promyelocytes	46,XX,der(6)t(6;8)(p23;q13),t(15;17)(q24;q21)[20]	positive for MPO, CD13, CD33, CD117, CD34, CD2, CD56 negative for HLA-DR	*PML::RARA* *BCR::ABL1*	ATRA + idarubicin	Yes	Alive, 10	2022 Cho *et al*.

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8	56	Female	6,86	90	70	APL	94.5% hypergranular promyelocytes	46,XX,t(9;22)(q34;q11),t(15;17)(q24;q21)[15]/46,XX[5]	positive for CD13, CD33, CD117, MPO negative for CD34 and HLA-DR	*PML::RARA* *MAPK1::BCR*	ATRA	Yes	Alive, >12	our case

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