Clinical and molecular features of acute promyelocytic leukemia with variant retinoid acid receptor fusions

Acute promyelocytic leukemia (APL) is a unique disease entity in acute myeloid leukemia (AML), characterized by the expansion of leukemic cell block at the promyelocytic stage. The vast majority of APL patients bear t(15;17)(q24;q21) involving the promyelocytic leukemia (*PML*) gene at chromosome band 15q24 and the retinoic acid receptor alpha (*RARA*) gene at 17q21, generating an aberrant *PML-RARA* fusion gene.[1,2] However, in a subset of APL patients, a t(15;17)(q24;q21) and *PML-RARA* fusion cannot be detected.[3] Many *RARA*, *RARB*, or *RARG* fusions have been reported so far, with APL patients presenting at least 17 alternative partner genes, including *PLZF, NPM1, NUMA, STAT5B, PRKAR1A, BCOR, FIP1L1, OBFC2A, GTF2I, TBLR1, IRF2BP2, NUP98, FNDC3B, PML, STAT3, CPSF6,* among others.[1,4-12] Whereas *RARA* fusions with *PML, NPM, NUMA, FNDC3B,* and *IRF2BP2* are sensitive to *ATRA, PLZF-RARA, STAT3-RARA, STAT5B-RARA, CPSF6-RARG* fusions are significantly resistant. Nevertheless, the molecular landscape of APL patients lacking classic t(15;17)(q24;q21)/*PML-RARA* remains to be delineated. Here, we report our investigations into the clinical and molecular features of APL patients lacking classic t(15;17)(q24;q21)/*PML-RARA*.

From January 2003 to December 2016, a total of 1401 patients with suspected APL were enrolled in this study. Patients were considered eligible for inclusion only if the following criteria were satisfied: morphological and immunophenotypic features were consistent with the diagnosis of APL. This study was approved by the ethics committee of the First Affiliated Hospital of Soochow University in Suzhou, P.R. China, in accordance with the Declaration of Helsinki.

We performed cytogenetic and molecular characterization on 1401 suspected APL patients for t(15;17)(q24;q21) and/or *PML-RARA via* karyotyping, fluoresecence *in situ* hybridization (FISH), RT-PCR, or RNA-seq (Figure 1A). Twenty patients negative for rearrangements of *RARA* genes were excluded. We identified t(15;17)(q24;q21) and/or *PML-RARA via* karyotyping, FISH, RT-PCR, or RNA-seq in 98.6% (1362 out of 1381) of cases (Figure 1A). In total, 19 patients with alternative RARA or RARG fusions were identified: *PLZF-RARA* fusions in 10 patients, *STAT5B-RARA* in 4, *STAT3-RARA* in 2, *CPSF6-RARG* in 2, and *TBLR1-RARA* in 1 patient, respectively (Figure 1A and B). We observed a significantly higher number of males with alternative *RARA* or *RARG* fusions when compared to APL with classic *PML-RARA* (84.2% *vs.* 52.6%; *P*=0.04). Primary patients' characteristics are summarized in Tables 1 and 2. Median white blood cell (WBC) count at presentation for the alternative *RARA* or *RARG* fusions cohort was significantly higher than the *PML-RARA* cohort ($19.7x10^9$/L *vs.* $2.5x10^9$/L; *P*=0.01) (Table 1). In addition, the median platelet count for the alternative *RARA* or *RARG* fusions cohort was significantly higher than that in the *PML-RARA* cohort ($78x10^9$/L *vs.* $25x10^9$/L; *P*<0.001).

Table 1. Clinical and laboratory features of 1381 patients with acute promyelocytic leukemia (APL).

	APL with t(15;17)/*PML-RARA*	APL with *X-RARA/RARG*	*P*
Cases	1362	19	
Age, years, median (range)	39(4-91)	42(24-70)	0.952
Gender, M/F	716/646 (1.1/1)	16/3 (5.3/1)	0.04
WBC, 10^9/L, median (range)	2.5(0.2-200)	19.7(1.34-72.7)	0.01
Hb, gd/L, median (range)	85(24-162)	83(45-125)	0.89
PLT, 10^9/L, median (range)	25(1-212)	78(24-282)	<0.001
Blast cell (%), median (range)	79(46~98)	77(48~96)	0.91
Karyotype			
Normal	78 (5.7%)	–	
t(15:17) alone	1012 (74.3)	–	
t(15:17) with ACAs	260 (19.1%)	–	
Failure	12 (0.9%)		
PML-RARA transcripts			
L type	838 (65.7%)	–	
S type	426 (33.4%)	–	
V type	12 (0.9%)	–	
Sanz risk stratification			<0.001
High	361 (26.5%)	13 (68.4%)	
Middle	685 (50.3%)	1 (5.26%)	
Low	316 (23.2%)	5 (26.31%)	
Complete remission (%)	791/835 (94.8%)	8/15 (53.3%)	<0.001
Relapse rate (%)	58/471 (12.3%)	5/8 (62.5%)	<0.001

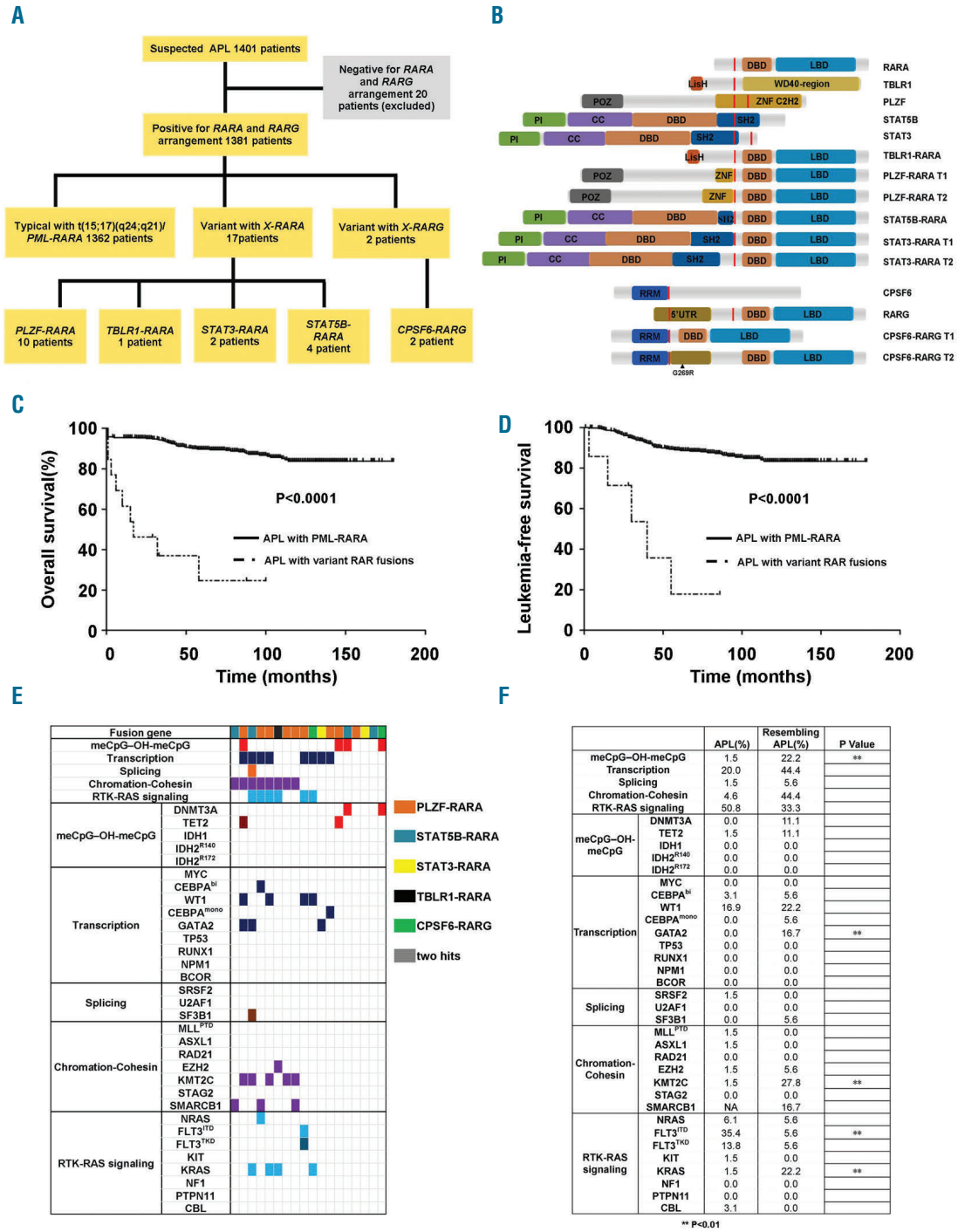
M: male; F: female; WBC: white blood cell count; Hb: hemoglobin level; PLT: platelet count; ACAs: additional chromosomal abnormalities.

Table 2. Clinical and laboratory features of acute promyelocytic leukemia (APL) patients with alternative *RARA* or *RARG* fusions.

Case	Gender/ Age	WBC, x10^9/L	HB, g/L	PLT, x10^9/L	Karyotype	FISH probe	WGS/ RNA-seq	Transcript type	*In vivo* ATRA response	Outcome
1	M/38	1.34	47	129	46,XY,t(11;17)(q23;q21)[20]	*PML-RARA* negative	ND	*PLZF-RARA* (exon3-exon3)	NA	Lost to follow up.
2	M/39	3.1	62	106	46,XY,t(11;17)[8]/45,X,-Y,t(11;17)[9]/45,X,-Y,t(3;6)(q26;p25),t(11;17)[3]	*PML-RARA* negative	ND	*PLZF-RARA* (exon4-exon3)	NA	Lost to follow up.
3	F/62	72.7	125	201	46,XX,t(11;17)(q23;q21)[10]	*PML-RARA* negative	ND	*PLZF-RARA* (exon3-exon3)	NA	Lost to follow up.
4	M/42	31.7	96	163	46,XY,del(9),t(11;17)(q23;q21)[10]	*PML-RARA* negative	ND	*PLZF-RARA* (exon3-exon3)	insensitive	Died in RP2 at 58 mo.
5	M/32	20	84	80	46,XY,t(11;17)(q23;q21)[9]/46,XY[1]	*PML-RARA* negative	ND	*PLZF-RARA* (exon4-exon3)	insensitive	Alive in CR2 at 72 mo (allo-HSCT in CR2 at 60 mo).
6	M/58	23.7	84	25	45,X,-Y,t(11;17)(q23;q21)[8]/46,XY[3]	*PML-RARA* negative	ND	*PLZF-RARA* (exon3-exon3)	NA	Died at 1 mo. (Patient decision to stop therapy).
7	M/46	10.54	111	31	45,X,-Y,t(11;17)(q23;q21)[10]	*PML-RARA* negative	ND	*PLZF-RARA* (exon3-exon3)	resistant	Died at day 4 (cerebral hemorrhage).
8	M/37	3.31	65	109	46,XY,t(11;17)(q23;q21)[5]/46,XY[5]	*PML-RARA* negative	ND	*PLZF-RARA* (exon3-exon3)	resistant	Died at 10 mo.
9	M/25	35	77	55	46,XY,t(11;17)(q23;q24)[10]	*PML-RARA* negative	ND	*PLZF-RARA* (exon3-exon3)	insensitive	Alive in CR1 at 24 mo (allo-HSCT in CR1 at 3 mo).
10	M/70	22.5	96	61	46,XY,t(11;17)(q23;q24)[10]	*PML-RARA* negative	ND	*PLZF-RARA* (exon3-exon3)	resistant	Lost to follow up.
11	M/32	3.8	83	28	46,XY[20]	*RARA* positive	*STAT5B-RARA*	*STAT5B-RARA* (exon15-exon3)	resistant	Alive in CR1 at 68 mo (allo-HSCT in CR1 at 19 mo).
12	M/49	19.7	74	24	46,XY[20]	*RARA* positive	*STAT5B-RARA*	*STAT5B-RARA* (exon15-exon3)	resistant	Died in RP1 at 13 mo
13	M/35	13	45	43	46,XY[20]	*RARA* positive	*STAT5B-RARA*	*STAT5B-RARA* (exon15-exon3)	resistant	Died at 1 mo.
14	M/46	17.18	77	78	45,X,-Y,1q-,11q+[8]/46,XY[2]	*RARA* positive	*STAT5B-RARA*	*STAT5B-RARA* (exon15-exon3)	resistant	Alive in RP2 at 14 mo.
15	M/26	6.6	73	94	46,XY[20]	*RARA* positive	*STAT5B-RARA*	*STAT5B-RARA* (exon23-exon3)	resistant	Died at 6 mo.
16	M/24	32.3	123	89	45,X,-Y[6]/46,XY[8]	*RARA* positive	*STAT5B-RARA*	*STAT5B-RARA* (exon21-exon3)	resistant	Died in RP1 at 36 mo (cerebral hemorrhage).
17	M/52	NA	NA	NA	46,XY[20]	*RARA* negative	*TBLR1-RARA*	*TBLR1-RARA* (exon5-exon3)	resistant	Lost to follow up.
18	F/48	22	69	73	92,XXXX[2]	*RARA* negative	*CPSF6-RARG*	*CPSF6-RARG* (exon4-exon4)	resistant	Refractory to ATRA, ATO, and chemotherapy, died of cerebral hemorrhage in 10 mo.
19	F/51	20.15	65	45	46,XX,del(12)(p12)[2]/46,XX[18]	*RARA* negative	*CPSF6-RARG*	*CPSF6-RARG* (exon4-exon1)	resistant	Alive in CR1 at 33 mo.

WBC: white blood cell count; Hb: hemoglobin level; PLT: platelet count; M: male; F: female; ND: not determined; NA: not available; allo-HSCT: allogeneic stem cell transplantation; ATO: arsenic trioxide; ATRA: all-trans retinoic acid; CR: complete remission; mo: months; RP: relapse.

We observed poor responses to ATRA in most patients with *PLZF-RARA, STAT3-RARA, STAT5B-RARA,* or *CPSF6-RARG* fusion transcripts (Tables 1 and 2). Only 8 out of 15 (53.3%) of cases with alternative *RARA* or *RARG* fusions acquired complete remission (CR) by chemotherapy combined with ATRA and/or arsenic trioxide. Furthermore, 62.5% of alternative *RARA* or *RARG* fusion cases acquiring CR eventually underwent relapse (Table 1). To further analyze the prognostic impact of APL with alternative *RARA* or *RARG* fusions, we compared the overall survival (OS) and leukemia-free survival (LFS) in APL with t(15;17)(q24;q21)/*PML-RARA*. APL patients with alternative *RARA* or *RARG* fusion showed poor outcomes: the 3-year OS rate was 26.7% compared



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		APL(%)	Resembling APL(%)	P Value
meCpG–OH-meCpG		1.5	22.2	**
Transcription		20.0	44.4	
Splicing		1.5	5.6	
Chromation-Cohesin		4.6	44.4	
RTK-RAS signaling		50.8	33.3	
meCpG–OH-meCpG	DNMT3A	0.0	11.1	
	TET2	1.5	11.1	
	IDH1	0.0	0.0	
	IDH2R140	0.0	0.0	
	IDH2R172	0.0	0.0	
Transcription	MYC	0.0	0.0	
	CEBPAbi	3.1	5.6	
	WT1	16.9	22.2	
	CEBPAmono	0.0	5.6	
	GATA2	0.0	16.7	**
	TP53	0.0	0.0	
	RUNX1	0.0	0.0	
	NPM1	0.0	0.0	
	BCOR	0.0	0.0	
Splicing	SRSF2	1.5	0.0	
	U2AF1	0.0	0.0	
	SF3B1	0.0	5.6	
Chromation-Cohesin	MLLPTD	1.5	0.0	
	ASXL1	1.5	0.0	
	RAD21	0.0	0.0	
	EZH2	1.5	5.6	
	KMT2C	1.5	27.8	**
	STAG2	0.0	0.0	
	SMARCB1	NA	16.7	
RTK-RAS signaling	NRAS	6.1	5.6	
	FLT3ITD	35.4	5.6	**
	FLT3TKD	13.8	5.6	
	KIT	1.5	0.0	
	KRAS	1.5	22.2	**
	NF1	0.0	0.0	
	PTPN11	0.0	0.0	
	CBL	3.1	0.0	

** P<0.01

Figure 1. The clinical and molecular characteristics of resembling acute promyelocytic leukemia (APL) with *RARA/RARG* fusions. (A) Flowchart of the patient cohort. (B) Schematic representation of alternative *RARA* and *RARG* fusions identified in this study. Breakpoints are indicated with red lines. Different patterns and colors are used to represent various functional regions of the RARA, RARG, TBLR1, PLZF, STAT5B, STAT3, and CPSF6 proteins. (C) Overall survival (OS) of APL compared with APL with alternative *RARA/RARG* fusions. (D) Leukemia-free survival (LFS) of APL compared with APL with alternative *RARA/RARG* fusions. (E) Mutational spectrum in APL with alternative *RARA* or *RARG* fusions. Each column represents one of the 18 resembling APL samples sequenced here. The rows in the graph represent individual genomic lesions. (F) Distribution of mutations in APL patients with *PML-RARA* or alternative *RARA/RARG* fusions.

to 92.1% in APL with *PML-RARA* (P<0.0001) (Figure 1C). The 3-year LFS was also clearly poorer than that of the APL cohort (20.0% *vs.* 86.5%; P<0.0001) (Figure 1D). The risk stratification of APL patients with alternative *RARA* or *RARG* was also significantly poorer than the *PML-RARA* cohort (P<0.001) (Table 1). Among the 13 patients with alternative *RARA* or *RARG* fusion with follow up, 8 (61.5%) patients received combinational induction therapy by all-trans retinoic acid (ATRA) and arsenic trioxide, 5 (38.5%) patients received combinational induction therapy by ATRA and chemotherapy. In addition, three patients who received allo-HSCT were still alive at 24, 68 and 72 months, respectively (Table 2). This suggests that allo-HSCT may be an effective way to improve the survival of the APL with alternative *RARA* or *RARG* fusions.

Next-generation sequencing (NGS) has identified novel genetic variants in many hematologic malignancies, including APL.[13] High frequency of *FLT3* and *WT1* mutations were identified in APL patients with *PML-RARA*. Nevertheless, the molecular landscape of APL patients with alternative *RARA* or *RARG* fusions remains to be delineated. To decipher the mutational spectrum of patients with alternative *RARA* or *RARG* fusions, we performed NGS on the target DNA with a panel of 382 genes in a cohort of 18 patients (*PLZF-RARA* n=9, *STAT5B-RARA* n=4, *STAT3-RARA* n=2, *CPSF6-RARG* n=2, and *TBLR1-RARA* n=1). Mutations were detected in 15 out of 18 patients (83.3%): 7 out of 18 (38.9%) patients carried 1 mutation, 3 out of 18 (16.7%) carried 2, 3 out of 18 carried 3, and 2 out of 18 carried 4, i.e. an average 1.7 mutations per sample (Figure 1E). We identified high frequencies of mutations, *KMT2C* (27.8%), *WT1* (22.2%), *K-RAS* (22.2%), *GATA2* (16.7%), *SMARCB1* (16.7%), followed by *DNMT3A* (11.1%), *TET2* (11.1%), *CEBPA* (11.1%), *SF3B1* (5.6%), *FLT3-TKD* (5.6%), *FLT3-ITD* (5.6%), *EZH2* (5.6%), and *N-RAS* (5.6%), etc. We further compared the mutational spectra of patients with alternative *RARA* or *RARG* to those with *PML-RARA* fusion.[13] APL with alternative *RARA* or RARG presented with more mutations of *KMT2C* (27.8% *vs.* 1.5%; P<0.01), *K-RAS* (22.2% *vs.* 0.5%; P<0.01), *GATA2* (16.7% *vs.* 0%; P<0.01), and fewer mutations of *FLT3-ITD* (5.6% *vs.* 35.4%; P<0.01) (Figure 1F).

Only 53.3% of APL with alternative *RARA* or *RARG* fusions achieved CR by chemotherapy combined with ATRA and/or ATO; the relapse rate was as high as 62.5%. The 3-year OS and LFS of APL with alternative *RARA* or *RARG* were worse than those of the *PML-RARA* cohort. Among the 19 patients in our study with suggested APL, 15.79% (3 out of 19) were insensitive and 63.16% (12 out of 19) were resistant to ATRA treatment. It has been reported that the three mutations in the *PML* part of *PML-RARA* could attenuate the negative regulation of arsenic on *PML-RARA*. The resistant effect can be overcome by either increasing the concentration of arsenic trioxide or by combination with ATRA.[14,15] The APL patients with alternative *RARA* or *RARG* fusions in our study were resistant to ATO and/or ATRA treatment. Fusion gene moiety was not involved in the NGS panel; however, we noticed a high proportion of mutation in signaling pathways, especially the *K-RAS* mutation, and epigenetics compared with APL patients. We speculated that the gene mutation might partly be the reason for these APL patients to be resistant or insensitive to ATRA and/or ATO.

In summary, we retrospectively analyzed 1381 patients with APL diagnosis and identified 1.4% patients with alternative *RARA* or *RARG*. We observed poor response to all-trans retinoic acid in most APL patients with *PLZF-RARA, STAT3-RARA, STAT5B-RARA*, or *CPSF6-RARG* fusion transcripts. NGS performed on APL patients with alternative *RARA* or *RARG* fusions revealed more mutations of *KMT2C, K-RAS,* and *GATA2*, but fewer mutations of *FLT3-ITD* when compared to APL patients with the *PML-RARA* fusion. We suggest that routine karyotypic analysis, FISH, and real-time polymerase chain reaction should be performed in patients with morphological and immunophenotypic features consistent with the diagnosis of APL. In suspected APL patients lacking a *PML-RARA* fusion, RNA sequencing should be performed to exclude variant fusion involving *RARA/RARB/RARG* genes. This study highlights the importance of combining multiple molecular techniques for the characterization and optimal management of APL lacking t(15;17)(q24;q21)/*PML-RARA* fusions.

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