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ARTICLE

iAMP21 in acute myeloid leukemia is associated with complex karyotype, *TP53* mutation and dismal outcome

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Abstract

Acute myeloid leukemia (AML) with intrachromosomal amplification of chromosome 21 (iAMP21) is rare and has not been well characterized. We report 13 patients, 7 men and 6 women, with a median age of 65 years. Eleven patients presented with AML with myelodysplasia-related changes, and two patients had therapy-related AML. Cytopenias were detected in all patients (11 pancytopenia and two bi-lineage cytopenia). Myelodysplastic changes were observed in all 11 patients with adequate cells to evaluate. Myelofibrosis was present in ten patients. All patients had a complex karyotype, including abnormalities of chromosomes 5, 7, 17, and hsr(21)(q22), and ten patients showed *TP53* deletion and/or mutation. Eleven patients received AML-based chemotherapy, one of whom also received hematopoietic stem cell transplant. By the end of the last follow-up, eight patients died with median survival of 3.2 months, four patients were alive with persistent AML, and one was in complete remission. The median overall survival was 6 months for all patients. We conclude that AML with iAMP21 is often associated with cytopenias, myelodysplasia, a complex karyotype, *TP53* mutation/deletion, and a poor prognosis despite current therapies.

Introduction

The Runt domain of transcription factor *RUNX1*, also known as *AML1* and *CBFA2*, located on chromosomal 21q22. *RUNX1* is frequently dysregulated in leukemias as results of chromosomal translocations, amplification, and point mutations [1]. The most frequent translocations involving *RUNX1* are t(8;21)(q22;q22)/*RUNX1T1-RUNX1* in acute myeloid leukemia (AML) and t(12;21)(p12.3;q22)/*ETV6-RUNX1* in B-lymphoblastic leukemia (B-ALL) [2–4]. Intrachromosomal amplification of chromosome 21 (iAMP21) is an uncommon finding in hematologic neoplasms that is most often detected by fluorescence in situ hybridization (FISH) analysis using a *RUNX1* probe. iAMP21 is recognized as ≥5 copies of *RUNX1* within one cell or ≥3 extra copies of *RUNX1* on a single abnormal chromosome 21 [5–7]. iAMP21 has been well characterized in B-ALL and recognized as a provisional entity by the current World Health Organization (WHO) classification [5]. B-ALL with iAMP21 accounts for ~2% of pediatric B-ALL, with a median age of 9 years, and is often associated with low white blood cell (WBC) count [5–8]. iAMP21 has been recognized as a poor risk factor in B-ALL and patients with B-ALL iAMP21 should be treated with more intensive therapy to overcome this adverse risk [7].

iAMP21 has been rarely reported in AML, with <15 cases being reported in the literature, mainly in single case reports [1, 2, 9–13]. Patients with AML iAMP21 have been mainly adults, who always had a complex karyotype. Clinicopathologic findings, molecular mutation profiles, and outcomes were largely unavailable in the reported cases.

In this study, we report 13 patients with AML iAML21 from our institution. We describe the clinicopathologic, immunophenotypical, and conventional cytogenetics findings in this study group and we performed next-generation sequencing (NGS) analysis in 11 patients.

Supplementary information The online version of this article (https://doi.org/10.1038/s41379-020-0494-3) contains supplementary material, which is available to authorized users.

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Materials and methods

Case selection

We searched the cytogenetics archives of our institution for cases of AML with *RUNX1* ≥ 5 copies during the years of January 2010 through December 2019. Thirteen cases of AML iAMP21 were identified. Clinical and laboratory data and patient outcomes were obtained by review of medical records. This study was conducted in accordance with the Declaration of Helsinki.

Morphologic examination

Wright–Giemsa-stained peripheral blood (PB) and bone marrow (BM) aspirate smears, and hematoxylin and eosin-stained sections of BM core biopsy specimens were assessed in all cases. Myelofibrosis was evaluated by reticulin and trichrome stains performed on the BM core biopsy when available. The grade of myelofibrosis was based on the European Consensus on grading of BM fibrosis [14].

Flow cytometric immunophenotyping

BM aspirate specimens were subjected to standard eight-color flow cytometry immunophenotypic analysis as described previously [15], using antibodies against the following antigens: CD2, CD3, CD4, CD5, CD7, CD9, CD10, CD11b, CD13, CD14, CD19, CD20, CD22, CD25, CD34, CD38, CD41, CD56, CD64, CD79b, CD117, CD123, Human Leukocyte Antigen-DR isotype (HLA-DR), myeloperoxidase (MPO), and terminal deoxynucleotide transferase (TdT; BD Biosciences, San Jose, CA). Cytochemical stains for MPO were performed on diagnostic BM aspirate smears.

Conventional cytogenetics and FISH analyses

Conventional G-banded chromosomal analysis was performed on unstimulated 24-h and 48-h BM aspirate cultures using standard techniques described previously [16]. Twenty metaphases from each sample were analyzed and results were reported according to the 2016 International System for Human Cytogenetics Nomenclature (ISCN 2016). A complex karyotype was defined as ≥3 chromosomal abnormalities.

FISH analysis was performed on BM aspirate smears or cultured cells with *RUNX1T1/RUNX1* dual-color dual-fusion FISH probes (Abbott Molecular) according to the manufacturer's instructions. The presence of ≥5 and/or clusters of *RUNX1* signals was considered as evidence of *RUNX1* amplification. Of a note, our laboratory started to perform FISH for *RUNX1T1/RUNX1* on all new AML patients presented at our institution in 2018.

Molecular mutation studies

Molecular analyses were performed as a part of the routine clinical work-up. Targeted NGS studies using panels of genes commonly altered in hematopoietic neoplasms were performed in nine patients, using a 28-gene, 53-gene, or 81-gene panel (Supplemental 1) as described previously [17].

Statistical analysis

The Kaplan–Meier method was used to estimate overall survival (OS) from the date of iAMP21 detection. The clinical follow-up end points included the date of death from any cause, or censored at time of hematopoietic stem cell transplant (SCT) or last follow-up for alive patients.

Results

Patients

We identified 25 patients with iAMP21 during the study period, 12 with B-ALL and 13 with AML, the later 13 patients constitute this study cohort, accounting for ~0.1% of total AML cases at our institution.

A summary of the clinical features of these patients is shown in Table 1. All patients were adults, six women and seven men, with a median age of 65 years (range, 34–83 years). Nine patients had a history of malignancy and had been treated at somewhere outside of our institutions (Table 1), the intervals from diagnosis of the first (prior) malignancy to the detection of iAMP21 were from 5 months to 294 months. For two patients who had therapy-related AML (t-AML), patient #12 received adriamycin, cytoxan, gemzar, paclitaxel, herceptin/trastuzumab, abraxane, and carboplatin for her breast cancer, and patient #13 was treated with revlimid, velcade, and dexamethasone for plasma cell myeloma. Of note, five patients had karyotypic and/or chromosomal information of the prior malignancies. Patient #6 had a karyotype of 46,XX,−5,del(7)(q22q36),+8,−21,+mar[7]/46,XX[8]; patient #13 had a normal diploid karyotype; patient #7 was reported to have a complex karyotype; patient #10 had monosomy 7 and add(11q); and patient #11 had del (5q). However, no detail karyotype was available for the latter three patients (cases #7, 10, and 11). None of the patients had FISH for *RUNX1* performed at outside hospitals. iAMP21 was detected in the first BM specimen at our institution for all patients.

Table 1 Clinical characteristics.

Case	Sex/age	Diagnosis		Int* (mon)	Prior malignancy		Peripheral blood				Treatment	Outcome	OS (mon)
		WHO	FAB		Diagnosis	Treatment	Blast	WBC 10^9/L	Hgb g/dL	PLT 10^9/L			
1	M/75	AML-MRC	M0		No	–	33%	2.8	9.6	107	Decitabine, vosaroxin	Deceased	0.9
2	M/62	AML-MRC	M1		No	–	67%	2.3	8.1	71	Azacitidine, vorinostat	Deceased	1.0
3	M/54	AML-MRC	M1		No	–	0	0.1	7.9	21	FLAG-IDA, guadecitabine	Deceased	6.1
4	F/83	AML-MRC	M1		No	–	20%	1.3	8.6	163	Venetoclax, decitabine	AWD	4.5
5	F/65	AML-MRC	M2	294	Breast cancer	Surgery, tamoxifen	3%	1.1	7.9	115	Guadecitabine SCT	ACR	(4) + 76
6	F/34	AML-MRC	M2	5	MDS	Decitabine	66%	0.3	8.3	4	ONC201 trial	Deceased	0.4
7	M/39	AML-MRC	M1	7	CMML-1	Decitabine	12%	0.5	8.6	19	Cladribine, idarubicin, cytarabine	AWD	0.5
8	M/75	AML-MRC	M2	120	MDS/MPN (*JAK2* mutated)	Vidaza, decitabine	17%	15.4	9.0	56	Chemo JAK2 inhibitor	Deceased	3.1
9	M/66	AML-MRC	M2	76	Prostate cancer	Radiation	5%	1.4	7.4	83	7 + 3	Deceased	12.5
10	F/50	AML-MRC (relapsed)	M1	38	Uterine sarcoma AML	Surgery chemo (AML)	12%	0.9	7.2	10	7 + 3	Deceased	3.0
11	F/74	AML-MRC (relapsed)	M2	6	AML	Chemo (AML)	0	1.9	9.3	20	NA	AWD	2.0
12	F/64	t-AML	M6	140	Breast cancer	Chemo	1%	6.1	7.8	13	No detail	Deceased	6.1
13	M/65	t-AML	M2	69	PCM	Chemo	0	0.8	8.2	13	NA	AWD	3.2

7 + 3 7 days of cytarabine +3 days of an anthracycline antibiotic or anthracenedione, *ACR* alive with complete remission, *AML* acute myeloid leukemia, *AWD* alive with disease, *CMML-1* chronic myelomonocytic leukemia-1, *F* female, *FLAG-IDA* fludarabine, cytarabine, filgrastim, and idarubicin, *Hgb* hemoglobin, *M* male, *mon* months, *MDS* myelodysplastic syndromes, *MPN* myeloproliferative neoplasms, *MRC* myelodysplasia-related changes, *OS* overall survival, *PCM* plasma cell myeloma, *PLT* platelet, *SCT* stem cell transplant, *t-* therapy-related, *WBC* white blood cell.

*Int interval period from the diagnosis of prior malignancy to the detection of iAMP21; +patient #5 received SCT at 4 months.

PB and BM findings

Complete blood count (CBC) data are available for all patients (Table 1). All patients had cytopenias, 11 with pancytopenia and 2 with bi-lineage cytopenia. The median WBC count was 1.3×10^9/L (range, 0.1–15.4×10^9/L), median hemoglobin level 8.2 g/dL (range, 7.2–9.6 g/dL), median platelet count 21×10^9/L (range, 4–163×10^9/L), and median blast count in the PB 8.5% (range, 0–67%).

The findings in BM core biopsy specimens and aspirate smears are summarized in Supplemental 2. The BM cellularity ranged from 5% to 95%. The median blast count was 40% (range, 20–80%). Blasts were medium sized to large with fine chromatin and distinct nucleoli. Cytoplasmic vacuoles were observed in seven cases. Auer rods were rare or absent. Dysplasia of one or multiple lineages was observed in 11 patients, and dysplasia could not be evaluated in the other 2 patients (#2 and #4) due to lack of adequate hematopoietic elements. Using the French–American–British (FAB) classification system [18], the neoplasms were classified as M0 ($n = 1$), M1 ($n = 5$), M2 ($n = 6$), and M6 ($n = 1$). Using the WHO classification, 11 neoplasms were classified as AML with myelodysplasia-related changes (AML-MRC), including two patients with relapsed AML; two were classified as t-AML.

Cytochemical staining for MPO was performed on BM aspirate smears of nine patients: unequivocally positive in two, rare positive blasts in two, and negative in five. Reticulin and trichrome stains were performed on BM biopsy specimens in all cases and ten patients showed variable degree myelofibrosis, MF-1 ($n = 6$), MF-2 ($n = 3$), MF-3 ($n = 1$), and MF-0 ($n = 3$), (Fig. 1, Supplemental 2).

Immunophenotypic findings

The immunophenotypes of the blasts are summarized in Table 2. The blasts were variably positive for following markers: CD13 (11/13), CD33 (11/13), CD117 (11/13), MPO (7/12), CD7 (6/13), CD4 (4/11), and TdT (4/12). The blasts were negative for CD2, sCD3, cCD3, CD5, CD14, and CD19 in all cases assessed

Cytogenetic and molecular findings

Karyotype and copies of *RUNX1* detected by FISH analysis are summarized in Table 3. All patients had a complex karyotype; 12 patients showed a high degree of genomic complexity as indicated by ≥5 cytogenetic alterations; and 10 had a monosomal karyotype. The most common additional cytogenetic alterations were: −5/del[5q] ($n = 9$), −7/del[7q] ($n = 6$), and −17/del[17p] ($n = 6$). High copy numbers of *RUNX1* were present as: hsr[21](q22) ($n = 4$), extra copies of chromosome 21 and/or i[21q], insertion of

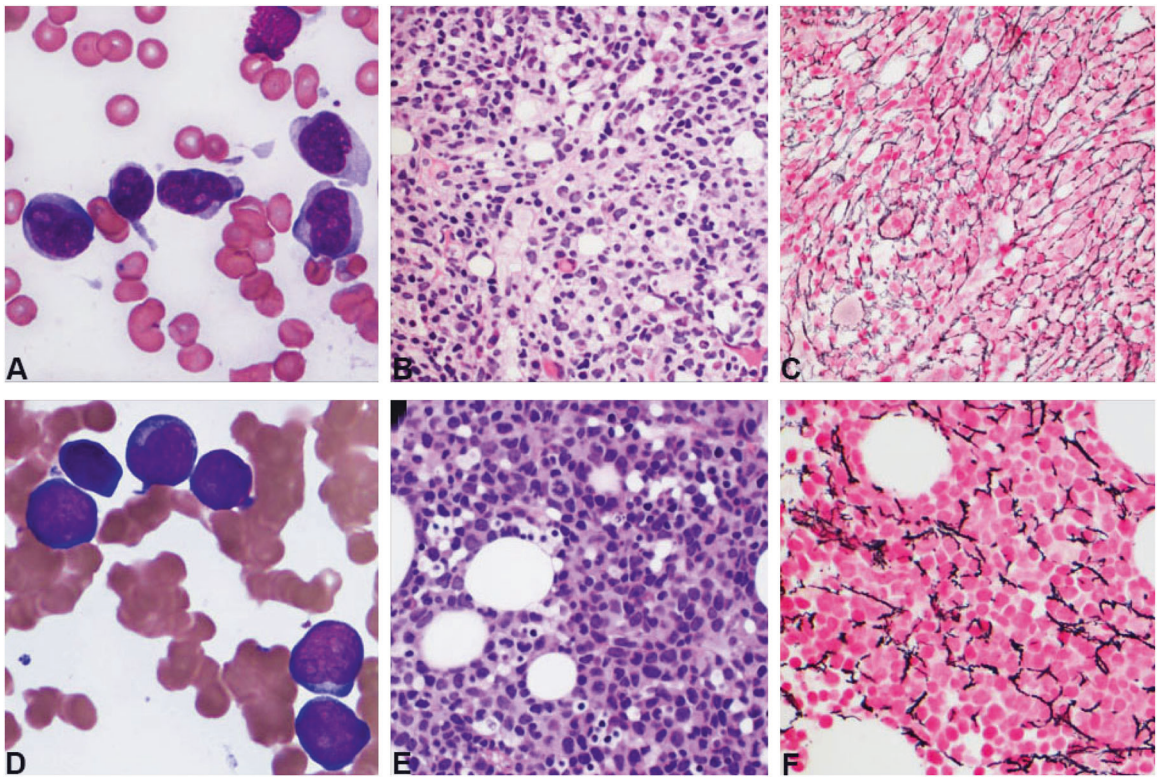

Fig. 1 Morphologic findings. a–c (Case #1), **a**: bone marrow (BM) aspirate smear showed blasts (1000×). **b**: BM biopsy specimen showed sheets of immature cells (400×). **c**: Reticulin stain showed increased reticulin fibers (400×). **d–f** (Case #5), **d**: BM aspirate smear showed blasts (1000×). **e** BM biopsy specimen showed sheets of immature cells (400×). **f**: Reticulin stain showed mild increase in reticulin fibers (MF-1) in a loose network (400×).

Table 2 Flow cytometric immunophenotype.

Marker	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10	Case 11	Case 12	Case 13	Pos cases
CD4	Neg	—	Pos (p)	Neg	Neg	Pos (p)	Neg	—	Neg	Neg	Pos	Pos	Neg	4
CD7	Neg	Neg	Pos (p)	Neg	Neg	Pos	Neg	Neg	Neg	Pos	Pos	Pos	Pos (p)	6
CD13	Pos	Pos	Pos	Pos	Pos (p)	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Pos	12
CD15	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Pos (p)	Neg	Neg	Neg	Neg	Neg	2
CD22	Pos (p)	—	Pos (p)	Pos (p)	Neg	Neg	Neg	—	Neg	Pos (p)	Neg	Neg	Neg	4
CD33	Pos	Pos	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Neg	11
CD34	Pos	Pos	Pos	Pos	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Neg	Pos	11
CD36	Pos	—	Neg	Neg	Pos (p)	Pos	Neg	—	Neg	Neg	Pos	Pos	—	5
CD38	Pos	Pos	Pos (p)	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	13
CD41	Pos	Neg	—	Neg	Neg	Neg	Neg	Neg	—	Neg	—	—	—	1
CD45	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	13
CD56	Neg	Neg	Pos	Pos (p)	Neg	Neg	Pos	Neg	Pos	Pos	Neg	Neg	Neg	5
CD64	Pos (p)	Neg	Pos (p)	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos (p)	3
CD117	Pos	Pos	Pos	Pos	Pos	Pos	Pos(p)	Pos	Pos	Neg	Pos	Neg	Pos	11
CD123	Pos	—	Pos	Pos	Pos	Neg	Pos	—	Pos (p)	Pos	Pos	Pos	Pos	10
HLA-DR	Pos	Pos	Pos	Pos	Pos (p)	Pos	Neg	Pos	Pos (p)	Pos	Pos	Neg	Pos	11
MPO	Neg	Neg	Pos	Pos	Pos	Neg	Neg	Pos	Pos	Pos	Pos	Neg	—	7
TdT	Pos	Neg	Neg	Neg	Pos (p)	Neg	Neg	Neg	Pos (p)	Pos	Neg	Neg	—	4

All cases are negative for CD2, CD3, cCD3, CD5, CD14, and CD19.

Neg negative, *Pos* positive, *Pos (p)* positive (partial).

Table 3 Cytogenetic and molecular findings.

Case	Cytogenetic findings		Molecular mutation findings		
	Karyotype	FISH	Positive	Negative	NGS panel
1	46,XY,del(5)(q23),del(7)(q22q34),der(21)t(5;21)(q32;q22)[cp6]/73~92 < 4n >, XXYY,der(5)t(5;21)(q23;q22)x2[cp2]/46,XY[8]	*RUNX1*x8[142]/*RUNX1*x4[33]/ *RUNX1*x9–12)[9]/*RUNX1*x5[8]	*RUNX1*	All others	28-gene
2	48,X,-Y,+6,+8,+13,del(13)(q12q22),+15,−17,−17,+21, add(21)(q22),+der(?)t (?;21)(?;q22)[cp3]/46,XY[17]	*RUNX1*x4–5[152]	None	*FLT3, CKIT, NPM1, RAS, CEBPA*	ND
3	45,XY,−2,−5,del(7)(q32q34),psu dic(11;14)(q23;p11.2), del(12)(p11.2),+r,+mar [7]/45,idem,del(17)(p11.2)[5]/46,idem,+8[3]/45~46,idem,+8,+1~3mar[cp5]	*RUNX1*x4[97]/*RUNX1*x5~8[42]	*TP53*	All others	81-gene
4	47~53,XX,add(2)(q31),der(2)add(2)(p12)del(2)(q34q36),t(3;16)(p13;q24),del(5) (q13q33),−16,+21,+21,+21, i(21)(q10),+1~6mar[cp10]/46,XX[6]	*RUNX1* amp[72]	*TP53, NF1, DNMT3A*	All others	81-gene
5	46~54,XX,del(5)(q22q35),+21,add(21)(q22)x2,+1~5mar[cp15]	*RUNX1*x4[22]/*RUNX1*x5~9[8]	None	All others	53-gene
6	41~47,XX,−4,add(4)(q27),−5,del(5)(q13q33),−7,−8,add(9)(p13), add(11)(p15), −13,−14,−16,der(17;21)(q10;q10),−18,−18,−19, der(19)t(19;21)(q13.3;q11.2), +add(21)(p11.2),add(21)(p11.2),−22, + 2~7mar[cp20]	*RUNX1*x4[154]/*RUNX1*x5[31]	*TP53*	*CEBPA, FLT3*	28-gene
7	40~47,XY,del(5)(q13q33),add(9)(q13),−16,−19,−21,−22,+1~2mar,1~4dmin [cp19]/46,XY[1]	*RUNX1* amp[139]	*TP53, PRPF40B*	All others	81-gene
8	46,XY,der(21)i(21)(q10)dup(21)(q11.2q22)[6]/46,idem,del(7)(q22q32)[6]/46,XY [8]	*RUNX1*x4–7[152]	None	*KIT, RAS, CEBPA, FLT3, NPM1*	ND
9	46,XY,del(5)(q13q33),+10,del(11)(q12),der(11;20)(q10;p10),del(17)(p11.2),−18, hsr(21)(q22)[13]/ 46~48,idem,add(3)(q29),+der(11;20),+mar[cp7]	*RUNX1*x4[94]/*RUNX1*x3[3]*	*TP53*	All others	81-gene
10	48~57,XX,+1,+2,+6,+8,+11,+12,+14,add(17)(p11.2),+19,+add(21)(p11.1),+i (17)(q10),+1~4mar[cp6]/61~64,XXX,−3,−4,−5,−12,−16,−17,+21,add(21) (p11.2),i(21)(q10),+1~2mar[cp4]	*RUNX1* amp[153]	*TP53, DNMT3A*	All others	81-gene
11	46,XX,i(5)(p10)[11]/42~45,XX,add(4)(p14),−5, del(5)(q13q33),−7,del(12) (q22q24.3),−19,hsr(21)(q22),+der(?)ins(?;21)(?;q21q22),+1~5mar[cp9]	*RUNX1*x4[20)/*RUNX1*x3[16]/ *RUNX1*x6 [8]	*TP53*	All others	81-gene
12	46,XX,del(17)(p12)[4]/42~46,XX,del(5)(q22q31),+del(7)(p15),der(7)add(7)(p12) add(7)(q11.2),der(7;17)(q10;q10)add(7)(q21)ins(7;21)(?;q22q22),−12,r(21)hsr(21) (q22),+1~2mar[cp6]/ 46,XX[10]	*RUNX1*x5–7[157]	*TP53*	All others	81-gene
13	46,XY,−9,+21,add(21)(q22),add(21)(q22)[7]/46,idem,der(4)add(4)(q21)hsr(21) (q22)[8]/46,XY[5]	*RUNX1* amp[122]	*TP53*	All others	81-gene

*Cases #9 showed hsr(21)(q22) and cluster of *RUNX1* signals on metaphases.

NGS next-generation sequencing, *FISH* fluorescence in situ hybridization.

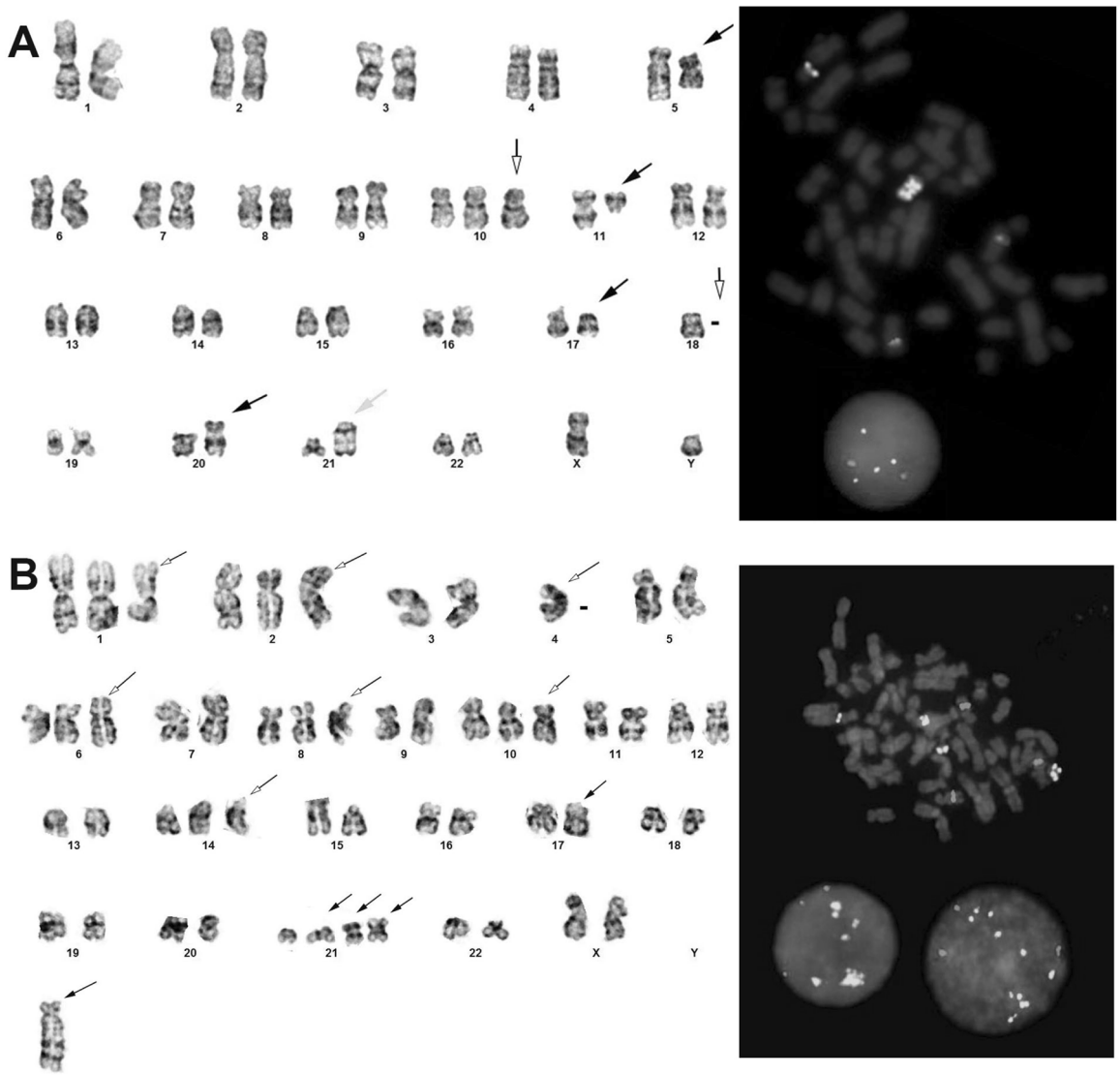

Fig. 2 Cytogenetic findings. Left side: conventional cytogenetic analysis; right side: FISH analysis with *RUNX1T1* (red)/*RUNX1* (green) dual-color dual-fusion probe. **a** Case #9, karyotype: 46,XY,del (5)(q13q33),+10,del(11)(q12),der(11;20)(q10;p10),del(17)(p11.2), −18,hsr(21)(q22). FISH showed two copies of *RUNX1T1*, four copies (interphase) or cluster (metaphase) of *RUNX1* signals. **b** Case #10, karyotype: 54,XX,+1,+2,−4,+6,+8,+10,+14,add(17)(p11.2),+add (21)(p11.1),+i(21)(q10),+mar. FISH showed three copies of *RUNX1T1* (+8) and >5 or cluster of *RUNX1* signals.

21q22, double minutes (dmin), and ring or marker chromosomes. FISH analysis revealed ≥5 *RUNX1* copies in interphase cells in 12 out of 13 cases, except case #9, in which 3–4 copies of RUNX1 were observed in interphases; but it showed hsr[21](q22) on chromosomal analysis and clusters of *RUNX1* signals on the metaphase FISH (Fig. 2).

Molecular mutation data are summarized in Table 3. Mutation of *TP53* was detected in 9 out of 11 patients tested, 6 of them showed only *TP53* mutation without any other gene mutations. Of a note, two patients (cases #6 and #7) showed *TP53* mutation at the initial diagnosis of MDS and chronic myelomonocytic leukemia (CMML-1), respectively. Other mutations in AML iAMP21 were uncommon and included *DNMT3A* ($n = 2$), *RUNX1* ($n = 1$), *PRPF40B* ($n = 1$), and *NF1* ($n = 1$). None of the fusion transcripts associated with AML, such as t(8;21), t(9;22), and inv [16], were detected in any of these cases. None of the 13 patients tested showed *FLT3, NRAS*, or *KRAS* mutation.

Outcomes

After detection of iAMP21, ten patients received multi-regimen chemotherapy. One patient achieved remission and received subsequent allogeneic SCT (at 4 months); the other nine patients were refractory to chemotherapy. At time of

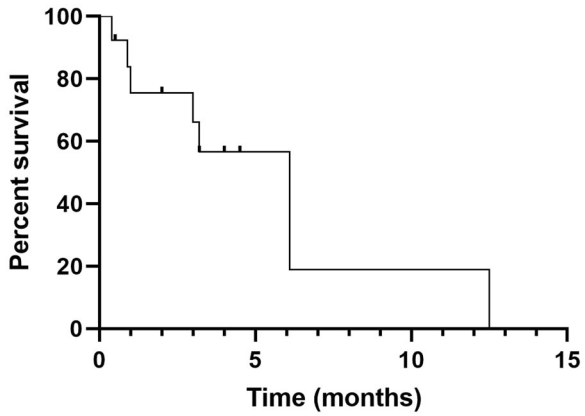

Fig. 3 Overall survival of 13 patients.

last follow-up, eight patients died from disease, four were alive with persistent disease, and one patient (case #5) was alive in complete remission after SCT. The median OS for this group of patients was 6 months (Fig. 3). Of a note, cases #4, #7, #11, and #13 had a short follow-up.

We also compared the OS of these 13 patients with iAMP21 to 20 patients who had AML with complex karyotype (14 had monosomal karyotype) but no iAMP21. Though patients with iAMP21 had a shorter OS, it was not significant (median OS, 6 months vs. 8.4 months, $p = 0.2624$).

Discussion

Amplification is a genomic alteration that typically results in overexpression of particular oncogenes located within the amplicon. Gene amplifications are uncommon events in AML and are often associated with refractoriness to chemotherapy and an aggressive clinical course [16, 19, 20]. *KMT2A* (*MLL*) and *MYC* are the most two frequently amplified genes in AML [16, 19]. Amplification of genes can locate extrachromosomally as dmin, intrachromosomally as homogeneously staining regions (hsr), or as a ring, marker/derivative chromosome, or isochromosome [12, 13, 16, 19, 21, 22]. Except hsr[21](q22) that can be easily recognized as iAMP21, other forms of amplification of *RUNX1* could be misinterpreted as unclarified ring, mark, or derivative chromosomes and "missed" by chromosomal analysis. Therefore, the true frequency of iAMP21 in AML may be underestimated. The most reliable assay to detect iAMP21 is FISH analysis with *RUNX1* probe. In our institution, we identified 13 cases in the past 10 years, 9 of them were from the recent 3 years. The "increased" rate in the recent 3 years is likely due to the initiation of FISH analysis for *RUNX1T1/RUNX1* in all newly diagnosed AML, which started 3 years ago.

RUNX1 is expressed in all hematopoietic lineages and acts to regulate the expression of various genes specific to hematopoiesis. *RUNX1* is one of the genes most frequently dysregulated in leukemia through different mechanisms, including translocations, amplifications, and mutation [1]. B-ALL with iAMP21 has been added as a new provisional entity in the 2017 WHO classification [23]. AML iAMP21 often exhibits several features that differ from B-ALL iMAP21. Patients with AML iAMP21 are almost all adults, with only one child reported who had constitutional r(21) [11]. AML iAMP21 is almost always associated with complex karyotype [2, 9–13], most of which being highly complex. AML iAMP21 also shows high frequency of *TP53* deletion and/or mutation: 3/3 patients reported in the literature with mutation information available [9, 10] and 10/13 patients in our cohort had *TP53* mutation and/or deletion. Patients with AML iAMP21 often present pancytopenia: 3/3 reported patients who had CBC information available [2, 9, 11] and 11/13 in this study group. Lastly, patients with AML iAMP21 are highly refractory to standard AML-based induction therapy and only one patient in this cohort (case #5) achieved remission after induction.

Outcome information was available in only two patients reported in the literature. One patient responded to treatment initially, but relapsed after 9 months and died 3 months later after SCT [2]. The other patient was reported to be in complete remission after SCT [11]. In our cohort, eight patients died with a median survival of 3.2 months; five patients were alive but four of them (#4, #7, #11, and #13) were recently diagnosed and had relatively short follow-up periods (<5 months), only one patient (#5) achieved remission after induction and has been in remission for ~6 year after SCT. Comparing to other patients in this cohort, this patient (#5) had a relatively less complex, non-monosomal karyotype, with a very low percentage (4%) of interphases showing *RUNX1* amplification, and no gene mutation (including *TP53*) by 53-gene NGS panel. All these features may contribute to the good responses to therapies and a better outcome.

The regions predominantly amplified in iAMP21 span chromosome 21q22.1q22.3 and ranged from 11.8 to 40.0 Mb [9, 22]. Three genes, *APP, ERG*, and *ETS2* are often amplified in patients with iAMP21 [9, 22, 24]. *APP* has been linked to Alzheimer's disease and dementia in adults with Down syndrome. *ETS2* and *ERG* are proto-oncogenic transcription factors that are involved in cell cycle development and regulation. *RUNX1* may be a bystander amplified with other significant genes [25]. Although the pathogenesis of AML iAMP21 is not clear, the *TP53* gene has important function in maintaining genomic stability and integrity. Mutational inactivation of *TP53* has been shown in experimental models to result in

gene amplification as well as in aneuploidy [26, 27]. Given that most patients with AML iAMP21 had *TP53* deletion/mutation and a very complex karyotype, genome instability and chromothripsis could be the underlying mechanisms of pathogenesis [26, 28]. Two patients (#6 and #7) showed *TP53* mutation at the initial diagnosis of MDS and CMML-1, supporting *TP53* mutation to be a primary event and likely play an important during the pathogenesis. Interestingly, two patients with AML iAMP21 had constitutional abnormality of r(21) [2, 11], suggesting the "instability" of structurally abnormal chromosome 21 r(21) and leads to iAMP21. Pathogenetic mechanisms in AML with iAMP21 may overlap with AML associated with *MLL* amplification, which shares some features, such as complex karyotype, *TP53* mutation/deletion, and aggressive outcomes [16]. A more comprehensive molecular study may help to understand the pathogenesis of this rare disease, especially for the cases with no or only *TP53* mutation.

In summary, AML iAMP21 is often associated with pancytopenia, morphological dysplasia, a complex karyotype, and *TP53* deletion/mutation. Patients with AML iAMP21 are often refractory to conventional AML therapy and have very poor outcomes. Early stem cell transplantation as well as novel therapies may improve patients' outcome.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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