Acute myeloid leukemia with cup-like blasts and *FLT3-ITD* and *NPM1* mutations mimics features of acute promyelocytic leukemia: a case of durable remission after sorafenib and low-dose cytarabine

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Some previous researches raised the possibility of a novel acute myeloid leukemia (AML) entity presenting cup-like cytomorphology with mutations of both *FLT3* and *NPM1* or one of them. However, the clinical implications of this subtype remain unknown. We describe a 63-year-old patient belonging to this distinct AML subtype, who presented similar features of acute promyelocytic leukemia (APL) including nuclear morphology, negative for CD34 and HLA-DR, and abnormal coagulation. He had no response to both arsenic trioxide and CAG regimen (cytarabine, aclarubicin, and G-CSF). Given that the patient carried the *FLT3-ITD* mutation, we switched to a pilot treatment of *FLT3* inhibitor sorafenib combined with low-dose cytarabine (LDAC). To date, the patient achieved durable complete remission over 58 months. These findings suggest that AML with cup-like blasts and *FLT3-ITD* and *NPM1* mutations mimic APL, and the prognosis of this subtype may be improved by sorafenib combined with LDAC. *Anti-Cancer Drugs* 33: e813–e817 Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc.

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Introduction

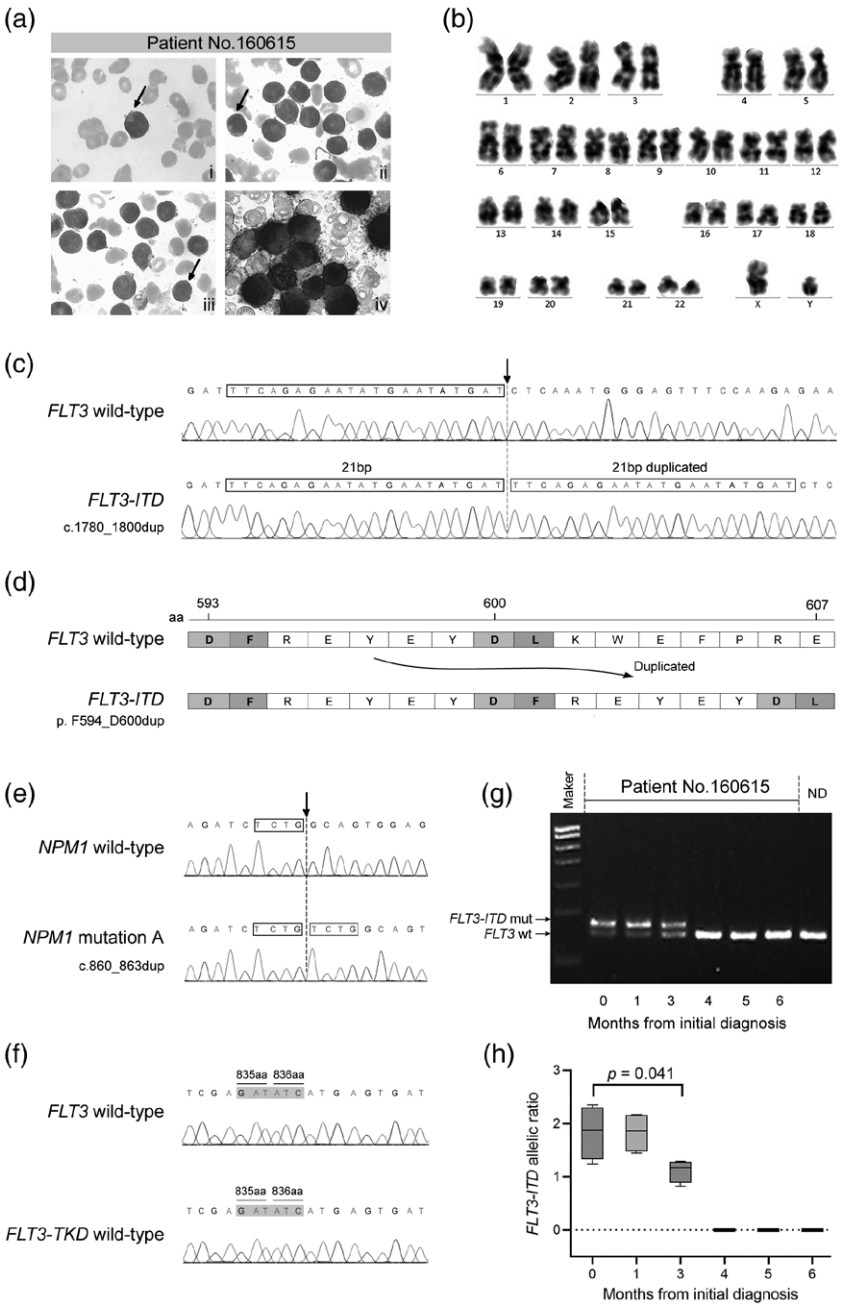
Acute myeloid leukemia (AML) is a molecularly and cytogenetically heterogeneous hematological malignancy. Previous studies have indicated a potential AML subtype characterized by a distinct cup-like blast phenotype with mutations of both *FLT3* and *NPM1* or one of them [1–5]. However, the clinical features, treatment and prognosis of this subtype remain to be depicted. Here, we report a case that was categorized into the abovementioned AML subtype exhibiting pathological features similar to acute promyelocytic leukemia (APL) and achieved durable complete remission by sorafenib combined with low-dose cytarabine (LDAC). Accordingly, we perform a literature review of similar studies to explore the clinical significance of this potential AML subtype.

Case presentation

Patient No. 160615, a 63-year-old man with a past medical history of hypertension, was admitted to our hospital for fever, sore throat, and fatigue over 2 weeks. He had no family genetic diseases. Physical examination showed no abnormalities.

A complete blood count revealed leukocytosis (17.92×10^9/L), anemia (hemoglobin 77 g/L), and thrombocytopenia (30×10^9/L), with 91% blasts in peripheral blood. An analysis of coagulation parameters suggested increased levels of D-dimer (15 750 ng/mL). The results of bone marrow aspiration revealed 99% of blasts that were peroxidase positive [Fig. 1a (iv)]. Some of blasts had 'cup-like' or 'fish-mouth' nuclear morphology [Fig. 1a (i)–(iii)]. Flow cytometry showed that the blasts were positive for CD117, CD13, CD33, CD64, and CD56 and negative for CD34, HLA-DR, and CD11b. Based on the morphological and immunotypical features, the microgranular variant of APL was suspected. We immediately began treatment with arsenic trioxide (ATO, 10 mg/day) before cytogenetic and molecular results were available. After 7 days of treatment, the patient exhibited a notable decrease in white blood cell count (1.88×10^9/L), no improvement in platelet count (25×10^9/L) and hemoglobin level (68 g/L). In addition, cytogenetics analysis revealed a normal karyotype (Fig. 1b) and fluorescence in-situ hybridization (FISH) failed to detect the *PML-RARA* rearrangement. Molecular studies using Sanger sequencing demonstrated

Fig. 1



Cup-like blasts with *FLT3-ITD* and *NPM1* mutations in an AML patient. (a) Morphological features of blasts derived from Patient No. 160615. Blasts with prominent nuclear invagination resulting in nuclei with a 'cup-like' or 'fish-mouth' appearance (as indicated by arrows) in both peripheral blood (i) and bone marrow smears (ii–iii), as detected with Wright-Giemsa staining. Blasts are myeloperoxidase positive (iv, POX stain). Images taken at 1000× magnification. (b) G-banding showed that the karyotype of the bone marrow cells derived from Patient No. 160615 was 46,XY. (c) Sanger sequencing chromatogram showing the mutation in *FLT3-ITD* in bone marrow cells derived from Patient No. 160615. The brown box indicates that the 21 bp fragment is duplicated in the DNA sequence. (iv) A schematic of the protein structure of the *FLT3-ITD* mutant. The c.1780_1800dupTTCAGAGAATATGAATATGAT variant results in the in-frame duplication of seven amino acid residues at codons 601 through 607. (e) Sanger sequencing chromatogram showing the mutation in *NPM1* in bone marrow cells derived from Patient No. 160615. The brown box indicates that the 4 bp fragment is duplicated in the DNA sequence (mutation A, c.860_863dupTCTG). (f) Sequencing chromatogram showing the wild-type *FLT3-TKD* in bone marrow cells derived from Patient No. 160615. The gray background confirms no mutations in codons 835 and 836 of the second tyrosine kinase domain (TKD) in *FLT3*. (g) 4% agarose gel electrophoresis of *FLT3-ITD* at different time points after the initial diagnosis of Patient No. 160615. The two bands (151 and 172 bp) correspond to wild-type and mutant *FLT3-ITD*, respectively. ND, DNA from healthy mucosa tissue of a normal donor; *FLT3-ITD* mut, *FLT3-ITD* mutation; *FLT3* wt, *FLT3* wild-type. (h) Boxplot showing significant *FLT3-ITD* allelic ratio changes over time (*FLT3-ITD* allelic ratio = optical density values of mutant/optical density values of wild-type). The *FLT3-ITD* allelic ratio gradually decreased and finally became negative. The experiments were repeated ≥3 times. An unpaired two-sided Student's *t*-test was used to compare two groups. AML, acute myeloid leukemia.

FLT3-ITD (c.1780_1800dup) and *NPM1* (c.860_863dup) mutations (Fig. 1c–e), while no mutation was detected in the second tyrosine kinase domain (TKD) in *FLT3* (Fig. 1f). Accordingly, we terminated the ATO treatment and switched to the CAG regimen (cytarabine 10 mg/m^2, q12h, days 1–14; aclarubicin 10 mg, days 1–10; and G-CSF 300 μg, days 0–14), but the patient developed acute respiratory distress syndrome and acute coronary syndrome during chemotherapy. The findings of bone marrow aspiration approximately 4 weeks following treatment suggested that remission had not been achieved, with 95.5% blasts. Given that Patient No. 160615 carried the *FLT3-ITD* mutation, we switched to a pilot treatment, 200 mg *FLT3* inhibitor sorafenib twice daily every day combined with LDAC at a dose of 20 mg q12h for 14 days every 4 weeks, with informed consent from the patient. Of note, at the time of treatment (2016), no *FLT3* inhibitors had been approved by the FDA specifically for AML patients with *FLT3* mutations. The treatment was well tolerated by the patient, and blasts in bone marrow were cleared after three cycles of treatment (Fig. 2). More importantly, at the molecular level, the *FLT3-ITD* allelic ratio gradually decreased and finally became negative with prolonged treatment time (Fig. 1g and h). Thereafter, LDAC was administered once every 2 months after completing the 8th course of treatment, once every 3 months after the 12th course of treatment, and once every 4 months after the 16th course of treatment.

Collectively, the patient received a total of 17 cycles of LDAC and over 32 months of treatment with sorafenib. At the time of writing, the patient has been free of leukemia for over 58 months.

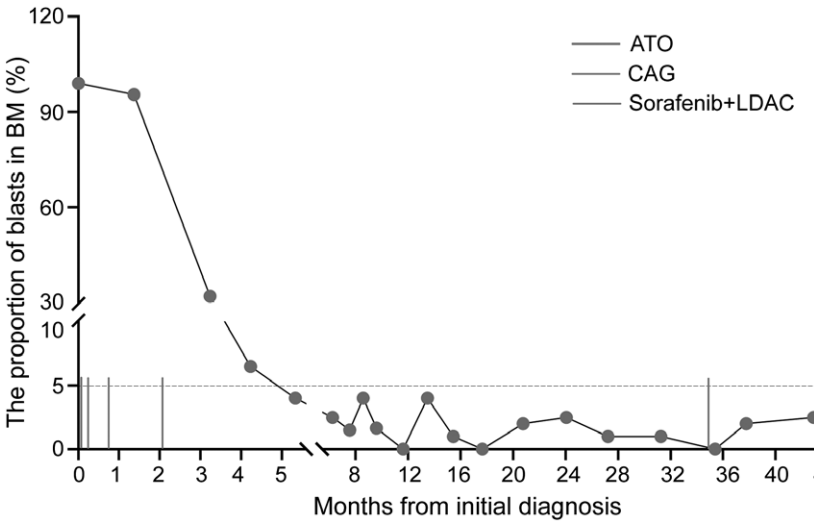
Discussion

To the best of our knowledge, eight retrospective studies and five cases regarding cup-like nuclei in AML with mutations of both *FLT3* and *NPM1* or one of them have been published thus far (Table 1). A summarized description of the previous studies and the present case is as follows: (1) morphology, the cup-like nuclear invagination spans at least 25% of the nuclear diameter in more than 5–10% of blasts [3,7]; (2) immunophenotype, mostly negative for HLA-DR and CD34; (3) molecular and cytogenetic parameters, a high incidence of *FLT3* or *NPM1* mutations with normal karyotype; and (4) clinical parameters, a high number of bone marrow blasts, high WBC counts, and high D-dimer levels.

Of note, these characteristics are more susceptible to confused with APL, including nuclear morphology, lacking CD34 and HLA-DR, and abnormal coagulation. FISH analysis for *PML-RARA* fusion gene is relevant in ruling out differential diagnoses in patients suspected of APL. Therefore, increasing awareness of these characteristics is warranted to avoid diagnostic delay and inappropriate treatment in AML patients who present with cup-like blasts with mutations of both *FLT3* and *NPM1* or one of them.

However, methods to effectively treat the AML subtype with cup-like blasts in patients with mutations of both *FLT3* and *NPM1* or one of them remain unknown. Two previous studies suggested that cytarabine-based intensive chemotherapy might be effective in such patients [1,5]. Moreover, there are few therapeutic options in elderly patients due to their age, performance status and complications, which make them inappropriate for intensive chemotherapy and stem cell transplantation.

Fig. 2



Clinical course of treatment and the change of bone marrow blasts over time in the Patient No. 160615. The brown vertical line indicates the treatment with CAG (cytarabine, aclarubicin, and G-CSF), and the pink vertical line indicates the treatment with sorafenib and low-dose cytarabine (LDAC). The dashed line indicates a normal blast cell proportion in bone marrow.

Sorafenib is an oral multi-kinase inhibitor that targets tumor cell proliferation, apoptosis, and angiogenesis by inhibiting the activity of FLT3, VEGFR-1/2/3, PDGFRβ, FGFR, KIT, RAF, and RET [14–16]. Sorafenib is categorized as type II inhibitor based on the form of interaction with receptor [17,18], and this property determines that sorafenib preformed more effective for *FLT3-ITD* than *FLT3-TKD* mutation [19]. The FDA approved sorafenib for advanced renal cell carcinoma, unresectable hepatocellular carcinoma and radioactive iodine refractory differentiated thyroid cancer [20]. Additionally, sorafenib is commonly applied in clinical trials for the treatment of AML [21–23]. Given the limited clinical effectiveness of sorafenib monotherapy for *FLT3*-mutated AML [21], combining sorafenib with LDAC is an appropriate alternative for less-intensive treatment in elderly AML patients. The recommended sorafenib dose approved by the FDA is 400 mg twice daily for solid tumors, but it is not well tolerated in AML patients [24,25]. 75% of AML patients were unable to adhere to treatment with 400 mg twice daily due to toxicity [26]. Liu *et al*. demonstrated that a low-dose administration of 200 mg twice daily showed similar pharmacological activities with 400 mg twice daily [27]. Considering the poor performance status of our patient, we finally chose 200 mg twice daily of sorafenib as a dosing regimen to minimize toxicities.

Furthermore, the correlation between cup-like morphology and prognosis in AML remains unclear and controversial. Several studies showed that there were no significant differences in complete remission rate or survival parameters between cup-like AML and cup-like negative group [1,7,9]. However, Chen *et al*. [6] revealed that the complete remission rate was higher in cup-like AML. As for our patient, he has experienced a leukemia-free survival of over 58 months until now, indicating that the prognosis of cup-like AML with *FLT3-ITD* and *NPM1* mutations may be improved by FLT3 inhibitors.

Conclusion

Our findings provide additional insight into a novel subtype of AML that presents a cup-like nuclear phenotype with mutations of both *FLT3* and *NPM1* or one of them that mimic APL. It also indicates that the combination of sorafenib with LDAC is an attractive treatment option for this AML subtype.

Materials and methods

Patient samples

All samples of patients were obtained by informed consent. The blast fraction in bone marrow was enriched by using density-gradient centrifugation. Genomic DNA was extracted from 5×10^6 cells using the silica-based

Table 1 **Historical review of studies of cup-like nuclei in acute myeloid leukemia with *FLT3* or *NPM1* mutation**

		Gene mutations (%)			Immunophenotype (%)										
Patients	No. cases	*FLT3*	*NPM1*	*FLT3* and *NPM1*	HLA-DR$^-$	CD34$^-$	Normal karyotype (%)	Bone marrow blasts (%)	Age	M/F ratio	Hg (g/L)	PLT ($\times 10^9$/L)	WBC ($\times 10^9$/L)	D-dimer (ng/mL)	Reference
NML and NPL	19	84	NA	NA	68	68	91.7	NA	66.5	0.46	NA	NA	NA	NA	Kussick *et al.* (2004) [3]
AML-M1	24	88	64	NA	50	71	70	NA	NA	0.33	NA	NA	NA	NA	Chen *et al.* (2006) [6]
NPL	55	72.7	60.4	49.1	33.5	93	81.5	78	56	0.83	NA	NA	77.4	NA	Kroschinsky *et al.* (2008) [7]
AML-M1	22	86	86	77.3	59	82	86	90	59	0.69	89	47	59.3	>5000	Chen *et al.* (2009) [1]
AML-M1	12	80	100	NA	67	100	100	NA	55.4	0.71	NA	NA	62.3	NA	Rakheja *et al.* (2011) [8]
AML-M0, M1, M2	15	71	50	33	40	53	82	82	62	0.87	95	75	3.3	NA	Carluccio *et al.* (2014) [9]
NPL	44	59.1	70.5	38.6	NA	72.7	NA	72.2	63	NA	89	74.9	NA	NA	Park *et al.* (2013) [10]
AML-M1, M2	43	50	73.8	36.6	100	100	84	NA	NA	1.15	NA	NA	NA	NA	Wang *et al.* (2018) [11]
AML	Case report	100	100	100	100	100	100	>90	73	F	92	27	148	61 608	Jalal *et al.* (2009) [12]
AML	Case report	100	100	100	NA	NA	100	93	68	M	90	50	139.3	NA	Jain *et al.* (2013) [2]
AML	Case report	0	100	0	100	100	100	79	75	M	76	15	354.1	NA	Robinson *et al.* (2015) [13]
AML	Case report	100	100	100	NA	NA	100	90	61	M	86	14	0.6	NA	Vidholia *et al.* (2015) [5]
AML	Case report	100	100	100	100	100	100	NA	58	M	107	45	126	12 010	Pepper *et al.* (2020) [4]
AML	Case report	100	100	100	100	100	100	99	63	M	77	30	17.9	15 750	Present case

AML, acute myeloid leukemia; F, female; M, male; NML, non-monocytic AML; NPL, non-promyelocytic AML; NA, not applicable.

procedure according to the manufacturer's protocols (TIANamp Genomic DNA kit; Tiangen, Beijing, China).

PCR for the detection of *FLT3* and *NPM1* mutations

PCR for *FLT3* and *NPM1* mutations were performed on genomic DNA using the previous published primers [28–30]. *FLT3-ITD* primer sequences: 5′-GCAATTTA GGTATGAAAGCCAGC-3′ and 5′-CTTTCAGCATT TTGACGGCAACC-3′. *FLT3-ITD* (c.1780_1800dup) primer sequences: 5′-GCAATTTAGGTATGAAAGCCAGC-3′ and 5′-GCACATTCCATTCTTACCAAACTCT-3′. *FLT3-TKD* primer sequences: 5′-CCGCCAGGAACGTGCTTG-3′ and 5′-GCAGCCTCACATTGCCCC-3′. *NPM1* primer sequences: 5′-TTAACTCTCTGGTGGTAGAATGAA-3′ and 5′-CAAGACTATTTGCCATTCCTAAC-3′.

Sequence analysis

The *FLT3* and *NPM1* mutations were identified by assessing independent PCR products which were cloned into the pMD18-T vector (Takara, Shiga, Japan) and sequenced by the Sanger method.

The analysis of *FLT3-ITD* allelic ratio

PCR products were electrophoresed on 4% agarose gels. The optical density values of the targeted bands were quantified with Image J software. The *FLT3-ITD* allelic ratio was calculated by the following formula: optical density values of mutant/optical density values of wild-type.

Acknowledgements

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This study has been approved by the ethics committee of Beijing Hospital. Informed consent was obtained from the patient.

Conflicts of interest

There are no conflicts of interest.

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