Acute myeloid leukemia with “cup-like” nuclear morphology, highlighting the electron microscopic features

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We present a 63-year-old male patient with acute myeloid leukemia (AML). His hemoglobin level was 10.0 g/dL, white cell count was 16.78 × 10³/µL with 94.6% blasts, and platelet count was 91 × 10³/µL. The bone marrow had 20% cellularity with 87.0% blasts. Approximately 20% of blasts in the peripheral blood exhibited cup-like nuclear morphology, and many mitochondria were concentrated within the nuclear pocket under electron microscopy. The blasts were negative for myeloperoxidase, but expressed CD13 and CD33. CD34 was positive and HLA-DR was weak. The cells demonstrated normal G-banding cytogenetics, and lacked FMS-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD) and nucleophosmin 1 (NPM1) gene mutation. The patient relapsed shortly after a response to initial chemotherapy and died 9 months after presentation. To date, we have encountered a total of 6 cases of cup-like AML in our institution. The ages ranged from 62 to 80 and male to female ratio was 3 to 3. Two had French-American-British M0, one had M1, two had myelomonocytic/monocytic leukemia, and the remaining one had hypoplastic leukemia. Two presented with marked leukocytosis, while the other two had leukopenia. All cases exhibited expression of myeloid antigens, and weak or negative expression of CD34 and/or HLA-DR. All cases carried a normal karyotype, and four had FLT3-ITD or NPM1 mutations, or both. In the nuclear indentation, a condensed collection of mitochondria was observed in all. At present, it remains to be determined whether cup-like nuclear morphology represents a distinctive AML subtype associated with particular clinical features, genetic abnormality, or treatment outcome.

Keywords: cup-like acute myeloid leukemia, CD34, HLA-DR, electron microscopy, FLT3-ITD, NPM1 mutation

INTRODUCTION

Morphological examination of Wright-Giemsa–stained smear slides under light microscopy is fundamental to the diagnosis of acute myeloid leukemia (AML), and French-American-British (FAB) leukemia.
Cup-like AML

experts introduced subtypes, M0 through M7, based on the morphology of leukemia cells after routine staining.\textsuperscript{1} As the FAB classification was subsequently found to correlate with cytogenetic abnormality and immunophenotype, e.g. FAB-M3 with t(15;17)(q24;q21) chromosomal translocation and loss of CD34 and HLA-DR expression, the World Health Organization (WHO) proposed the currently used classification scheme of AML in 2008 by combining the morphology with cytogenetic, genetic, and immunophenotypical information.\textsuperscript{2}

On the other hand, in 2004, Kussick et al. described a total of 19 AML cases, in which the leukemia blasts exhibited prominent nuclear invagination, i.e. “cup-like” nuclear indentation.\textsuperscript{3} The blasts demonstrated little cytoplasmic granularity and no evidence of t(15;17) (q24;q21), despite low-to-absent expression of CD34 and HLA-DR. As initial reports suggested that this characteristic nuclear morphology was closely associated with FMS-like tyrosine kinase 3-internal tandem duplication (\textit{FLT3-ITD}) and nucleophosmin 1 (\textit{NPM1}) gene mutation,\textsuperscript{3,4} cup-like AML and the potential morphology-genomic-immunophenotypical association have drawn the attention of many investigators.\textsuperscript{5-12}

Here, we first present an illustrative case of cup-like AML and describe the clinical, morphological, immunophenotypical, cytogenetic, and genetic features. We next summarize a total of 6 cup-like AML cases treated at our institution focusing upon the electron microscopic features. The aim of this report was to verify the characteristics of cup-like AML that have been described in earlier studies.

\textbf{MATERIALS AND METHODS}

\textit{Phase Contrast Microscopy}

Buffy coat suspensions prepared from peripheral blood (PB) or mononuclear cells from bone marrow (BM) were subjected to phase contrast microscopic examination. The images were captured by an ECLIPSE TS100 inverted microscope and a digital camera system (Nikon Corporation, Tokyo, Japan).

\textit{Flow Cytometry}

Cell surface antigen expression of leukemia blasts was analyzed by single- or multicolor flow cytometry (NAVIOS 3L flow cytometer; Beckman Coulter Inc., Fullerton, CA, USA). Blasts were gated by the side-scatter/forward-scatter and/or CD45 expression/side-scatter characteristics, and then subjected to flow cytometry analysis using fluochrome-labeled monoclonal antibodies specific for the following antigens: CD4, CD7, CD11b, CD11c, CD13, CD14, CD19, CD33, CD34, CD36, CD38, CD45RA, CD45RO, CD56, CD66c, CD117, and HLA-DR.

\textit{Cytogenetic Study}

Cells were incubated overnight under standard conditions, and then cultured in the presence of 0.1 µg/mL colcemid for 2 hr. After harvesting, the cells were treated with hypotonic solution and fixed in methanol:acetic acid (3:1). Chromosomes were banded by trypsin-Giemsa staining.

\textit{FLT3-ITD and NPM1 Mutation}

Genomic DNA was isolated from leukemia cells by means of proteinase K and phenol/chloroform. Polymerase chain reaction (PCR) amplification encompassing exons 14 and 15 of the \textit{FLT3} gene and exon 12 of the \textit{NPM1} gene was performed in a Veriti 96 Well Thermal Cycler (Applied Biosystems, Inc., Forester City, CA), and the PCR products were separated by ethidium bromide-stained agarose gel electrophoresis. For \textit{FLT3-ITD}, aberrant-sized PCR products were excised from the gel and cloned into the plasmid (pGEM®-T Easy; Promega, Fitchburg, WI), and 3 independent cloned DNAs were sequenced by an ABI 310 automated sequencer (Applied Biosystems) in order to avoid PCR artifacts. For \textit{NPM1} mutation, the PCR products were subjected to direct sequencing. The sequences of the primers were: \textit{FLT3}-forward, 5’-GCAATTTAGGTAGGAAAGCCAGC-3’; \textit{FLT3}-reverse, 5’-CTTTCTCAGCATTATGACCGCAACC-3’; \textit{NPM1}-forward, 5’-TTA-
ACTCTCTGGTGTTAGAATGAA-3’; and NPM1-reverse, 5’-CAAGACTATTTGCCATTCTAAC-3’.

**Electron Microscopy**

Mononuclear cells prepared from PB were fixed for 2 hr in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer, and dehydrated through a graded series of ethanol. Dehydrated samples were embedded in epoxy resin. Thin sections were cut on an ultramicrotome, mounted on copper grids, and stained with uranyl acetate and lead citrate. To detect ultrastructural localization of myeloperoxidase (MPO), cells were fixed for 1 hr in 2.5% glutaraldehyde in 0.1 M cacodylate buffer and then incubated for 1.5 hr in a reaction solution containing 0.1% diaminobenzidine tetrahydrochloride and 0.02% hydrogen peroxide. Specimens were post-fixed in buffered osmium tetroxide. Leukemia blasts were investigated under a transmission electron microscope (JEM-1200EX; JEOL, Tokyo, Japan). In each case, a minimum of 250 blast cells was viewed.

**RESULTS**

**Case Report (Case 1)**

A 63-year-old man was referred to our department with a diagnosis of acute leukemia. On the day before admission, the patient saw his primary care physician due to general fatigue and exertional dyspnea, when a blood test showed leukocytosis and thrombocytopenia. On examination, there was no surface lymphadenopathy or splenomegaly. The hemoglobin level was 10.0 g/dL, white cell count was $16.78 \times 10^3/\mu$L containing 94.6% blasts, and platelet count was $91 \times 10^3/\mu$L. Fibrinogen was 358 mg/dL and D-dimer was 0.8 μg/mL. Blood chemistry values were unremarkable except for lactate dehydrogenase (LDH) of 340 IU/L. The mRNA level of the Wilms’ tumor-1 (WT-1) gene was $1.8 \times 10^5$ copies/μg RNA.

Leukemia blasts in PB were medium-sized and exhibited a high nuclear-cytoplasmic ratio with scanty basophilic cytoplasm. Cytoplasmic granules were not apparent under light microscopy. Approximately 20% of blasts demonstrated cup-like nuclear morphology (Figure 1) and more than 90% of blasts had spherical nuclear indentation containing organelles under examination by phase contrast microscopy (Figure 2). BM aspirates showed 20% cellularity with blasts comprising 87.0% of nucleated cells. Cup-like blasts in BM were not as prominent as those in PB. The blasts were negative for MPO, but expressed CD13 and CD33 by flow cytometry (Figure 3), classified into the FAB-M0 category. Other antigen expressions were: CD7^−, CD14^−, CD19^−, CD36^−, CD38^+, CD117^+, CD11b^dim, CD11c^dim, CD45RA^+, CD45RO^−, CD56^−, and CD66c^−. CD34 was positive and HLA-DR was weak (Figure 3). Cytogenetic study demonstrated a normal male karyotype. FLT3-ITD and NPM1 mutations were negative (Table 1).

Although the patient was refractory to the ‘7 + 3’ induction chemotherapy consisting of cytarabine and idarubicine, MEC (mitoxantrone, etoposide, and cytarabine) salvage regimen led to complete remission (CR) of leukemia. However, he developed severe pneumonia requiring mechanical ventilation during the second cycle of MEC and declined further treatments. On the 223rd day after first presentation, blasts appeared in PB. Although PB blasts promptly disappeared in response to additional MEC therapy, he developed pneumonia, and died of hypoxia and septic shock 9 months after first presentation.

**Electron Microscopic Examination (Case 1)**

Leukemia blasts in PB were 5 to 10 μm in diameter and the nuclear-cytoplasmic ratio ranged from 0.5 to 0.9. Nuclei were round or indented, and had dispersed chromatin and small to medium-sized nucleoli. Twenty-one percent of the blasts per section had a cup-like nuclear indentation spanning >25% of the nuclear diameter and many mitochondria were concentrated within the nuclear pockets (Figure 4A and B). Rough endoplasmic reticulum (ER) and Golgi apparatus were inconspicuous.
Figure 1. Wright-stained smears of PB (cases 1 and 5) and BM (cases 2, 3, 4, and 6) demonstrating the appearance of leukemia blasts. Myeloperoxidase (MPO)-stained pictures are aligned at bottom right in each panel. Nuclear indentations are indicated by arrows. An Auer rod is shown in case 2 (top, left). Original magnification, ×100 objective lens.

Figure 2. Phase-contrast microscope images of PB of cases 1, 2, 3, and 5, and BM of case 6. Arrows indicate spherical nuclear indentation containing organelles.
Figure 3. Single-color flow cytometry of leukemia blasts (case 1). Positive cell populations for each antigen are indicated by horizontal bars, and their percentages are shown. Leukemia cells appear to be composed of two populations with regard to the intensity of CD33, CD38, and CD117 expression.

| Case 1  | Case 2  | Case 3  | Case 4  | Case 5  | Case 6  |
|---------|---------|---------|---------|---------|---------|
| **Age/sex** | 63/male | 82/male | 80/female | 62/male | 77/female | 70/female |
| **Classification** | FAB-M0 | Hypoplastic leukemia | FAB-M5b | FAB-M4/AML-MRC | FAB-M1 | FAB-M0 |
| **Hemoglobin (g/dL)** | 10.0 | 11.0 | 9.6 | 7.1 | 11.0 | 11.3 |
| **Platelets (×10^3/µL)** | 91 | 120 | 125 | 49 | 60 | 55 |
| **White cells (×10^9/µL)** | 16.78 | 0.9 | 338.94 | 1.68 | 16.20 | 60.04 |
| **PB blasts (%)** | 94.6 | 2.0 | 93.0 | 12.0 | 61.0 | 98.8 |
| **BM blasts (%)** | 87.0 | 28.1 | 69.1 | 69.1 | 92.5 | 93.8 |
| **MPO (%)** | − | + (95.0) | + (16.5) | + (12.5) | + (82.5) | − |
| **α-NBE** | − | − | + | + | − | − |
| **D-dimer (µg/mL)** | 0.8 | 0.8 | 15.2 | 0.8 | 88.6 | 1.5 |
| **LDH (IU/L)** | 340 | 226 | 1,764 | 225 | 503 | 1,152 |
| **CD13*** | + | + | + | −/+ | + | + |
| **CD33*** | + | + | + | + | + | − |
| **CD34*** | + | −/+ | − | dim | dim | + |
| **HLA-DR** | dim | + | −/+ | + | − | dim |
| **Karyotype** | 46,XY[10] | 46,XY[10] | 46,XX[10] | 46,XY[10] | 46,XX[10] | 46,XX[10] |
| **FLT3-ITD** | − | − | + | − | + | + |
| **NPM1 mutation** | − | − | − | + | − | − |
| **WT-1 (copies/µg RNA)** | 1.8 × 10^3 | 5.6 × 10^3 | 1.5 × 10^4 | 2.1 × 10^4 | Not tested | Not tested |
| **Initial treatment** | IDR + AraC, MEC | BSC | BSC | DNR + AraC | DNR + AraC | DNR + AraC |
| **Survival (months)** | 9 | 16 | 0 | 5 | 13 | 2+ |

*Flow cytometry: The antigen expression was scored as − (expressed in <20% of the cells analyzed relative to control staining), dim, or +; the presence of two symbols separated by a slash indicates that the cells of interest were composed of two populations.

**The limit of detection was 5 × 10 copies/µg RNA (WT1 mRNA assay kit; Otsuka Pharmaceutical Co. Ltd., Tokyo).

Abbreviations: AML-MRC, acute myeloid leukemia with myelodysplasia-related changes; MPO, myeloperoxidase; α-NBE, α-naphthyl butyrate esterase; BSC, best supportive care; IDR, idarubicin; DNR, daunorubicin; AraC, cytarabine.
Occasional cells had primary granules of <250 nm in diameter, exhibiting MPO activity (Figure 4A, right).

**Characteristics of Cup-like AML Cases Treated at Our Institution (Cases 1 to 6)**

To date, we have encountered a total of 6 cases of cup-like AML at our institution (cases 1 to 6), and their characteristics are summarized in Table 1. Figure 1 shows Wright- and MPO-stained PB or BM blasts, and their phase contrast pictures, except for case 4, are shown in Figure 2, featuring the nuclear indentation with cytoplasmic organelles.

![Figure 4. Electron microscopic appearance of leukemia blasts (case 1). (A) Lower magnification pictures, showing three blasts with variable degree of nuclear indentation (left), and focusing upon a blast with prominent nuclear indentation containing many mitochondria (middle). MPO-positive primary granules are shown on the right. (B) Higher magnification pictures, showing the longitudinal (top) and transverse section (bottom) of the nuclear indentation. Mitochondria concentrated in the indentation are highlighted on the right.](image-url)
The ages ranged from 62 to 80 and the male to female ratio was 3 to 3. Classification of AML included FAB-M0 in 2, M1 in 1, myelomonocytic/monocytic leukemia in 2, and the remaining one had hypoplastic leukemia. Two patients presented with marked leukocytosis and high blast counts associated with high LDH values, while two had leukopenia. Cases 2, 3, 4, and 5 were MPO-positive, and cases 3 and 4 exhibited sodium fluoride-sensitive α-naphthyl butyrate esterase positivity, confirming monocytic characteristics. D-dimer was elevated in 2. Leukemia cells in all cases expressed myeloid-associated antigens and lacked T and B lineage markers. Four showed weak or negative expression of CD34, and expression of HLA-DR was weak or negative in 4 (Figure 5, Table 1). All cases carried a normal karyotype. Four patients achieved CR after intensive chemotherapy and two patients declined chemotherapy.

FLT3-ITD was detected in 3 cases by the presence of aberrant-size PCR products on agarose gel electrophoresis. Cloning and sequencing of the PCR products in cases 3 and 5 revealed duplication of 24 nucleotides with insertion of 3 nucleotides and duplication of 27 nucleotides, respectively, within the FLT3 exon 14. Cases 4 and 5 demonstrated the type A NPM1 mutation, i.e. duplication of TCTG tetranucleotide at positions 956 through 959 (c.956_959dupTCTG) within the NPM1 exon 12, resulting in a frameshift altering the C-terminal portion of the NPM1 protein.13

![Figure 5](image-url)

**Figure 5.** Single-color flow cytometry of expression of CD13, CD33, CD34, and HLA-DR in cases 2, 3, 4, 5, and 6. Positive cell populations for each antigen are indicated by horizontal bars, and their percentages are shown.
Figure 6 shows electron microscopic images of leukemia blasts in PB of cases 2 through 6, and the features of all 6 cases are summarized in Table 2. The cells exhibited immature morphology with high nuclear-cytoplasmic ratio, dispersed or mildly condensed nuclear chromatin, and small to medium-sized nucleoli. MPO-positive granules were present in the cytoplasm in 1 to 88% of the blasts. Cup-like cells spanning ≥25% of the nuclear diameter comprised 4 to 25% of the blasts per section. In the nuclear indentation, condensed collection of mitochondria and fibrillary formation were observed in all cases, and MPO-positive primary granules and rough

![Image](image_url)

**Figure 6.** Electron microscopic images showing the cup-like nuclear indentation of leukemia cells in cases 2 through 6. Higher magnification pictures showing the components of the nuclear invagination are aligned at the bottom. An Auer rod in case 2 is indicated by an open arrow.
ER were concentrated in 3 and 2 cases, respectively. Occasional cells in case 2 had Auer rods in the pocket (Figure 6).

**DISCUSSION**

We described here a representative case of cup-like AML and summarized a total of 6 AML cases with cup-like nuclear morphology treated at our institution. Although the classification of these leukemia types based on the standard criteria and laboratory features at presentation were variable, all cases were characterized by weak or negative expression of CD34 and/or HLA-DR, normal karyotype, either NPM1 mutation or FLT3-ITD, or both, and most recently, IDH1 (isocitrate dehydrogenase [NADP(+)]) gene mutation. However, these correlations were not always significant, and not all features were verified in our 6 cases. These controversial results in part may be attributable to the reliability and reproducibility of the criterion of cup-like morphology under light microscopy. A literature review found a total of 9 large series of cup-like AML; 3-7,9-12 some series included all FAB categories of AML, except for M3, 9,11 whereas other series excluded M4 and M5, 3,6 as myelomonocytic/ monocytic leukemia cells usually exhibit nuclear indentation. By comparison with the control group of AML that lacked this characteristic nuclear morphology, cup-like AML has been shown to be correlated with female predominance, 4,6 high PB and BM blast%, 7,11 high MPO positivity, 7 FAB-M1 subtype, high D-dimer levels, 7 low-level or lack of expression of CD34 and HLA-DR, normal or abnormal chromosome karyotype, either NPM1 mutation or FLT3-ITD, or both. Accumulation of MPO-positive primary granules within the nuclear indentation, providing the ultrastructural basis for the cup-like morphology, 13,16 and normal G-banding cytogenetics. Three had FLT3-ITD or NPM1 mutations and one was double-positive (IDH1 (isocitrate dehydrogenase [NADP(+)]) gene mutation. 12 However, these correlations were not always significant, and not all features were verified in our 6 cases. These controversial results may be attributable, in part, to the reproducibility of the criterion of cup-like morphology under light microscopy.

**Table 2. Electron microscopic appearance of leukemia blasts in PB**

| Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 |
|--------|--------|--------|--------|--------|--------|
| Cell size | 5-10 µm | 5-10 µm | 5-9 µm | 6-9 µm | 5-9 µm | 6-10 µm |
| Nuclear/cytoplasmic ratio | 0.5-0.9 | 0.5-0.9 | 0.5-0.9 | 0.6-0.9 | 0.6-0.8 | 0.6-0.9 |
| Nucleus | Round/indented | Round/indented | Round/indented | Round/indented | Round/indented | Round/indented |
| Chromatin | Dispersed | Dispersed | Dispersed | Mildly condensed | Mildly condensed | Mildly condensed |
| Nucleolus | Small to medium | Small to medium | Small to medium | Small to medium | Small to medium | Small to medium |
| Cytoplasm | Rough ER and Golgi apparatus | Scarce | Scarce | Scarce to abundant | Scarce to abundant | Scarce |
| MPO- primary granules | 1% | 87% | 76% | 48% | 88% | 3% |
| Cup-like nucleome | 21% | 9% | 9% | 4% | 25% | 5% |
| Contents of the pocket | Mitochondria, FF | Mitochondria, FF, Auer rods, primary granules | Mitochondria, FF, primary granules | Mitochondria, FF, primary granules, rough ER | Mitochondria, FF, primary granules, rough ER | Mitochondria, FF |
| Other organelles | MB, MF, FF, lipid droplet | MB, MF, FF, lipid droplet | MB, MF, FF, lipid droplet | MB, MF, FF | MB, MF, FF | MB, MF, FF |

Abbreviations: ER, endoplasmic reticulum; FF, fibrillary formation; MB, multivesicular body; MF, myelin figure.
AML, i.e. the presence of prominent cup-like nuclear invagination spanning at least 25% of the nuclear diameter in ≥10% of the blast population in Wright-Giemsa–prepared smears, as this criterion is artificial and inter/intra investigator discordance in the diagnosis is inevitable. We suggest in this study that phase contrast microscopy examination may provide information indicative of aggregation of organelles within the invagination, and that cup-like AML may be applicable to equivocal cases when cytoplasmic organelles are found to accumulate within the nuclear pocket under electron microscopy, despite their percentage below 10% (Table 2; cases 4 and 6). At present, it remains to be determined whether cup-like nuclear morphology represents a distinctive AML subtype associated with particular clinical features and genetic abnormality.

The mechanism for the formation of cup-like nuclear indentation is speculative. One possibility is that the nuclear invagination simply represents a property of myeloid cells/granulocytes that have lobulated and deformable nuclei to enhance migration through tight tissue spaces to the site of bacterial or fungal infection. Another possibility is that cytoplasmic organelles are condensed within the indentation, the indentation is not a primary nuclear deformity, but rather compression of the nuclear contour by the aggregate of organelles such that the mechanism for maintaining the distribution of cytoplasmic organelles may be disrupted. Kroschinsky et al. suggested that localization of mutant NPM1 protein in the cytoplasm instead of the nucleolar localization of wild type NPM1 is a cause of nuclear deformity.

Although the initial series of cup-like AML indicated close association with FLT3-ITD, the authors did not exclude the possibility that cup-like AML may have a better prognosis than other FLT3-ITD-positive AMLs. In the series of Chen et al., in which 64% and 88% cases carried NPM1 mutation and FLT3-ITD, respectively, the CR rate was 71% and the rate was significantly higher than that of the control group. However, in another study from the same group, in which patients were treated with cytarabine-based chemotherapy, the authors did not find any difference in the CR rate or overall and/or progression-free survivals between the cup-like and control groups. Two other studies failed to find an impact of cup-like morphology on the response rate or survival parameters. Thus, to determine whether cup-like AML is associated with treatment outcome and survival, prospective studies of uniformly-treated AML focusing upon the nuclear morphology, preferably with the aid of electron microscopy examination, are required.

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我々は、まず、cup-like 核形態を認めた急性骨髄性白血病 (AML) の 1 例を提示する。症例は 63 歳男性。ヘモグロビン 10.0 g/dL、白血球数 16.78 × 10^3/μL (芽球 94.6%)、血小板数 91 × 10^3/μL。骨髄は細胞密度 20%、芽球 87%。末梢血中の芽球の 20%が cup-like 核形態を示し、電子顕微鏡下で核嵌入部にミトコンドリアが集簇していた。芽球はペルオキシダーゼ陰性であったが、CD13、CD33 陽性で、CD34 陽性、HLA-DR の発現は減弱していた。染色体は正常核型。FMS-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD) と nucleophosmin 1 (NPM1) 遺伝子変異は認めなかった。化学療法に反応したが短期間で再発し、初診から 9 か月で死亡した。

次に、当院では、これまでに合計 6 症例の cup-like AML を診療した。年齢は 62 から 80 歳、男女比は 3:3。2 例は French-American-British 分類 M0、1 例は M1、2 例は骨髄単球性/単球性白血病、1 例は低形成白血病であった。2 例は高度の白血球増多で、2 例は白血球減少で発症した。全例で骨髄球系の抗原を発現していた。全例で正常核型。4 例で FLT3-ITD と NPM1 遺伝子変異のいずれかまたは両者の発現が減弱していた。全例で正常核型、4 例で FLT3-ITD と NPM1 遺伝子変異のいずれかまたは両者が認められた。全例で、核嵌入部のミトコンドリアの集簇を認めた。現時点では、cup-like AML が特定の臨床病態、遺伝子変異、治療アウトカムと関連するかどうかは不明である。

キーワード：cup-like 急性骨髄性白血病、CD34、HLA-DR、電子顕微鏡、FLT3-ITD、NPM1 遺伝子変異