MLL-SEPT6 Positive Acute Myeloid Leukemia Patients Often Co-occur With NRAS Mutations?

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Research

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Abstract

Background
The MLL-SEPT6 fusion gene is a relatively rare genetic event in leukemia. Its clinical characteristics, prognosis, especially the profile of co-occurring gene mutations remain unclear.

Methods
We retrospectively analyzed four rare leukemia cases carrying MLL-SEPT6 in our hospital from laboratory examination, diagnosis, treatment and prognosis, and provided a comprehensive and detailed description on clinical profile of MLL-SEPT6-positive AML patients in the literature.

Results
All the four patients were diagnosed with acute myeloid leukemia (AML) and harbored X chromosome and 11 chromosome rearrangements. Three of four cases occurred NRAS mutation while the rest one with congenital AML did not. Of the four cases, one developed drug-resistant, one suffered relapse after bone marrow transplantation (BMT) and one died. Combined with other cases reported in literatures, we found that of all patients diagnosed with AML, 90.9% were children (≤ 9 years old) and 54.5% were infants (≤ 1 year old). The survival time between infant group (≤ 1 year old) and pediatric group (>1 and <18 years old), patients that received BMT and that received chemotherapy alone did not show significant differences (P > 0.05).

Conclusions
MLL-SEPT6 was more commonly observed in pediatric AML patients, some of which may co-occur with NRAS mutations. The prognosis was inconclusive and may not be related to age or BMT. More information needs to be accumulated and summarized from additional cases to confirm the underlying connection between NRAS mutations and MLL-SEPT6 in order to better understand the profile in MLL-SEPT6-positive AML.

Background
Acute myeloid leukemia (AML) is a malignant tumor that originates from the myeloid blood cells, characterized by abnormally increased leukemic cells in bone marrow or peripheral blood [1]. Cytogenetic and molecular abnormalities occur commonly in AML patients. Based on genetic mutations and specific chromosomal rearrangements, the World Health Organization (WHO) divides AML with recurrent genetic abnormalities into 11 subgroups [2]. The mixed-lineage leukemia (MLL) gene rearrangements is one of the most common chromosomal abnormalities in AML [3, 4].

The MLL gene at 11q23 has many partner genes, of which over 80 have been identified [5]. Among them, the SEPTIN6 (SEPT6) gene located at Xq24 involving in the formation of MLL arrangement t(X;11)(q22-24;q23) is extremely rare in AML [6], a limited number of cases have been documented in literatures. To our knowledge, most cases are children while only one adult case has been reported [6–16]. The exact role of MLL-SEPT6 in hematopoietic cells and its effect on leukemogenesis are still unknown. There is little information on clinical features, treatment strategies and prognosis of such patients, accompanied gene mutations carried by such patients have not been described.

We retrospectively analyzed data on four acute leukemia patients carrying the MLL-SEPT6 fusion gene that treated in our hospital, especially of the gene mutations information. Additionally, we reviewed cases in literatures together with our cases to provide evidence for potential therapeutic strategies.

Materials And Methods

Case selection
We collected four patients harboring MLL-SEPT6 gene from a pool of 1656 leukemia patients within our hematological diseases database in the past four years, which were referred as case 1-4. The diagnostic criteria were according to WHO classification of tumors of haematopoietic and lymphoid tissues [17]. We conducted a retrospective analysis and systematic summary with information of morphology, flow cytometric analysis, cytogenetics, molecular biology and other related laboratory tests results.

Literature review
We conducted literature search on PubMed with the keywords “MLL-SEPT6”, “MLL-SEPTIN6” or “t(X;11)” to gather related case reports.

Statistical Analysis
Kaplan–Meier method was used for survival evaluation. Log-rank test was used to assess the difference between groups. P < 0.05 was considered statistically significant. All data were analyzed with SPSS Statistics, version 21 (StatSoft).

Results
Clinical presentation
All MLL-SEPT6 positive cases (case 1-4) were male with ages ranging from 0 to 57 years and a median of 5.2 years, their detailed information was summarized in Table 1. All cases had manifestations of fever and pale complexion. The three pediatric cases (cases 2, 3, and 4) were accompanied by hepatosplenomegaly, and two cases (cases 3 and 4) had scattered petechiae and ecchymoses. The case 1 is an elderly male patient with perianal abscess and diabetes in addition to the above symptoms. The case 2 was accompanied by pain in both lower limbs, tenderness of the sternum and hypertrophy of tonsils. In the case 3, multiple lymph nodes were palpable on bilateral neck, he was positive of sternal tenderness, he also had hyperuricemia and acute bronchial pneumonia. The case 4 is a newborn delivered by cesarean section due to “decreased fetal heart rate”. The birth weight was 3100g. The infant had no spontaneous breath at birth and was generally cyanotic, he restored spontaneous respiration under assisted ventilation, he developed persistent pulmonary arterial hypertension and neonatal pneumonia. His parents were healthy and with no history of genetic disease.

| Items                      | Case 1                      | Case 2                      | Case 3                      | Cs  |
|---------------------------|-----------------------------|-----------------------------|-----------------------------|-----|
| Sex                       | Male                        | Male                        | Male                        | Mt  |
| Age                       | 57 years                    | 9 years                     | 16 months                   | 0 r |
| Physical examination      | Perianal abscess            | Lower limbs pain, sternal tenderness and tonsil hypertrophy | Scattered petechiae on the neck, cervical lymphadenopathy and sternal tenderness (+) |     |
| Hepatomegaly/Splenomegaly | No/No                       | Yes/Yes                     | Yes/Yes                     | Ye  |
| CNS involvement           | No                          | Yes                         | Yes                         | Ye  |
| WBC/Hb/PLT (×10⁹/L/g/L/×10⁹/L) | 12.3/79.0/105.0             | 3.0/93.0/245.0              | 123.8/41.0/39.0             | 11  |
| Serum LDH (U/L)           | NA                          | 966                         | 2080                        | N/A |
| D-Dimer (ug/L/DDU)        | 13117.0                     | 3149.0                      | 2469.0                      | 62  |
| Blood blasts (%)          | 69.0                        | 2.0                         | 52.0                        | 17  |
| Bone marrow blasts (%)    | 92.0                        | 27.5                        | 56.0                        | 20  |
| Morphological diagnosis   | AML-M5                      | AML-M2                      | AML-M4                      | AA  |
| Immunophenotype           | The leukemic cells expressed HLA-DR, CD117, CD33, CD13, CD38, CD15, CD64 and CD4 | The leukemic cells expressed HLA-DR, CD33, CD38, CD15, CD64 | The leukemic cells expressed HLA-DR, CD33, CD13, CD38, CD15 and CD64 | Th |}

Laboratory results showed that three cases (cases 1, 3, and 4) had high white blood cells (WBC) count, anemia, and low platelet count. One case (case 2) had low level of hemoglobin and WBC count but normal platelet count, and two cases (case 2 and 3) had increased level of serum lactate dehydrogenase. The D-dimer level of all four patients were increased.

Morphological evaluation
All cases exhibited morphological characteristics of AML (Figure 1). The French American British (FAB) morphological classification of each case was M5 (case 1 and 4), M2 (case 2) and M4 (case 3). Three cases (cases 1, 3, and 4) showed hypercellular bone marrow and the other case (case 2) had severe hypocellular bone marrow. The case 1 and case 4 were evaluated as M5 and revealed of 92.0% and 20.4% blasts in marrow aspirate, 69.0% and 17.0% in peripheral blood respectively (Table 1, Figure 1A and 1D). The case 2 was evaluated as M2, the marrow aspirate revealed 27.5% myeloblasts and the peripheral blood exhibited 2.0% blasts (Table 1, Figure 1B). The case 3 was evaluated as M4, the marrow aspirate showed 56.0% blasts and the peripheral blood exhibited 52.0% blasts (Table 1, Figure 1C).

Flow cytometric analysis

The flow cytometric analysis revealed the presence of myeloid blasts in bone marrow samples from all four patients (Table 1, Figure 2). The percentage of blasts was highest of 92% in the case 1 and lowest of 4.2% in the case 3. All cases were positive for CD33, CD15, CD64 indicating myeloid lineage, CD13 was positive only in the case 1 and case 2. All the cases were positive for CD38 and HLA-DR. CD117 was positive only in the case 3, CD34 was negative in all the four cases.

Cytogenetic analysis

The results showed that all four cases had clonal abnormalities of X chromosome and chromosome 11 or complex karyotype abnormalities (Table 1, Figure 3). In case 1, the metaphase cells exhibited abnormalities of t(X;11)(q24;q23) (Figure 3A). The metaphase cells collected in the case 2 showed 45, Y, del(X)(q21), der(11)t(X;11)(q24;q23), -20, add(22)(q13) (Figure 3B). In the case 3, in addition to the abnormal karyotype of t(X;11)(q24;q23), thirteen metaphase cells had del(7)(q21q31) (Figure 3C). The metaphase cells in case 4 showed abnormal karyotype of 46, Y, ins(X;11)(q23;q24q12) (Figure 3D).

Molecular analysis

We performed molecular biology tests including screening of fusion genes and next-generation sequencing (NGS) analysis on all cases (Table 1). The MLL-SEPT6 fusion gene was detected in all four cases. The NGS panel included a total of 20 frequently mutated genes in AML which were ASXL1, CEBPA, DNMT3A, EZH2, FLT3, IDH1, IDH2, KIT, NPM1, PHF6, RUNX1, TET2, TP53, BCR, MLL, KRAS, NRAS, PDGFRα and WT1, the sequencing depth was 2000x. The results showed that three cases (cases 1-3) harbored NRAS(NM_002524) mutations, the mutation sites of the case 1 and 2 were both NRASG12V, with variant allele frequencies (VAF) of 0.3585 and 0.0300 respectively. In addition, the case 1 was also accompanied by an insertion mutation of ASXL1 D821 with a VAF of 0.3585. The reexamination results on the third month showed that the MLL-SEPT6 gene remained positive but its expression level dropped from 100–24.4%. The ASXL1 D821 VAF dropped to 0.0068 and NRASG12V gene mutation was not detected. Four month later, the patient achieved complete remission (CR), the ASXL1 D821 VAF dropped to 0.0032 and this time both the NRAS mutation and MLL-SEPT6 fusion gene were negative. The NRASA146T mutation in the case 3 occurred in exon 4 with a VAF of 0.4411. MLL-SEPT6 gene and NRAS gene mutations both turned negative on the ninth month after diagnosis. The patient relapsed on the twenty-seventh month with positive MLL-SEPT6 and NRAS A146T mutation (VAF = 0.2662). In the case 4, no pathogenic gene mutations were detected.

Clinical Course

The treatment and follow-up information of all cases (case 1-4) was shown in Table 1. Three cases (case 1-3) received chemotherapy, the case 2 subsequently received a bone marrow transplantation (BMT), the case 4 was a newborn and did not receive any chemotherapy. The clinical follow-up period ranges from 0.5 to 38 months with a median of 21.5 months. The case 1 initially received IA (idarubicin + cytosine arabinoside) regimens, which didn't make him reach CR. Then he began a new chemotherapy regime with decitabine combined with half dose CAG (cytarabine + aclacinomycin + granulocyte colony stimulating factor) and achieved CR. He also received an intrathecal injection (cytarabine + dexamethasone + methotrexate) for central nervous system infiltration prevention. After the chemotherapy, the bone marrow aspiration of the patient revealed a normal karyotype and negative molecular results of MLL-SEPT6 rearrangement and NRAS mutation. The case 2 received MAE (mitoxantrone + cytarabine + etoposide) regimens and reached CR one month later. In the following six months, the patient received BMT. Nineteen months after the transplantation he was re-admitted for headache. A flow cytometry analysis of the cerebrospinal fluid revealed 81.9% leukemia cells which indicated central nervous system leukemia, following five courses of intrathecal injection, the child's headache relieved and no leukemia cells were detected in his cerebrospinal fluid. The case 3 received HA (homoharringtonine + cytarabine) regimens and intrathecal injection (cytarabine + dexamethasone + methotrexate), he reached CR four weeks after the diagnosis. This patient relapsed 27 months later, after receiving HAI (homoharringtonine + cytarabine + idarubicin) regimen he reached and remained CR status. The case 4 had dyspnea at birth, he was on assisted ventilation and given blood infusion to improve anemia and thrombocytopenia. The newborn's condition did not improve during the treatment. His parents refused the follow-up treatment, and the child died a week later.

Literature review

A total of 22 MLL-SEPT6 positive cases were included in this literature review, including four cases in our report and eighteen cases from literatures [6–16]. Table 2 and Table 3 listed the detailed laboratory results and clinical information of all cases.
| Characteristic                        | N (%)   |
|--------------------------------------|---------|
| Gender                               | N=22    |
| Male                                 | 16 (72.7%) |
| Female                               | 6 (27.3%)  |
| Age (years)                          | N=22    |
| ≤1                                   | 12 (54.5%) |
| >1 and <18                           | 8 (36.4%) |
| ≥18                                  | 2 (9.1%)  |
| WBC count (×10⁹/L)                   | N=15    |
| ≥20.0                                | 9 (60.0%) |
| <20.0                                | 6 (40.0%) |
| Symptom at presentation              | N=10    |
| Hepatosplenomegaly                   | 5 (50.0%)|
| CNS involvement                      | 3 (30.0%)|
| Lymphadenopathy                      | 3 (30.0%)|
| Skin involvement                     | 2 (20.0%)|
| FAB classification                   | N=22    |
| M1                                   | 1 (4.5%)  |
| M2                                   | 8 (36.4%) |
| M4                                   | 5 (22.7%) |
| M5                                   | 5 (22.7%) |
| Unknown                              | 3 (13.6%)|
| Chromosomal abnormalities            | N=22    |
| Translocations                       | 11 (50.0%)|
| Insertions                           | 9 (40.9%) |
| Complex abnormalities                | 3 (13.6%)|
| Treatment protocol                   | N=19    |
| Chemotherapy alone                   | 8 (42.1%)|
| BMT                                  | 9 (47.4%) |
| No chemotherapy                      | 2 (10.5%)|
| Survival outcome                     | N=19    |
| Alive                                | 13 (68.4%)|
| Died                                 | 6 (31.6%) |

Footnotes: WBC, white blood cells; CNS, central nervous system; BMT, bone marrow transplantation.

The age of the patients ranged from 0 to 57 years (median = 1 year), with a male–female ratio of nearly 3:1 (16 males vs. 6 females). Twenty patients (90.9%) were children (≤ 9 years old), including twelve (54.5%) infants (≤ 1 year old). The majority of the patients manifested leukocytosis (range 1-608 × 10⁹/L), anemia (range 41-109 g/L) and low platelet counts (range 9-254 × 10⁹/L). According to the high WBC index [18], nine (60.0%) of the fifteen cases with WBC count information were defined as high WBC levels. Twelve cases were not provided with description of clinical features. Of the remaining ten cases, five children (50.0%) were observed of splenomegaly and hepatomegaly and three patients (30.0%) had lymphadenopathy. Central nervous system involvement was observed in three children (30.0%) and skin involvement was observed in two (20.0%).

All patients were diagnosed with AML (twenty children and two adults) according to the former FAB classification: five patients (three children and two adults, 22.7%) of M5, five children (22.7%) of M4, eight children (36.4%) of M2, one child (4.5%) of M1, and three children (13.6%) unknown.
All the cases had available cytogenetic information, chromosomal translocations (eleven cases) were the most common chromosomal rearrangements, followed by chromosomal insertions (nine cases). Among them, Xq24 (nine cases) and 11q23 (fourteen cases) were the most frequently involved chromosomal bands. Seven cases (31.8%) demonstrated complex abnormalities.

Of all 22 cases, 18 cases had clinical follow-up with median period of 27.7 months (0.5–101.5 months). Table 3 showed the clinicopathologic features of evaluable patients. Eight patients (42.1%) received chemotherapy alone. Nine patients (50%) received BMT. Six of eighteen patients died during the period of follow-up. Kaplan-Meier survival analysis was performed on eighteen cases with complete follow-up information (Figure 4A). As of the final follow-up, median survival time has not been reached. In order to understand the impact of age and BMT on survival time, the patients were divided into infant group (≤1 year old, n=10), pediatric group (>1 and <18 years old, n=7), and adult group (≥18 years old, n=1). At the time of the last observation, there was no statistically significant differences in survival time between infant group and pediatric group (hazard ratio for infant-pediatric = 0.26, 95% confidence interval = 0.07 to 1.67, P = 0.1822, Figure 4B). The adult group was not included in the statistical analysis because there was only one case with complete follow-up information in this group. Meanwhile, the patients were also divided into receiving chemotherapy alone (n=6, one without survival information was excluded) and receiving BMT (n=9) treatment groups according to the treatment protocol. Survival time of the patients received chemotherapy alone and BMT did not show significant differences neither (hazard ratio for chemotherapy alone-BMT = 1.04, 95% confidence interval = 0.18 to 6.19, P = 0.9647, Figure 4C).

### Discussion

The **MLL** gene is a frequent target of rearrangement in human leukemia, especially in infant and pediatric leukemia [9, 19, 20]. It is well established that the rearrangement heralds poor prognosis [5, 21]. These rearrangements include fusions with many partner genes, but rarely involve the X chromosome. **SEPT6** is a member of the septin family of GTPases. Members of this family are involved in cell polarity, cytokinesis and oncogenesis [22, 23]. The **MLL** gene and **SEPT6** gene are vulnerable to damage to form translocations associated with infant AML.

In this study, we described four cases of AML with **MLL-SEPT6** fusion gene. The FAB subtypes were mainly M2, M4 and M5, which was consistent with literatures. Most cases that have been reported were children. As far as we know, only two adult patients have been reported including one case in our series and 54.5% (12/22) were infant patients (≤1 year old). Among these cases, 60.0% were with high level of WBC, and 30% manifested central nervous system involvement, which were similar to the clinical features of **MLL**-rearranged AML patients. The findings of Balogobind BV et al. showed that **MLL**-rearranged AML patients usually exhibit high tumor burden, including organomegaly, high median WBC and central nervous system involvement [24]. The present study included the largest number of **MLL-SEPT6** cases to date. The patients' NGS test results were not provided except ours, and we also tracked the patients' molecular biological examination results. Three of four cases in our series occurred **NRAS** mutation while the rest one with congenital AML did not.

The **NRAS** mutations has very important roles in pathogenesis and progression of human leukemia, which have been frequently reported in AML patients [25, 26]. **NRAS** G12V is required in leukemia self-renewal process, independent of its effects on growth and survival [27]. Compared with other subtypes of leukemia, acute leukemia with **MLL**-translocations (such as **MLL-AF4** and **MLL-AF9**) harbored the fewest number of mutations, in which **NRAS** mutations commonly co-occur [27, 28]. In our series, we identified **NRAS** mutations in **MLL-SEPT6** positive AML patients for the first time, and most of the mutation sites appeared at codon 12 and 145. The former site is a hotspot mutant of **NRAS** and the latter site has also been reported [25, 29]. The VAF of **NRAS** mutation decreased as the patients condition improved. When the patients achieved CR, it also turned negative. The underlying connection between **NRAS**mutations and **MLL-SEPT6** and whether non-congenital **MLL-SEPT6**-positive AML patients all have **NRAS** mutations remain to be further studied in a larger cohort in the future.

**MLL** gene rearrangement in AML usually indicates poor prognosis. The prognostic significance of **NRAS** mutations in AML patients remains unclear [30–33]. Of the four cases in our series, one developed drug-resistant at first, one suffered relapse after **BMT** and one died, showing unsatisfactory therapeutic effect. However, whether the outcomes of patients with **MLL-SEPT6** were aggravated by the concurrence of **NRAS** mutations needs a follow-up study. Kaplan–Meier curve demonstrated that pediatric group (>1 and <18 years old) did not show better survival time compared with infant group (≤1 year old). Age may not be an independent prognostic factor for survival. Most of the patients received chemotherapy, nine of them received **BMT**, but three of them eventually died. The survival time of patients between the chemotherapy alone group and the **BMT** group did not show a significant difference, which suggested that **BMT** may not improve the survival time of patients between the chemotherapy alone group and the **BMT** group did not show a significant difference, which suggested that **BMT** may not improve the survival time of such patients. This was consistent with several studies and meta-analyses that suggesting BMT does not improve survival in patients receiving chemotherapy alone [22, 23, 24]. As of the final follow-up, median survival time has not been reached. In order to understand the impact of age and **BMT** on survival time, the patients were divided into infant group (≤1 year old), pediatric group (>1 and <18 years old) and adult group (≥18 years old). At the time of the last observation, there was no statistically significant differences neither (hazard ratio for infant-pediatric = 0.26, 95% confidence interval = 0.07 to 1.67, P = 0.1822, Figure 4B). The adult group was not included in the statistical analysis because there was only one case with complete follow-up information in this group. Meanwhile, the patients were also divided into receiving chemotherapy alone (n=6, one without survival information was excluded) and receiving **BMT** (n=9) treatment groups according to the treatment protocol. Survival time of the patients received chemotherapy alone and **BMT** did not show significant differences neither (hazard ratio for chemotherapy alone-BMT = 1.04, 95% confidence interval = 0.18 to 6.19, P = 0.9647, Figure 4C).

### Conclusions

In conclusion, the **MLL-SEPT6** fusion gene was more commonly observed in pediatric patients diagnosed with AML. **NRAS** mutations were observed in these patients, most frequently of the **NRAS** G12V hotspot mutation. Whether **NRAS** mutations are related to the occurrence of **MLL-SEPT6**-positive AML is currently unclear. The prognosis was inconclusive and may not be related to age. **BMT** may not improve survival in these patients. More cases should be accumulated and summarized to better understand the profile in **MLL-SEPT6**-positive AML. Our findings provide a basis for better understanding the mechanisms of leukemogenesis and the development of potential therapeutic targets for **MLL-SEPT6**-positive AML.
Abbreviations

AML: acute myeloid leukemia
BMT: bone marrow transplantation
WHO: World Health Organization
WBC: white blood cells
FAB: French American British
NGS: next-generation sequencing
VAF: variant allele frequency
CR: complete remission
IA: idarubicin + cytosine arabinoside
CAG: cytarabine + aclacinomycin + granulocyte colony stimulating factor
MAE: mitoxantrone + cytarabine + etoposide
HA: homoharringtonine + cytarabine
HAI: homoharringtonine + cytarabine + idarubicin
CNS: central nervous system
Hb: hemoglobin
PLT: platelets
LDH: lactate dehydrogenase
NA: not available

Declarations

Ethics approval and consent to participate
This study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Shengjing Hospital of China Medical University (No. 2021PS122K). All patients provided written informed consents.

Availability of data and materials
The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Consent for publication
Written informed consent for publication of their clinical details and clinical images was obtained from the patient/parent of the patient.

Competing interests
The authors declare that they have no competing interests.

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Author's contributions
Shuang Fu and Fang Chen performed study concept and design; Shuang Fu, Fang Chen and Ying Yang performed development of methodology and writing, review and revision of the paper; Fang Chen and Ying Yang provided acquisition, analysis and interpretation of data, and statistical analysis. All authors read and approved the final paper.

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References

1. Culp-Hill R, D'Alessandro A, Pietras EM. Extinguishing the Embers: Targeting AML Metabolism. Trends Mol Med. 2021;27(4):332-344. doi: 10.1016/j.trends.2020.08.011

2. De Kouchkovsky I, Abdul-Hay M. Acute myeloid leukemia: a comprehensive review and 2016 update. Blood Cancer J. 2016;6(7):e441. doi: 10.1038/bcj.2016.50

3. Matsu H, Yoshioka K, Fukumura K, Nakatani K, Noguchi Y, Takasaki S, et al. Recurrent CCND3 mutations in MLL-rearranged acute myeloid leukemia. Blood Adv. 2018;2(21):2879-2889. doi: 10.1182/bloodadvances.2018019398

4. Pandey R, Arentsen-Peters ST, Kim H, Kim S, Park Y, et al. MLL-SEPT6 fusions in childhood acute myeloid leukemia: a high-throughput genomic and transcriptomic study. Blood. 2021;140(2):132-134. doi: 10.1182/blood.2021005162

5. Meyer C, Burmeister T, Gröger D, Tsauro G, Fehina L, Renneville A, et al. The MLL recombinome of acute leukemias in 2017. Leukemia. 2018;32(2):273-284. doi: 10.1038/leu.2017.213

6. Ono R, Taki T, Taketani T, Kawaguchi H, Taniwaki M, Okamura T, et al. SEPT6N, a human homologue to mouse Sept6N, is fused in MLL to infant acute myeloid leukemia with complex chromosomal abnormalities involving 11q23 and Xq24. Cancer Res. 2002;62(2):333-337. PMID: 11865373

7. Borkhardt A, Teigler-Schlegel A, Fuchs U, Keller C, König M, Harbott J, et al. An ins(X;11)(q24;q23) fuses the MLL and the Septin 6/KAIA28 gene in an infant with AML-M2. Genes Chromosomes Cancer. 2001;32:82-88. doi: 10.1002/gcc.1169

8. Slater DJ, Hilgenfeld E, Rappaport EF, Shah N, Meek RG, Williams WR, et al. MLL-SEPT6N fusion occurs in rare translocation of chromosomes 3, X, and 11 in acute myelogenous leukaemia and in (t(X;11)(11q23) in acute myeloid leukemia, and MLL genomic breakpoint in complex MLL-SEPT6N rearrangement is a DNA topoisomerase II cleavage site. Oncogene. 2002;21(20):4706-4714. doi: 10.1038/sj.onc.1205572

9. Kim HJ, Ki CS, Park Q, Koo KH, Yoo KH, Kim EJ, et al. MLL/SEPT6N chimeric transcript from inv ins(X;11)(q24;q23)13 in acute monocytic leukemia: report of a case and review of the literature. Genes Chromosomes Cancer. 2002;38(1):8-12. doi: 10.1002/gcc.10235

10. Fu JF, Liang DC, Yang CP, Hsu JJ, Shih LY. Molecular analysis of t(X;11)(q24;q23) in an infant with AML-M4. Genes Chromosomes Cancer. 2003;38(9):253-259. doi: 10.1002/gcc.10272

11. Harrison C, J., Cuneo A., Clark R., Johansson B., Lafage-Pochitaloff M., Mugneret F., et al. Ten novel 11q23 chromosomal partner sites. European 11q23 Workshop participants. Leukemia (Baltimore). 1998;12(5):811-822. doi: 10.1038/sj.leu.2401017

12. Nakata Y, Mori T, Yamazaki T, Suzuki T, Okazaki T, Kurosawa Y, et al. Acute myeloid leukemia with hypergranular cytoplasm accompanied by t(X;11) (q24;q23) and rearrangement of the MLL gene. Leuk Res. 1999;23(1):85-88. doi: 10.1016/s0145-2126(98)00131-3

13. Cerveira N, Lisboa S, Correia C, Bizarro S, Santos J, Torres L, et al. Genetic and clinical characterization of 45 acute leukemia patients with MLL gene rearrangements from a single institution. Mol Oncol. 2012;6(5):553-564. doi: 10.1016/j.molonc.2012.06.004

14. De Braekeleer E, Meyer C, Douet-Guilbert N, Basinko A, Le Bris MM, Morel F, et al. Identification of MLL partner genes in 27 patients with acute leukemia rearrangements from a single cytogenetic laboratory. Mol Oncol. 2011;5(6):555-563. doi: 10.1016/j.molonc.2011.08.003

15. Cerveira N, Micci F, Santos J, Pinheiro M, Correia C, Lisboa S, et al. Molecular characterization of the MLL-SEPT6 fusion gene in acute myeloid leukemia: identification of novel fusion transcripts and cloning of genomic breakpoint junctions. Haematologica. 2008;93(7):1076-1080. doi: 10.3324/haematol.12594

16. Kadkol SS, Bruna A, Oh S, Schmidt ML, Lindgren V. MLL-SEPT6 fusion transcript with a novel sequence in an infant with acute myeloid leukemia. Cancer Genet Cytogenet. 2006;168(2):162-167. doi: 10.1016/j.cancergynto.2006.02.020

17. Arb D, Bruning RD, Le Beau MM, et al. Acute myeloid leukemia and related precursor neoplasms. In: Swerdlow SH, Campo E, Harris NL, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. Revised 4th edition. France: IARC Press; 2017. p. 130-171.

18. Nguyen S, Leblanc T, Fenaux P, Witz F, Blaise D, Pigneux A, et al. A white blood cell index as the main prognostic factor in t(8;21) acute myeloid leukemia. Cancer Res. 2002;62(2):333-337. doi: 10.1182/blood.v99.10.3517

19. Antunes ETB, Ottersbach K. The MLL/SET family and haematopoiesis. Biochim Biophys Acta Gene Regul Mech. 2020;1863(8):194579. doi: 10.1016/j.bbagrm.2020.10.001

20. Rice S, Roy A. MLL-rearranged infant leukaemia: A ‘thorn in the side’ of a remarkable success story. Biochim Biophys Acta Gene Regul Mech. 2020;1863(8):194579. doi: 10.1016/j.bbagrm.2020.10.001

21. Wong NM, So CWE. Novel therapeutic strategies for MLL-rearranged leukemias. Biochim Biophys Acta Gene Regul Mech. 2020;1863(9):194584. doi: 10.1016/j.bbagrm.2020.10.001

22. Ivanov AI, Le HT, Naydenov NV, Rieder F. Novel Functions of the Septin Cytoskeleton: Shaping Up Tissue Inflammation and Fibrosis. Am J Pathol. 2021;191(1):40-51. doi: 10.1016/j.ajpath.2020.09.007

23. Macara IG, Baldarelli R, Field CM, Glotzer M, Hayashi Y, Hosu SC, et al. Mammalian septins nomenclature. Mol Biol Cell. 2002;13(12):4111-4113. doi: 10.1091/mbc.02-07-0438

24. Balgobind BV, Zwaan CM, Pieters R, Van den Heuvel-Eibrink MM. The heterogeneity of pediatric MLL-rearranged acute myeloid leukemia. Leukemia. 2011;25(8):1239-1248. doi: 10.1038/leu.2011.90

25. Bacher U, Haferlach T, Schoch C, Kern W, Schnittger S. Implications of NRAS mutations in AML: a study of 2502 patients. Blood. 2006;107(10):3847-3853. doi: 10.1182/blood-2005-08-3522

26. Pomeroy EJ, Lee LA, Lee RDW, Schirm DK, Temiz NA, Ma J, et al. Ras oncogene-independent activation of RALB signaling is a targetable mechanism of escape from NRAS(V12) oncogene addiction in acute myeloid leukemia. Oncogene. 2017;36(23):3263-3273. doi: 10.1038/onc.2016.471
27. Sachs Z, LaRue RS, Nguyen HT, Sachs K, Noble KE, Mohd Hassan NA, et al. NRASG12V oncogene facilitates self-renewal in a murine model of acute myelogenous leukemia. Blood. 2014;124(22):3274-3283. doi: 10.1182/blood-2013-08-521708

28. Trentin L, Bresolin S, Giarin E, Bardini M, Serafin V, Accordi B, et al. Deciphering KRAS and NRAS mutated clone dynamics in MLL-AF4 paediatric leukaemia by ultra deep sequencing analysis. Sci Rep. 2016;6:34449. doi: 10.1038/srep34449

29. Wang S, Wu Z, Li T, Li Y, Wang W, Hao Q, et al. Mutational spectrum and prognosis in NRAS-mutated acute myeloid leukemia. Sci Rep. 2020;10(1):12152. doi: 10.1038/s41598-020-69194-6

30. Welch JS, Ley TJ, Link DC, Miller CA, Larson DE, Koboldt DC, et al. The origin and evolution of mutations in acute myeloid leukemia. Cell. 2012;150(2):264-278. doi: 10.1016/j.cell.2012.06.023

31. Liu X, Ye Q, Zhao XP, Zhang PB, Li S, Li RQ, et al. RAS mutations in acute myeloid leukaemia patients: A review and meta-analysis. Clin Chim Acta. 2019;489:254-260. doi: 10.1016/j.cca.2018.08.040

32. Damm F, Heuser M, Morgan M, Wagner K, Görlich K, Grosshennig A, et al. Integrative prognostic risk score in acute myeloid leukemia with normal karyotype. Blood. 2011;117(17):4561-4568. doi: 10.1182/blood-2010-08-303479

33. Sano H, Shimada A, Taki T, Murata C, Park MJ, Sotomatsu M, et al. RAS mutations are frequent in FAB type M4 and M5 of acute myeloid leukemia, and related to late relapse: a study of the Japanese Childhood AML Cooperative Study Group. Int J Hematol. 2012;95(5):509-515. doi: 10.1007/s12185-012-1033-x

34. Winters AC, Bernt KM. MLL-Rearranged Leukemias-An Update on Science and Clinical Approaches. Front Pediatr. 2017;5:4. doi: 10.3389/fped.2017.00004.

35. Balgobind BV, Raimondi SC, Harbott J, Zimmermann M, Alonzo TA, Auvrignon A, et al. Novel prognostic subgroups in childhood 11q23/MLL-rearranged acute myeloid leukemia: results of an international retrospective study. Blood. 2009;114(12):2489-2496. doi: 10.1182/blood-2009-04-215152

Table

Due to technical limitations, table 2 docx is only available as a download in the Supplemental Files section.

Figures
Figure 1

Morphologic evaluation of leukemic cells at diagnosis (Wright–Giemsa stain, ×1000). (A), (B), (C) and (D) represented the case 1, 2, 3 and 4, respectively. BM, bone marrow; PB, peripheral blood.

Figure 2

Flow cytometry results of bone marrow. (A), (B), (C) and (D) represented the case 1, 2, 3 and 4, respectively.
Figure 3

Karyotype analysis results of bone marrow. (A), (B), (C) and (D) represented the case 1, 2, 3 and 4, respectively.

Figure 4

Kaplan–Meier survival analysis of eighteen cases with complete follow-up information. These included fourteen reported cases with clinical follow-up, and four cases in our series. (A) Survival months of eighteen cases. (B) Infant group (≤ 1 year) vs. pediatric group (>1 and <18 years old). Hazard ratio for infant-pediatric = 0.26, 95% confidence interval = 0.07 to 1.67, P = 0.1822. (C) Chemotherapy alone vs. BMT. Hazard ratio for chemotherapy alone-BMT = 1.04, 95% confidence interval = 0.18 to 6.19, P = 0.9647.

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