Outcome and Prognostic Features in Pediatric Acute Megakaryoblastic Leukemia Without Down Syndrome

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Abstract

Background: Acute megakaryoblastic leukemia (AMKL) is a biologically heterogeneous subtype of acute myeloid leukemia (AML) that originates from megakaryocytes. Despite improvements in the accuracy of diagnosing AMKL as well as increased amount of data available on this rare subtype, clear prognostic factors and treatment recommendations remain undefined.

Methods: We performed a retrospective study on 40 patients (age ≤18 years) with non–Down syndrome AMKL and assessed the effect of different prognostic factors on the outcomes

Results: The complete remission (CR) rate of the patients was 57.9% and 81.1%, respectively, at the end of induction therapy and . The overall survival (OS) and event-free survival (EFS) rate at 2 years was 41±13% and 41±10%, respectively. An analysis of the cytogenetic features showed that patients with +21 or hyperdiploid (50 chromosomes) had significantly better OS than those in other cytogenetic subgroups (Plog-rank=0.048 and Plog-rank=0.040, respectively). Besides cytogenetics, an excellent early treatment response (CR and minimal residual disease1% after induction therapy) also provided a significant survival benefit in univariate analysis in our study. However, multivariate analysis indicated that allogeneic hematopoietic stem cell transplantation (allo-HSCT) was the only independent prognostic marker (RR=11.192; 95 % CI, 2.045-61.241; P=0.005 for OS and RR=5.400; 95 % CI, 1.635-17.832; P=0.006 for EFS, respectively).

Conclusion: AMKL in patients with non-Down syndrome has a poor outcome and allo-HSCT may be a better option for post-remission therapy than conventional chemotherapy especially for those having a poor response to induction therapy.

Background

Acute megakaryoblastic leukemia (AMKL) is a biologically and clinically heterogeneous subtype of acute myeloid leukemia (AML), accounting for approximately 5-15% of newly diagnosed childhood AML cases [1-8]. It occurs mainly in children with Down syndrome (DS) who have an excellent prognosis even with lower intensity therapy[9,10]. Moreover, patients with DS-AMKL often have a GATA1 gene mutation, which is preceded by a transient myeloproliferative disease (TMD) [11-14]. In contrast, non-DS-AMKL represents a biologically heterogeneous disease with a poorer prognosis[3,7,15]. Recent advances in enhanced diagnostics, intensified treatment protocols, and supportive care have improved outcomes for patients with non-DS-AMKL. However, clear prognostic indicators and treatment recommendations for this subgroup remain controversial.

While some groups treat non-DS-AMKL as high-risk subtype, recommending hematopoietic stem cell transplantation (HSCT) with first complete remission (CR), others treat as standard risk in the absence of unfavorable cytogenetics and/or a poor response to induction therapy and obtain superior survival rates with intensive chemotherapy alone[2,3,16-18].

Hence, a detailed understanding of non-DS-AMKL biology, individual patient genetics, and phenotypic complexity is necessary to improve the disease outcomes[19]. In this study, we reported the clinical, cytogenetic, and therapeutic data in a relatively homogeneous group of pediatric patients with non-DS-AMKL. Next, we assessed the effects of different prognostic factors and explored the role of HSCT in the treatment of this AML subtype.

Methods

Study population

We collected data on the clinical characteristics and outcomes of pediatric patients (those aged between 1 and 18 years) who were newly diagnosed with non-DS-AMKL.

Ascertainment of non-DS AMKL Cases

The diagnosis of AMKL was established based on the FAB criteria by studies of cell morphology and cytochemistry[20]and required confirmation by immunologic methods. Immunophenotyping is negativity of lymphoid antigens together with either the positivity of 2 megakaryoblastic markers (CD41, CD42, or CD61) or the expression of one megakaryoblastic marker associated with CD36 positivity[21]. Cytogenetic analysis was performed as previously described[22]. MLL-r, WT1, and EVI1 were detected by reverse transcriptase PCR (RT-PCR), and ABL1 was evaluated as a control gene. The high expression of EVI1 and WT1 was determined based on whether the EVI1/ABL and WT1/ABL ratios of each patient were higher than 8% and 0.6%, respectively. The sequencing of GATA1 was also prospectively performed from 2018[23]. Chromosome which were performed on bone marrow blasts with the G-banding technique were described according to the International System of Human Cytogenetic Nomenclature[24]. Exclusion criteria were AMKL as secondary malignancy and DS-AMKL. This study was approved by the ethics committee of Peking University People's Hospital after written informed consent was obtained from their parents or guardians.

Treatment plan

Patients received chemotherapy with induction therapy (induction) of cytarabine (Ara-C), idarubicin, and etoposide(AIE). After that, a second induction therapy (induction)) of AIE or HA (cytarabine and homoharringtonine), followed by consolidation and intensification therapy (i.e., HDAra-C [high-dose cytarabine and idarubicin], AIE or HA, which were given sequentially). Patients who achieved CR after induction received second induction therapy with AIE, whereas others received HA protocol (Fig. 1 and Table 1). The entire course of treatment without HSCT was 12 to 18 months. Prophylaxis for central nervous system leukemia (CNSL), intrathecal chemotherapy with methotrexate, cytarabine, and dexamethasone were administered 6 to 8 times to the patients in the nontransplant cohort and at least 4 times in the transplant cohort. After at least 2 rounds of induction therapy, allogeneic HSCT in CR1 was recommended for all patients with an HLA compatible sibling donor such as a matched sibling donor (MSD) or a haplo family donor or a matched unrelated donor (MUD)according to their guardians' wishes

in our institution. Unrelated cord blood (UCB) unit with a minimum of 4/6 HLA loci match transplantation was also considered as an alternative. Busulfan and cyclophosphamide were used as the conditioning regimen.

Minimal residual disease assessment and definitions

MRD was assessed using multicolor flow cytometry (MFC) according to the leukemia-associated immunophenotyping (LAIP). CR was defined as bone marrow blasts of < 5% and no localized leukemic infiltrates. Early treatment response was evaluated according to the CR rate and level of MRD after induction therapy. Relapse was defined as the recurrence of ≥ 5% blasts in bone marrow aspirates or extramedullary leukemia at any site.

Statistical analysis

Overall survival probability (OS) was defined as the time from diagnosis to death by any cause, and event-free survival probability (EFS) was defined as the time from diagnosis to relapse, death by any cause, or induction failure. Survival analysis was performed using the Kaplan-Meier method with differences compared *via* the log-rank test. Multiple regression analysis for EFS and OS was conducted using a multiple Cox regression model. The chi-square test or Fisher exact test was used to analyze the differences in the distribution of individual parameters among patient subgroups. All statistical analyses were performed using SPSS software, version 23.0 (SPSS Inc., Chicago, IL) and GraphPad Prism 7.00. Statistical significance was defined as $P < 0.05$.

Results

Patient characteristics

From February 2011 to February 2019, non-DS-AMKL was diagnosed in 40 patients at our institution. The last follow-up was November 2019, and the median follow-up period was 16.1 months (range, 4.6 to 71.8). Patients with AMKL frequently presented with a long disease history. The median interval to diagnosis at our institution was 1 month (range, 0-12 months). Additionally, there were no patients with initial CNS involvement in our cohort. Patient characteristics are summarized in Table 2.

Cytogenetics analysis

Cytogenetic data were available for 37 (92.3%) patients: hypodiploid (n=0), normal karyotype (n=9, 24.3%), pseudodiploid (n=8, 21.6%), 47 to 50 chromosomes (n=12, 32.4%), and >50 chromosomes (n=7, 18.9%). Among 28 patients with chromosomal aberrations, 5 (17.9%) had numerical aberrations only, 6(21.4%) had structural aberrations only, 17 (60.7%) had both, and 13 (46.4%) had complex karyotypes (three or more independent abnormalities including at least one structural abnormality). Chromosome gain such as +21, +8, +19 occurred in 13(35.1%), 12(32.4%), and 8(21.6%) patients, respectively. Three patients (8.1%) had 11q23 aberrations and only 1 patient had t (1;22) (p13; q13). Monosomy 7/7q- and -5/5q- were found in 3(8.1%) and 2(5.4%) patients, respectively.

Molecular analysis

Sequencing of the hematopoietic transcription factor GATA1 was performed on 22 patients. Four patients (18.2 %) were positive for a GATA1-mutation. None of these children was previously diagnosed with DS or had a history of TMD during the neonatal period. Interestingly, the leukemic blasts of all four patients showed CD7 surface marker expression, a common feature of DS-AMKL. Three patients were treated by chemotherapy alone and are in continuous CR. Only one child received HSCT due to the high level of MRD after induction . Each child with GATA1-mutation was maintained in continuous CR.

Rearrangements of the MLL gene on 11q23 *via* RT-PCR detection were detected in 4(10.0%) patients, and one of them was not detected by G-banding. The MLL translocation in this group was MLL-AF4 (n=1), MLL-AF9 (n=2) and MLL-PTD (n=1).

WT1 and EVI1 overexpression were identified in 33 of 37 (89.2%) and 17 of 29 (58.6%) patients, respectively.

Early treatment response

Two of the 40 patients transferred to another hospital after induction without evaluation of the BM status. One and two patients abandoned treatment with no CR at the end of induction and , respectively. Of the remaining patients, 22 (57.9%) and 30 (81.1%) were in CR after induction and , respectively. Twenty-six (68.4%) had an MRD < 1% and 12 (31.6%) had an MRD > 1% after induction (Table 2).

Outcomes

Ultimately, 35 patients were included for survival analysis. The transplant and nontransplant cohorts comprised of 26 and 9 patients, respectively. Of the 26 patients in the transplant cohort, 16 had haplo-HSCT, 7 had HLA-MSDT (including 3 had a 6/6 HLA-MSDT), 1 had an HLA-MUDT, and the other 2 had a UCB donor (1 with a 6/6 HLA-matched donor, 1 with a 4/6 HLA-matched donor). Twenty-three patients opted for HSCT at CR1. The remaining 3 patients underwent transplantation without achieving CR after relapse for further salvage treatment and just one of them achieved CR2 for only 2 months after HSCT. Besides the 3 patients, 7 patients relapsed ultimately after transplantation and the median time between HSCT and relapse was 3 months range , 0-16 months.

The estimated 2-year probability of OS was 41±13% among 35 patients, 42±15% in the transplant cohort, and 41±17% in the chemotherapy cohort. The estimated 2-year probability of EFS was 41±10% in all patients, 49±12% in the transplant cohort, and 21±17% in the chemotherapy cohort. (Fig. 2 and 3)

Factors associated with long-term survival

The patients' clinical characteristics, including gender, age, WBC count at diagnosis, did not affect survival. Analysis of cytogenetic features for OS demonstrated that patients with +21 or hyperdiploid (50

chromosomes) had significantly better OS than those in other cytogenetic subgroups ($P_{log\text{-}rank}$=0.048 and $P_{log\text{-}rank}$=0.040, respectively).

We also compared the response to induction therapy and survival outcomes between EVI1 high patients and patients with low/undetectable EVI1. EVI1 high patients had a CR rate of 57.1% after induction as compared to 90.9% for the remaining patients (P =0.053). Besides, patients in the EVI1 high cohort tended to have a lower rates of 2-year OS (54±14% *vs* 89±10%, $P_{log\text{-}rank}$=0.089) and EFS (54±14% *vs* 79±13%, $P_{log\text{-}rank}$=0.179); but, the data were not statistically significant. However, EVI1 high patients had a better OS in transplant cohort than in chemotherapy cohort ($P_{log\text{-}rank}$=0.009).

Early treatment response also had an impact on the survival rates of non-DS-AMKL patients. The poor responders had an estimated 2-year OS of only 8±11 % and 2-year EFS of 18±12 %, while good responders (<5 % BM blasts after induction) showed a 2-year OS of 74±12 % ($P_{log\text{-}rank}$=0.023) and 2-year EFS of 60±14 % ($P_{log\text{-}rank}$=0.003). Besides, patients with MRD < 1% tended to have a favorable OS and EFS compared to those with a high level of MRD at the end of induction (Table 3).

Next, we assessed the therapeutic effect of HSCT. The baseline characteristics were similar between the nontransplant and transplant cohorts (Table 2). Patients who did not receive HSCT and had an event before the median time until transplantation (4 months) were excluded. The estimated 2-year OS and EFS were significantly higher in the transplant cohort than in the chemotherapy cohort ($P_{log\text{-}rank}$=0.042 and 0.044, respectively).

In the multivariate analysis for 2-year OS and EFS, which included hyperdiploid, +21, early treatment response as well as post-remission therapy as risk factors, only post-remission therapy (transplant *vs* chemotherapy; RR=11.192; 95 % CI, 2.045-61.241; P=0.005 for OS and RR=5.400; 95 % CI, 1.635-17.832; P=0.006 for EFS, respectively) had independent prognostic significance. For poor early treatment response, which was statistically significant in the univariate analysis, multivariate analysis indicated a trend towards poor prognosis (Table 4).

Discussion

The retrospective single-institution study reported the clinical characteristics and outcomes of pediatric non-DS-AMKL. The 2-year OS and EFS were 41±13% and 41±10%, respectively, similar to outcomes reported from other studies[6,8,25]. Besides, we assessed the effect of different prognostic factors on the outcomes of this subtype.

AMKL is characterized by various chromosomal abnormalities, and the focus on cytogenetic analyses remains of high prognostic importance[8,15,26-28]. Although multivariate analysis did not reveal significant differences, univariate analysis showed that patients with hyperdiploid had significantly better OS than those in other cytogenetic subgroup. Another good prognostic factor in our study was +21, which was especially represented in patients with GATA1-mutation. GATA1-mutation is a characteristic of DS-

AMKL who have a good prognosis. Similarly, our study showed that all of the 4 cases with GATA1-mutation remained in remission. Moreover, the research found that GATA1-mutant cases represented a distinct subset, which was strongly correlated with the signature found in DS-AMKL at the gene expression level[5]. These patients were not only biologically similar but also clinically similar, which suggested that they might benefit from the less intensive chemotherapy regimens given to DS-AMKL patients[4,6,29,30]. However, it should be pointed out that all patients except 2 with hyperdiploid had +21 in our study and the role of +21 in those patients could not be ignored. Further, we would detect the prognostic effect of hyperdiploid without +21 in a larger cohort.

Specific chromosomal abnormalities and gene mutations could be useful for stratification of patients to develop risk-adapted therapeutic strategies. However, some cases are devoid of known cytogenetic or molecular prognostic markers. Aberrant expression of specific genes may define clinically relevant biological subsets. For example, EVI1 overexpression was significantly associated with inferior survival in AML-M4/M5 subtype with MLL rearrangements[31]. In our study, nearly half of AMKL had high EVI1 expression and those patients tended to have low CR and survival rates. Other studies also reported that EVI1 overexpression was often observed among the AMKL patients, but was not prognostic in this subtype[31,32], suggesting a difference in biology based on cell type of origin of leukemia. However, the result that patients with EVI1 overexpression having a better survival rate after HSCT indicated that transplantation might eliminate this adverse factor. Unfortunately, we did not analysis the impact of fusion genes such as NUP98-JARID1A (t(11;15)(p15;q35)) and CBFA2T3-GLIS2 (inv(16)) as relevant technology has not carried out in our institution.

An assessment of early treatment response is also an essential prognostic factor in pediatric AML. Poor early treatment response detected by morphology was an adverse prognostic marker in our study which was in accordance with AML-BFM 04 trial[6]. Additionally, the MRD monitoring is used to improve risk stratification[18,33-34]. Several studies described the effect of MRD status in patients with AMKL after induction therapy[7,25]. Herein, we observed that patients with MRD >1% had an adverse OS and EFS compared to patients with MRD <1%. However, some of the patients in our cohort with good MRD response to induction therapy subsequently relapsed. Failure to detect residual leukemia cells by MFC due to immunophenotypic shifts might have contributed to disease recurrence[34]. Thus, advances in MFC integrated with other technology are necessary to improve the sensitivity of MRD testing and identification of patients who are likely to have a poor treatment response in non-DS-AMKL patients.

At present, the recommendation for allo-HSCT in pediatric non-DS-AMKL patients is still controversial. AML-BFM 04 trial reported the survival rates between patients, who were transplanted in CR1 or who received chemotherapy alone, did not differ significantly (5-year OS 70±11 % vs. 63±6 %; *P*=0.85) [6]. French ELAM 02 trial based on intensive chemotherapy protocols followed by consolidation reported that a 5-year OS was 54%[35]. However, a retrospective study by the European Group for Bone Marrow Transplant (EBMT) revealed that the 3-year OS was 82% for patients undergoing allo-HSCT[36]. Inaba H. *et al.* found that transplantation in CR1 did not improve survival, but some patients were salvaged by allo-HSCT in second CR or had evidence of residual disease[8]. In our study, patients with non-DS-AMKL had

more than 80% probability of achieving CR at the end of induction therapy, but they had a high rate of relapse with chemotherapy, especially those without GATA1-mutation. Patients who underwent allo-HSCT had significantly higher estimated OS and EFS rates than those who received chemotherapy alone. Besides, poor early treatment response which was a significant predictor of inferior survival outcomes in univariate, but not multivariate, analysis in our study, indicated that HSCT might have a more significant antileukemic potential as post-remission treatment than chemotherapy alone. Although improvements in induction therapy are reported to be beneficial, the major improvement in the treatment of AMKL must come in post-remission therapy[37]. Still, the results were interpreted carefully due to the small number of AMKL patients with allo-HSCT in our study.

Conclusion

In summary, non-DS-AMKL is a rare subtype of AML with a poor prognosis. More comprehensive studies are needed to optimize the risk stratification, such as cytogenetic analysis and early treatment response in this subtype. With poor OS but CR rates comparable to other AML subtypes, allo-HSCT could be a better option for post-remission therapy than conventional chemotherapy especially for those having a poor response to induction therapy.

Abbreviations

AMKL: acute megakaryoblastic leukemia; AML: acute myeloid leukemia; CNS:, central nervous system leukemia; CR: complete remission; DS: Down syndrome; EBMT: European Group for Bone Marrow Transplant; EFS: event-free survival; HSCT: hematopoietic stem cell transplantation; LAIP: leukemia-associated immunophenotyping; MFC: multicolor flow cytometry; MRD: minimal residual disease; MSD: matched sibling donor; MUD: matched unrelated donor; OS: overall survival; RT-PCR: reverse transcriptase PCR; TMD: transient myeloproliferative disease; UCB: unrelated cord blood ; WBC: white blood cell.

Declarations

Competing interests

The authors declare that they have no conflict of interest.

Authors' contributions

Yu Wang performed the data analysis and interpretation and was the main author of the study; Aidong Lu, Yueping Jia, Yingxi Zuo and Jun Wu participated actively in the study conception and design; Leping Zhang carried out the study, reviewed the results, and approved the final manuscript.

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References

1. Pagano L, Pulsoni A, Vignetti M, Mele L, Fianchi L, Petti MC, et al. Acute megakaryoblastic leukemia: experience of GIMEMA trials. Leukemia. 2002; 16(9):1622-6. https://www.nature.com/articles/2402618.
2. Barnard DR, Alonzo TA, Gerbing RB, Lange B, Woods WG. Comparison of childhood myelodysplastic syndrome, AML FAB M6 or M7, CCG 2891: report from the Children's Oncology Group. Pediatr Blood Cancer. 2007; 49(1):17–22. https://onlinelibrary.wiley.com/doi/abs/10.1002/pbc.20951.
3. Athale UH, Razzouk BI, Raimondi SC, Tong X, Behm FG, Head DR,et al. Biology and outcome of childhood acute megakaryoblastic leukemia: a single institution's experience. Blood. 2001; 97(12):3727-32. https://ashpublications.org/blood/article-lookup/doi/10.1182/blood.v97.12.3727.
4. Gruber TA, Downing JR. The biology of pediatric acute megakaryoblastic leukemia. Blood. 2015; 126(8):943-50. https://ashpublications.org/blood/article-lookup/doi/10.1182/blood-2015-05-567859.
5. De Rooij JDE, Branstetter C, Ma J, Li Y, Walsh MP, Cheng J, et al. Pediatric non-Down syndrome acute megakaryoblastic leukemia is characterized by distinct genomic subsets with varying outcomes. Nat Genet. 2017; 49(3):451-6. https://www.nature.com/articles/ng.3772.
6. Schweitzer J, Zimmermann M, Rasche M, von Neuhoff C, Creutzig U, Dworzak M, et al. Improved outcome of pediatric patients with acute megakaryoblastic leukemia in the AML-BFM 04 trial. Ann Hematol. 2015; 94(8):1327–36. https://link.springer.com/article/10.1007/s00277-015-2383-2.
7. O'Brien MM, Cao X, Pounds S, Dahl GV, Raimondi SC, Lacayo NJ, et al. Prognostic features in acute megakaryoblastic leukemia in children without Down syndrome: a report from the AML02 multicenter trial and the Children's Oncology Group Study POG 9421.Leukemia. 2013;27(3):731-4. https://www.nature.com/articles/leu2012223.
8. Inaba H, Zhou Y, Abla O, Adachi S, Auvrignon A, Beverloo HB, et al. Heterogeneous cytogenetic subgroups and outcomes in childhood acute megakaryoblastic leukemia: a retrospective international study. Blood. 2015;126(13):1575-84. https://ashpublications.org/blood/article-lookup/doi/10.1182/blood-2015-02-629204.
9. Hama A, Yagasaki H, Takahashi Y, et al. Acute megakaryoblastic leukaemia (AMKL) in children: a comparison of AMKL with and without Down syndrome.Br J Haematol. 2008; 140(5):552-61. https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-2141.2007.06971.x.
10. Xavier AC, Ge Y, Taub JW. Down syndrome and malignancies: a unique clinical relationship—a paper from the 2008 William Beaumont Hospital symposium on molecular pathology. J Mol Diagn. 2009;

11(5):371-80. https://jmd.amjpathol.org/article/S1525-1578(10)60254-1/fulltext.

11. Hitzler JK, Cheung J, Li Y, Scherer SW, Zipursky A. GATA1 mutations in transient leukemia and acute megakaryoblastic leukemia of Down syndrome. Blood. 2003; 101(11): 4301–4. https://ashpublications.org/blood/article-lookup/doi/10.1182/blood-2003-01-0013.
12. Malinge S, Izraeli S, Crispino JD. Insights into the manifestations, outcomes, and mechanisms of leukemogenesis in Down syndrome. Blood. 2009; 113(12): 2619–28. https://ashpublications.org/blood/article-lookup/doi/10.1182/blood-2008-11-163501.
13. Malinge S, Chlon T, Doré LC, Ketterling RP, Tallman MS, Paietta E, et al. Development of acute megakaryoblastic leukemia in Down syndrome is associated with sequential epigenetic changes. Blood. 2013; 122(14): e33–43. https://ashpublications.org/blood/article-lookup/doi/10.1182/blood-2013-05-503011.
14. Roy A, Roberts I, Norton A, Vyas P. Acute megakaryoblastic leukaemia(AMKL) and transient myeloproliferative disorder (TMD) in Down syndrome: a multi-step model of myeloid leukaemogenesis. Br J Haematol. 2009; 147(1):3-12. https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-2141.2009.07789.x.
15. de Rooij JD, Masetti R, van den Heuvel-Eibrink MM, Cayuela JM, Trka J, Reinhardt D, et al. Recurrent abnormalities can be used for risk group stratification in pediatric AMKL: a retrospective intergroup study. Blood. 2016;127(26):3424–31. https://ashpublications.org/blood/article-lookup/doi/10.1182/blood-2016-01-695551.
16. Creutzig U, Zimmermann M, Lehrnbecher T, Graf N, Hermann J, Niemeyer CM, et al. Less toxicity by optimizing chemotherapy, but not by addition of granulocytecolony-stimulating factor in children and adolescents with acute myeloid leukemia: results of AML-BFM 98. J Clin Oncol. 2006; 24(27): 4499–506. https://ascopubs.org/doi/full/10.1200/JCO.2006.06.5037?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200pubmed.
17. Reinhardt D, Diekamp S, Langebrake C, Ritter J, Stary J, Dworzak M et al. Acute megakaryoblastic leukemia in children and adolescents, excluding Down's syndrome: improved outcome with intensified induction treatment. Leukemia. 2005;19(8): 1495–6. https://www.nature.com/articles/2403815.
18. Rubnitz JE, Inaba H, Dahl G, Ribeiro RC, Bowman WP, Taub J et al. Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: results of the AML 02 multicentre trial. Lancet Oncol. 2010; 11(6): 543–52. https://www.nature.com/articles/2403815.
19. De Marchi F, Araki M, Komatsu N. Molecular features, prognosis, and novel treatment options for pediatric acute megakaryoblastic leukemia. Expert Rev Hematol. 2019;12(5):285-93. https://www.tandfonline.com/doi/full/10.1080/17474086.2019.1609351.
20. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR et al. Criteria for the diagnosis of acute leukemia of megakaryocyte lineage (M7). A report of the French-American-British Cooperative Group. Ann Intern Med. 1985; 103(3):460–2. https://www.acpjournals.org/doi/abs/10.7326/0003-4819-103-3-460.

21. Garand R, Robillard N. Immunophenotypic characterization of acute leukemias and chronic lymphoproliferative disorders: practical recommendations and classifications. Hematol Cell Ther. 1996;38(6):471-86.
22. Qin YZ, Zhu H, Jiang B, Li JL,Lu XJ,Li LD, et al. Expression patterns of WT1 and PRAME in acute myeloid leukemia patients and their usefulness for monitoring minimal residual disease. Leukemia Res. 2009;33(3):384-90. https://doi.org/10.1016/j.leukres.2008.08.026.
23. Alford KA, Reinhardt K, Garnett C, Norton A, Bohmer K, von Neuhoff C, et al. Analysis of GATA1 mutations in Down syndrome transient myeloproliferative disorder and myeloid leukemia. Blood. 2011; 118(8):2222–38. https://pubmed.ncbi.nlm.nih.gov/21715302/.
24. ISCN. In: Shaffer LG, Slovak ML, Campbell LJ, eds.An International System for Human Cytogenetics Nomenclature. Basel: S. Karger; 2009.
25. Maarouf N, Mahmoud S, Khedr R, Lehmann L, Shaaban K, Ibrahim S, et al. Outcome of Childhood Acute Megakaryoblastic Leukemia: Children's Cancer Hospital Egypt 57357 Experience. Clin Lymphoma Myeloma Leuk. 2019;19(3):e142-e152. https://www.clinical-lymphoma-myeloma-leukemia.com/article/S2152-2650(18)31525-8/fulltext.
26. Masetti R, Guidi V, Ronchini L, Bertuccio NS, Locatelli F, Pession A. The changing scenario of non-Down syndrome acute megakaryoblastic leukemia in children. Crit Rev Oncol Hematol. 2019; 138:132-8. https://www.sciencedirect.com/science/article/abs/pii/S1040842819300666?via%3Dihub.
27. Chilton L, Hills RK, Harrison CJ, Burnett AK, Grimwade D, Moorman AV. Hyperdiploidy with 49-65 chromosomes represents a heterogeneous cytogenetic subgroup of acute myeloid leukemia with differential outcome. Leukemia. 2014;28(2):321-8. https://www.nature.com/articles/leu2013198.
28. Sandahl JD, Kjeldsen E, Abrahamsson J, Ha SY, Heldrup J, Jahnukainen K, et al. Ploidy and clinical characteristics of childhood acute myeloid leukemia: a NOPHO-AML study. Genes Chromosomes Cancer. 2014;53(8):667-75. https://onlinelibrary.wiley.com/doi/abs/10.1002/gcc.22177.
29. Reinhardt D, Reinhardt K, Neuhoff C, Sander A, Klusmann JH, Pekrun A, et al. GATA1-mutation associated leukemia in children with trisomy 21 mosaic. Klin. Padiatr. 2012; 224(3): 153–5. https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-0032-1308988.
30. Kudo K, Hama A, Kojima S, Ishii R, Morimoto A, Bessho F, et al. Mosaic Down syndrome-associated acute myeloid leukemia does not require high-dose cytarabine treatment for induction and consolidation therapy. Int J Hematol. 2010;91(4):630-5. https://link.springer.com/article/10.1007%2Fs12185-010-0549-1.
31. Jo A, Mitani S, Shiba N, Hayashi Y, Hara Y, Takahashi H,et al. High expression of EVI1 and MEL1 is a compelling poor prognostic marker of pediatric AML. Leukemia. 2015; 29(5):1076-83. https://www.nature.com/articles/leu20155.
32. Ho PA, Alonzo TA, Gerbing RB, Pollard JA, Hirsch B, Raimondi SC, et al. High EVI1 Expression Is Associated with MLL Rearrangements and Predicts Decreased Survival in Paediatric AML: A Report

From the Children's Oncology Group.Br J Haematol. 2013;162(5):670-7. https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.12444.

33. Tierens A, Bjorklund E, Siitonen S, Marquart HV, Wulff-Juergensen G , Pelliniemi TT, et al. Residual disease detected by flow cytometry is an independent predictor of survival in childhood acute myeloid leukaemia: results of the NOPHO-AML 2004 study. Br J Haematol. 2016;174(4):600–9. https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.12444.
34. Buldini B, Rizzati F, Masetti R, Fagioli F, Menna G, Micalizzi C et al. Prognostic significance of flow-cytometry evaluation of minimal residual disease in children with acute myeloid leukaemia treated according to the AIEOP-AML 2002/01study protocol. Br J Haematol. 2017; 177(1):116-26. https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.14523.
35. Teyssier AC, Lapillonne H, Pasquet M, Ballerini P, Baruchel A, Ducassou S,et al. Acute megakaryoblastic leukemia (excluding down syndrome) remains an acute myeloid subgroup with inferior outcome in the French ELAM02 trial. Pediatr Hematol Oncol. 2017;34(8):425–7. https://doi.org/10.1080/08880018.2017.1414905.
36. Garderet L, Labopin M, Gorin NC, Polge E, Baruchel A,Meloni G, et al. Hematopoietic stem cell transplantation for de novo acute megakaryocytic leukemia in first complete remission: a retrospective study of the European Group for Blood and Marrow Transplantation (EBMT). Blood. 2005; 105(1):405–9.
37. Hahn AW, Li B, Prouet P, Giri S, Pathak R, Martin MG. Acute megakaryocytic leukemia: What have we learned. Blood Rev. 2016; 30(1):49-53. https://www.sciencedirect.com/science/article/abs/pii/S0268960X15000600?via%3Dihub.

Tables

Table 1 AML protocols in our institution

Treatment and medicine	Dose	Time
AIE		
cytarabine	100-150mg/(m^2·d)	D1-7
idarubicin	10mg/(m^2·d)	D3,5
etoposide	100-150mg/(m^2·d)	D6-8
HA		
homoharringtonine	4-6mg/(m^2·d)	D1-7
cytarabine	100-150mg/(m^2·d)	D1-7
HDAra-C		
cytarabine	2g/(m^2·d)	D1-4
idarubicin	10mg/(m^2·d)	D2,4

Table 2 Characteristics of patients in non-DS-AMKL

Parameter	Patients No.	Chemotherapy Cohort(n=9	Transplant Cohort(n=26	*P*-value
Age,mon				.327
Median(range)	21(3-72)	19(3-48)	21(10-72)	
12	8(20.0%)	3(33.3%)	4(15.4%)	
12	32(80.0%)	6(66.7%)	22(84.6%)	
Sex				.349
male	21(52.5%)	6(66.7%)	13(50.0%)	
female	19(47.5%)	3(33.3%)	13(50.0%)	
WBC count,×10^9/L				.840
Median(range)	13.95(2.25-121.41)	8.42(4.46-37)	14.99(2.25-121.41)	
<20	27(67.5%)	6(66.7%)	18(69.2%)	
>20	13(32.5%)	3(33.3%)	8(30.8%)	
Hemoglobin,g/L				.210
Median(range)	86(41-125)	72(52-109)	89(41-125)	
Platelet count,×10^9/L				.339
Median(range)	27(3-359)	31(5-339)	25(3-359)	
Cytogenetics				
+21	13(35.1%)	4(44.4%)	9(39.1%)	.791
Hyperdiploid	7(18.9%)	2(22.2%)	5(27.1%)	.977
CR after induction 1				.847
Yes	22(57.9%)	6(66.7%)	16(61.5%)	
No	16(42.1%)	3(33.3%)	10(38.5%)	
CR after induction 2				.761
Yes	30(81.1%)	8(88.9%)	22(84.6%)	
No	7(18.9%)	1(11.1%)	4(15.4%)	
MRD after induction 1				.678
<1%	26(68.4%)	7(77.8%)	18(69.2%)	
>1%	12(31.6%)	2(22.2%)	8(30.8%)	

CR complete remission; *MRD* minimal residual disease; *WBC* white blood cell.

Table 3 Univariate analysis of factors associated with long-term outcomes in non-DS-AMKL

Factors	No. Of Cases	2-Year OS(%)	*P*-value	2-Year EFS(%)	*P*-value
Sex			.1		.199
Male	19	30±15		31±12	
Female	16	60±22		58±15	
Age(mon)			.470		.731
<12	7	64±21		64±21	
>12	28	41±13		40±11	
WBC count,10^9/L			.137		.329
<20	24	50±14		46±12	
>20	11	0		30±18	
Cytogenetics					
Complex	12	62±15	.909	55±15	.967
t(v;11q23)/MLL rearrangements	4	60±22	.832	60±22	.705
+21	13	76±16	.048	53±16	.358
+8	11	64±15	.217	49±20	.434
Hyperdiploid	7	100	.040	49±27	.327
EVI1 overexpression			.089		.179
Yes	14	54±14		54±14	
No	11	89±10		79±13	
CR after induction			.023		.003
Yes	22	74±12		60±14	
No	13	8±11		18±12	
CR after induction			.135		.086
Yes	30	57±14		50±11	
No	5	0		13±17	
MRD after induction			.007		.001
<1%	25	61±15		57±12	
>1%	10	24±16		7±10	
Transplant			.027		.044
Yes	26	46±16		49±12	
No	9	41±17		21±17	

CR complete remission; *MRD* minimal residual disease; *WBC* white blood cell.

Table 4 Multivariable Cox regression analysis for OS and EFS

	OS			EFS		
	HR	95%CI	*P*-value	HR	95%CI	*P*-value
Hyperdiploid	1.001	.257-3.897	.961	2.287	.424-12.330	.336
+21	2.792	.420-18.561	.288	0.865	.237-3.162	.826
CR after induction I	1.010	.175-5.811	.991	3.483	.838-14.470	.086
MRD after induction I	1.802	.364-8.918	.471	2.986	.813-10.969	.099
Transplant	11.192	2.045-61.241	.005	5.400	1.635-17.832	.006

CI confidence interval; *CR* complete remission; *EFS* event-free survival; *HR* high risk; *MRD* minimal residual disease; *OS* overall survival.

Figures

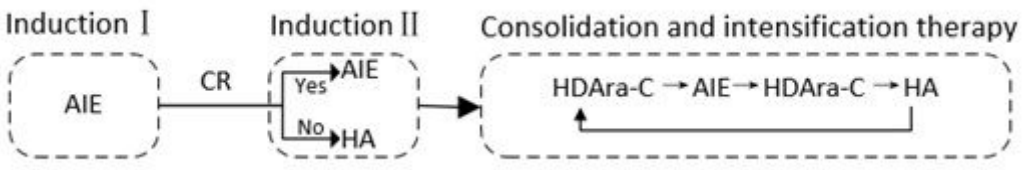

Figure 1

Flow chart of AML protocol in our institution.

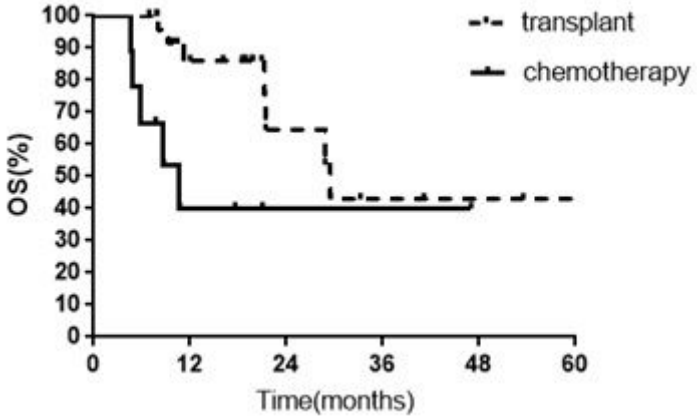

Figure 2

Kaplan-Meier estimates of 2-year OS in the transplant and chemotherapy cohort.

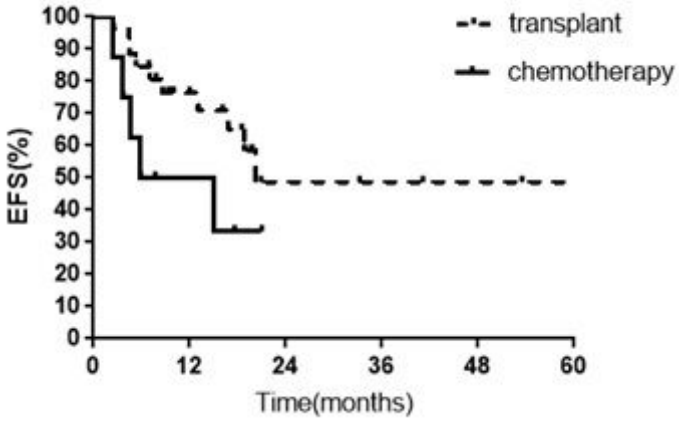

Figure 3

Kaplan-Meier estimates of 2-year EFS in the transplant and chemotherapy cohort.