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# TREATMENT OUTCOME AND CHALLENGES OF PEDIATRIC AML IN A LMIC: A SINGLE- INSTITUTION EXPERIENCE

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## ABSTRACT

**Background**: Despite improvements in pediatric acute myeloid leukemia (AML) outcomes, results remain poor in low- and middle-income countries (LMICs).

**Objectives**: We aimed to evaluate the survival outcome of pediatric AML patients, with special emphasis on various prognostic factors.

**Methods**: This prospective study included all newly diagnosed pediatric AML patients (<18 years) treated at the National Cancer Institute, Egypt (2020–2022) using a protocol adapted from MyeChild 01.

**Results**: 100 patients were enrolled. By the end of follow-up, sustained remission was achieved in 34 cases while 28 cases relapsed or were refractory, and 38 died during supportive care. The 1-year and 2-year overall survival (OS) rates were 51% and 38% while the 1-year and 2-year event-free survival (EFS) rates were 44% and 33%, respectively. Patients <2 years of age and those with KMT2A-rearrangements showed worse EFS (P=0.026 and P=0.049, respectively). WBC count at diagnosis $<100\times10^9$/L and Core-binding factor leukemias had been associated with better OS (P=0.031 and P=0.032, respectively) and EFS (P=0.021 and P=0.020, respectively). Post-induction remission and being classified in the standard-risk group were both significantly associated with prolonged OS and EFS compared to patients who were refractory (p< 0.001,each), as well as those in the high-risk group (p< 0.001,each). High-risk patients undergoing allogeneic HSCT had better OS and EFS (P=0.001, each). Morphological response to induction was identified as independent prognostic factor (P< 0.001).

**Conclusion**: Treatment-related mortality and high relapse rate remain major barriers to improve outcomes in LMICs.

**KEYWORDS**: Pediatric, acute myeloid leukemia, LMICs, overall survival, event free survival, ,KMT2A.

## INTRODUCTION

Acute leukemia (AL) is the most prevalent form of cancer in the pediatric population. Among childhood leukemias, acute lymphoblastic leukemia (ALL) represents 80% of cases, while acute myeloid leukemia (AML) accounts for approximately 15–20% (1).

AML is a clonal hematopoietic disorder resulting from the malignant transformation of bone marrow (BM)–derived self-renewing stem cells or progenitor cells. This transformation leads to the proliferation and accumulation of immature, non-functional myeloid blasts in the BM and peripheral tissues (2).

Over recent decades, the outcome for children with AML has improved markedly. Currently, complete remission (CR) occurs in approximately 80% of patients, with event-free survival (EFS) and overall survival (OS) rates reaching around 60% and 70%, respectively (3). Despite these improvements, outcomes for pediatric AML in low- and middle-income countries (LMICs) continue to lag due to resource constraints and limited access to advanced diagnostics and treatments (4).

This study aimed to evaluate OS and EFS in newly diagnosed pediatric AML patients, treated at the National Cancer Institute, Egypt (2020–2022) using a modified chemotherapy regimen based on the MyeChild 01 protocol (5). In this protocol, etoposide was removed from induction chemotherapy, anthracyclines were omitted from consolidation therapy in standard-risk patients, while fludarabine and cytarabine were used for high-risk patients. Additionally, we investigated various prognostic factors and their associations with OS and EFS.

## PATIENTS AND METHODS

### 2.1 Patients

This was a single -center prospective cohort study included all newly diagnosed AML pediatric patients younger than 18 years who were presented to National Cancer Institute (NCI), Egypt, from January 1, 2020, to December 31, 2022.

Patients with AML Down syndrome, Acute promyelocytic leukemia (APL/AML M3), relapsing or secondary AML, mixed phenotype acute leukemia, isolated extramedullary disease and children with documented myelodysplastic syndrome (MDS) were excluded due to difference in treatment protocols.
Collected data included patients' demographics, presenting symptoms and signs. Initial disease evaluation comprised complete blood counts (CBC), liver and renal function tests, and cerebrospinal fluid (CSF) analysis, bone marrow aspiration (BMA), immunophenotyping, cytogenetics and molecular studies. Slides of BMA were examined using Leishman stain, supplemented with cytochemical stains such as MPO or SBB, esterases, acid phosphatase and PAS when indicated for determination of French American British (FAB) subtype.
Treatment response was monitored using morphological assessment of BM and minimal residual disease (MRD) evaluation by flow cytometry (FC) and by real-time quantitative PCR (RT-qPCR) in patients with a leukemia-specific molecular marker. Monitoring was performed post induction 1 (between day 21 to day 35) and induction 2 for all patients and post intensification 1 for those with residual disease post induction 2. MRD levels of <0.1 and >3 log reduction of PCR was considered negative.
The study was approved by the Institutional Review Board of NCI-Cairo. Informed consent was obtained from all guardians.

**2.2 Methods**

**Immunophenotyping**

Immunophenotyping on BM or PB samples was performed by flow cytometry to verify the diagnosis of AML. A complete fluorescein-labelled mouse monoclonal antibodies panel of myeloid markers (MPO, CD13, CD33, CD117, CD14, CD15), B- and T-Lymphoid markers, stem cell marker CD34, as well as CD56 and HLADR was performed on six colors Navios cytometer (Beckman Coulter Diagnostics, USA). Sub-classification of AML was done by a secondary antibody panel of markers; CD4, CD14, CD64, CD11c, CD41, CD61, and Glycophorin A.

**Cytogenetics analyses**

Conventional karyotyping by G-banding technique was routinely performed for all cases. In the majority of the cases, at least 20 metaphases were analyzed using an IKAROS imaging system (MetaSystems, Altlussheim, Germany). The karyotypes were interpreted using the International System for Human Cytogenetic Nomenclature (ISCN) 2016. Fluorescence in situ hybridization (FISH) was performed to detect KMT2A and ETV6 genes rearrangements and aberrations using Locus specific XL KMT2A and XL ETV6 break apart probes (Metasystems, Altlussheim, Germany), respectively, according to the manufacturer's instructions .For each slide, at least 200 interphase nuclei and 10 metaphases were evaluated by 2 analysts, using a fluorescence microscope (AxioImager.Z1 mot, Carl Zeiss Ltd., Hertfordshir, UK) and ISIS imaging system (Metasystems, Altlussheim, Germany) (6).

**Molecular detection of fusion gene transcripts and mutational analysis**

Reverse transcription polymerase chain reaction (RT-PCR) was performed for all samples for the detection of fusion transcripts t(8,21)(q22;q22) RUNX1::RUNX1T1, inv(16)(p13q22) CBFß::MYH11, t(4;11) (q21;q23), and t(9;22)(q34;q11) p190 and p210 BCR::ABL1 fusion genes, according to the BIOMED-1 guidelines. Analysis of mutation status of internal tandem duplication of FLT3 (FLT3-ITD) by Sanger sequencing were done for all samples.

**2.3 Treatment Protocol and Risk Stratification**

Patients were stratified into risk groups based on cytogenetic/molecular characteristics as illustrated in **Table 1** and treatment response (morphological and MRD) according to MyeChild 01.
Standard risk (SR) patients, defined as patients with GR or IR cytogenetics who achieve morphological CR after course 1 and a MRD level of <0.1% or >3 log reduction in transcript level after course 1(IR) and course 2 (GR), receive: MA → MA →HD Ara-C →HD Ara-C.
Intermediate risk patients are defined as patients with GR or IR cytogenetics who have a MRD level of >0.1% and/ or <3 log reduction in transcript level after course 2 (GR) or course 1 (IR) and fall to <0.1 or >3 log reduction after course 3 for GR or course 2 for IR patients. These patients will have their treatment intensified and receive FLA/mito as course 3 and HD Ara-C as course 4 (MA → MA → FLA/mito →HD Ara C).
High risk (HR) patients, defined as patients with PR cytogenetics and those with GR or IR cytogenetics who fail to achieve morphological CR after course 1 of chemotherapy, receive: MA →FLA/mito →FLA/mito→FLA →HSCT.
Of note, patients with GR or IR cytogenetics who achieve morphological CR after course 1 may become high risk if their MRD level remains >0.1% or the reduction in transcript levels is <3log reduction after course 2 for IR or after course 3 for GR patients despite treatment intensification with FLA-mito. The treatment courses are detailed in **Table S1.**

**CNS directed therapy**

In an atraumatic tap, CNS disease is defined as CNS1 with <5 x$10^6$ /L WBC in CSF and no blasts, CNS2: <5 x$10^6$ /L WBC in CSF with blasts or CNS3: >5 x$10^6$ /L WBC in CSF with blasts or clinically significant neurological

deficits (such as cranial nerve lesions) and/or radiological evidence of an intracranial or intradural mass. If the lumbar puncture is traumatic (>10 RBC/ml) and contains >5 WBC/ml, Steinherz/Bleyer algorithm (CSF WBC/CSF RBC >2x blood WBC/RBC) should be used to distinguish between CNS2 and CNS3.
CNS1: Two triple intrathecal, one with each of the first two courses
CNS2 disease or traumatic tap at diagnosis: Two triple intrathecal per week (minimum of three in course 1), followed by triple intrathecal at the start of cycle 2
CNS disease (CNS3): Two triple intrathecal per week until the CSF is clear plus a further two (minimum of six in three weeks from diagnosis), followed by triple intrathecal with each cycle of chemotherapy.
Doses of triple intrathecal are illustrated in **Table S2.**

### 2.4 Clinical endpoints

Complete Remission (CR) was defined as less than 5% blasts in the BM; no evidence of extra medullary disease; and evidence of hematopoietic recovery (ANC ≥ 1000/µl and rising; platelet count ≥ 80,000/µl and rising). Patients who failed to achieve CR after completion of initial treatment, with >5% blast in the BM were defined as having resistant or refractory disease (RD). Relapse was defined by reappearance of more than 5% blasts in the BM or evidence of extra medullary disease following CR. Early deaths comprise death during first 42 days of induction 1 before response status is assessed while treatment related mortality (TRM) is defined as death from any cause other than progressive disease that occurs after 42 days from starting therapy (7).
OS was estimated from the date of entry of study till the date of death or last follow up. EFS was measured from the date of entry of the study to the date of induction treatment failure, relapse or death (4).

### 2.5 Statistical Methods

Statistical analysis was done using IBM SPSS® Statistics version 26 (IBM® Corp., Armonk, NY, USA). Numerical data was expressed as mean and standard deviation or median and range as appropriate. Comparison of quantitative variables between two groups was done using Mann-Whitney test (non-parametric t-test) for not normally distributed numerical data. Comparison between 3 groups was done using Kruskal-Wallis test (non-parametric ANOVA) then post-Hoc test was used for pair-wise comparison.
Survival analysis was done using Kaplan-Meier method and comparison between two survival curves was done using log-rank test. Hazard ratio (HR) with its 95% confidence interval (CI) was used for risk estimation. All tests were two-tailed. A p-value < 0.05 was considered significant (8).

## RESULTS

### 3.1 Patient characteristics

A total of 127 AML patients were observed over the study period. After excluding 17 patients with APL, 4 cases were down syndrome (<4 years), 2 cases AML on top of Fanconi and 4 cases died within 48 hours of admission,100 patients were enrolled in this study.
Sixty-two patients (62%) were males, with a male/female ratio of 1.6:1. The median (range) age at diagnosis was 9 years (2 months to 18 years). Seven cases (7%) presented with chloroma, and 10 cases (10%) showed CNS infiltration with blast cells (CNS 3). The median (range) WBC count at diagnosis was 39.5 (1-545) x $10^9$/L.The most common FAB subtype was AML M2 in 34 %, followed by AML- M4 in 30%. The other patients' demographic, clinical, and laboratory' characteristics are illustrated in **Table 2**.

### 3.2 Genetic Features

Cytogenetic analysis was carried out to all patients, the karyotyping was successfully performed in 94 patients, but six cases (6%) had a failure of mitosis. Eighty-four (84%) patients had an abnormal cytogenetics result. Core binding factor (CBF) leukemia was identified in 33 patients (33%), including 23 (23%) with t(8;21)(q22;q22) RUNX1::RUNX1T1 and 10 (10%) with inv(16)(p13q22) CBFβ::MYH11. Notably, only one patient (1%) had t(9;22)(q34;q11.2) BCR::ABL1 fusion gene. The median modal chromosomal number (MCN) was 46 (range 38–89). Common gains involved chromosomes 8 (17%) and 21 (8.5%), while chromosome 7 abnormalities were seen in 5.3% of cases of which 3 (3.2%) had -7, 1 (1.1%) had del(7q) and 1 (1.1%) had unbalanced translocation involving 7q. Deletions of TP53 and 5q occurred in 2.1% and 1.1% of patients, respectively, and 6.4% had complex karyotypes. KMT2A abnormalities were detected in 19 patients (19%), mainly rearrangements (13%), including t(9;11) (4%), t(10;11) (2%), t(11;14) (1%), and t(2;11) (1%), with five cases having unidentified partner chromosomes. Six (6%) cases showed other KMT2A aberrations i.e gain or loss of KMT2A. KMT2A-r was the sole abnormality in seven cases while 6 cases (6%) harbored additional numerical aberrations (e.g.+8 in 4 cases). Chromosomal aberrations are illustrated in Fig 1. Analysis for FLT3 mutation was carried out on 70 patients only revealing 7 cases had mutant FLT3/ITD with high allelic ratio>0.4.

**Response to treatment**

At end of induction 1, 71 patients (71%) had morphologic CR, 8 patients (8%) were refractory while 21 patients died during supportive care without assesment of remission status. Out of 71 who achieved CR after induction I (Fig 2), MRD was performed by FC for 30 patients only according to the adequacy of the sample and the presence of leukemia-associated immunophenotypes. MRD < 0.1% was achieved in 14 cases (19.7%) and >0.1 in 16 cases (22.5%).

There were 48 patients (48%) out of 100 cases treated as SR ,31 patients (31%) treated as HR while 21(21%) cases died whithout know remission status for final risk assesment. No patients was classified in intermediate risk group due to unavailable MRD for many cases with intermediate risk cytogenetics and also discrepency between MRD by flow and by PCR for patients with good risk cytogenetics.

Only six high risk underwent allogeneic HSCT—five of whom are currently alive, while one patient died post-transplant. The remaining HR patients did not proceed to HSCT due to various reasons, including lack of a fully HLA-matched donor, death during supportive care, refractory disease, or disease relapse while awaiting transplantation.

Of the total cohort, 64 patients died during the study period. The leading causes of death were related to supportive care complications (38 cases), the majority were attributed to infections and gram negative bacteria were the most common isolated pathogens. Additionally, 3 patients died from refractory disease, and 23 of the 25 patients who experienced relapse subsequently died.

**3.4 Impact of prognostic factors on patients' outcomes**

The follow up period of the studied group ranged from 0.36 to 50 months, with a median of 12.2 months, The 1- and 2-year OS were 51% and 38, while the 1- and 2-year EFS of the whole group were 44% and 33%, respectively with a median of 8.7 months (Fig 3A).

Patients under 2 years of age showing worse EFS compared to those older than 2 years (P= 0.026). Patients with WBC<100 × $10^9$/L at diagnosis had significantly better survival outcome for both OS (P=0.031) and EFS (P=0.021)(Fig 3B).

Initial cytogenetics grouping significantly influenced the patients'outcome. Patients with PR cytogenetics had worse OS and EFS compared to those with IR and GR groups (P= 0.004 and P= 0.005, respectively)(Fig 3C). As regard cytogenetic subgroups, patients with CBF-leukemias [inv(16)(p13q22) and t(8;21)(q22;q22) RUNX1::RUNX1T1] demonstrated superior outcomes, affecting both OS (P=0.032) and EFS (P=0.020)(Fig 3D). Among CBF patients, although inv(16) had been associated with better survival compared to t(8;21), but failed alone to reach statistical significance for OS (P = 0.081) and EFS (P = 0.058). Moreover, t(8;21) alone did not show a statistically significant impact on survival.

Aberrations of KMT2A genes were asoociated with worse OS and EFS compared to patients with normal KMT2A gene (P= 0.028 and P = 0.018, respectively)(Fig 3E). Moreover, patients with KMT2A-r had significantly worse EFS compared to KMT2A-r negative patients (P= 0.049). Contrary to previous literature, FLT3 mutations with a high allelic ratio did not show a significant impact on survival, possibly due to unavailability of data in 30 patients. Other cytogenetic abnormalities—such as +8, +21, del(9q), and loss of sex chromosomes—had no significant effect on survival. Additionally, rare abnormalities like t(9;22), del(5q), and p53 deletion were too infrequent to assess their prognostic value. Notably, the most important prognostic factor identified was the morphological response to induction therapy ($p < 0.001$). While MRD is recognized as a critical factor post-induction, this study could not confirm its prognostic value, likely due to a high proportion of patients lacking MRD data. Final risk stratification significantly impacted the outcome as high risk group was significantly associated with worse survival ($p < 0.001$)(Fig 3F). High risk patients subjected to allogenic HSCT had better OS and EFS (P= 0.001, each)**.** No other clinical or laboratory parameters were found to significantly impact OS or EFS. **Table 3** outlines the prognostic factors and their associations with OS and EFS.

Multivariate analysis was performed using the Cox proportional hazards model, the only independent predictor of OS was the morphological response to first induction, where patients achieving CR had significantly better OS (Hazard Ratio: 7.9; 95% CI: 4.5–13.6; $p < 0.001$), highlighting the critical importance of early treatment response in predicting long-term outcomes.

**DISCUSSION**

Over the past decade, survival outcomes in pediatric AML have improved markedly in high-income countries.These advances are attributed to improvements in supportive care, response-based therapy adaptation, intensified chemotherapy, incorporation of novel agents, selective use of HSCT, and more effective salvage regimens (9). However, in LMICs, the progress has been limited, with survival rates often below 40% (4). Contributing factors include late disease presentation, and inadequate access to intensive supportive care and lack of recources (10).

In this study, gender did not significantly affect OS or EFS, in agreement with previous studies (11,12). The best outcomes were observed in children aged 2–10 years, while patients under 2 years and over 10 years had inferior survival .This age-related survival pattern is consistent with the AML-BFM (11), although not universally observed (13,14).

A high WBC count at diagnosis is generally related to a worse prognosis (14). However, this varied across different trials (15). In our study, high initial WBC count (≥ 100 x $10^9$/L), was associated with worse survival compared to

low WBC. Similar results were observed in Medical Research Council (MRC) AML12 trial (16) and in another study in Korea (17).
The most common FAB subtypes were M2 followed by M4, consistent with other LMIC studies (12). FAB subtype was not significantly associated with survival in our cohort, corroborating data from the MRC AML10/12 trials (18). However, some evidence suggests M2 may have a favorable outcome compared to M0 and M7 (12).
Chromosomal abnormalities are identified in approximately 75–80% of pediatric AML. Advances in molecular diagnostics and understanding of the genetics have significantly refined classification systems and informed the development of risk-adapted treatment strategies over the past three decades (19).In this study cytogenetic analysis revealed that CBF leukemias [t(8;21) and inv(16)] were the most frequent abnormalities (33%) and were associated with superior outcomes. Patients with inv(16) had better outcomes than those with t(8;21), in line with previous studies (20).
KMT2A-r, observed in 13%, were associated with worse EFS ($p = 0.049$), similar to prior pediatric AML studies (21). The prognostic value of KMT2A-r may vary depending on the fusion partner (7,22). FLT3/ITD mutations with high allelic ratio (>0.4) were detected in 7 patients, but due to missing data in 30% of the cohort, its impact on survival could not be clearly established. Nevertheless, high allelic ratio FLT3/ITD has been widely recognized as an adverse prognostic factor (23,24).
Patients with CNS1 status had slightly better OS compared to those with traumatic lumbar puncture (TLP) or CNS3 disease with near significance ($p = 0.069$). These findings are somewhat consistent with COG data, which suggest that CNS disease at diagnosis may negatively affect outcomes (25). However, other studies, including the French ELAM02 and earlier COG trials, reported no significant difference in outcomes based on CNS status (26,27).
The 1-year OS and EFS were 51% and 44%, respectively. These results show mild improvement over a previous National Cancer Institute -Egypt study (2014–2016) using ADE protocol (cytarabine/doxorubicin/etopside) where 1-year OS and EFS were 45.1% and 39%(28). Compared to other LMICs, our outcomes were superior to studies in Ethiopia and Vietnam (12,29), but slightly below results reported from Brazil (30). In contrast, outcomes in high-income countries remain significantly better. Studies such as COG AAML0531 and AML1031 reported 3-year OS of 69.4% and 65.4%, respectively (31,32). European and Japanese trials (BFM, AIEOP, SJCRH, JPLSG) consistently report 3-year OS rates exceeding 70–80% and 3 -year EFS 60-70% (33–36). The survival differences may be attributed to lack of supportive care, difficulties in risk stratification with limited access to molecular examination, limited resources and prolonged time to HSCT in developing countries.
Therapeutic response after induction therapy is a key predictor of pediatric AML outcome and is evaluated using morphology, MRD detection, PCR, and more recently a next-generation sequencing (NGS) targeting AML-specific markers to identify high-risk patients needing additional treatment like HSCT (37).
Complete remission (CR) was achieved in 71% of patients post-induction 1 and 87.3% after induction 2, comparable to other LMIC studies (38–40). Morphological response after induction I was the only independent prognostic factor affecting outcome in multivariate analysis ($p < 0.001$). This finding is supported by several large trials (10,41).
Minimal residual disease (MRD) by FC is now considered a superior method for risk stratification (42). However, in our study, not all cases could be followed with MRD and thus could not be evaluated for prognostic significance. Future efforts should focus on integrating MRD into routine evaluation.
Infection-related mortality remains a major challenge. Among the 64 deaths recorded, 38 cases were related to supportive care complications, including 33 cases due to infections. Early death occurred in 21%, and treatment-related mortality (TRM) was 21.5%.These figures reflect a significant improvement compared to earlier data from our center, where early death occurred in 43.8% and TRM reached 61.8% (43). Still, they remain higher than those reported in high-income countries, where TRM and ED typically fall below 10% (44,45).
Our data support the role of HSCT in improving outcomes for high-risk and relapsed patients. Patients who underwent HSCT showed significantly better OS and EFS compared to those who did not ($p = 0.001$), consistent with international guidelines (46).

## CONCLUSION

Despite modifications to chemotherapy protocol, survival rates for pediatric AML remain unsatisfactory. Factors such as response to induction, favorable cytogenetics (particularly CBF leukemias), and HSCT were associated with better outcomes, while treatment-related mortality and high relapse rate remain significant barriers. Strengthening supportive care, timely risk stratification, integration of molecular diagnostics, MRD monitoring, incorporation of novel agents, selective use of hematopoietic stem cell transplantation (HSCT), are critical next steps toward improving outcomes in LMIC settings.

**Declaration of Interest**
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**TABLE 1 Cytogenetic/Molecular Risk Categories**

| Good risk (GR) | Intermediate Risk (IR) | Poor risk (PR) |
|---|---|---|
| • t(8;21)(q22;q22)<br>• inv(16)/t(16;16)(p13q22)<br>• NPM1 mutant without FLT3-ITD<br>• Double mutation of CEPBA without FLT3-ITD | • t(9;11)(p21;q23)/MLL-MLLT3<br>• t(11;19)(q23;p13.3)/MLL-MLLT1<br>• All other MLL rearrangements not classified as high risk<br>• CN-AML without a good or poor risk molecular mutation<br>• All other abnormalities which are neither good nor poor risk | • inv(3)(q21q26)/t(3;3)(q21;q26)/ abn(3q26)<br>• -5/del(5q)<br>• -7<br>• t(6,9)(p23;q34)/DEK-NUP214<br>• t(9;22)(q34;q11)/BCR-ABL-1<br>• 12p abnormalities<br>• t(6,11)(q27;q23)/MLL-MLLT4<br>• t(4;11)(q21;q23)/MLL-AFF1<br>• t(10;11)(p11~14;q23)/MLL-MLLT10<br>• t(5;11)(q35;p15.5)/NUP98-NSD1<br>• t(7;12)(q36;p13)/MNX1-ETV6<br>• FLT3-ITD without NPM1 or CBF |

**TABLE 2 Demonstrating demographic, clinical, and laboratory characteristics of 100 pediatric patients with AML**

| Parameter (Total N, 100) | Value (%) | Parameter | Value (%) |
|---|---|---|---|
| **Age (years)** | 9 years (2 months to 18 years) * | **KMT2A Aberrations** | 19/100 (19) |
| < 2 | 22 (22) | Rearrangements | 13 (13) |
| 2-10 | 39 (39) | Gain/deletion | 6 (6) |
| > 10 | 39 (39) | **Recurrent Translocations** | 47/100 (47) |
| **Gender** | | t(8;21) (q22;q22) | 23 (23) |
| Male | 62 (62) | inv. 16 (p13q22) | 10 (10) |
| Female | 38 (38) | KMT2A rearrangements | 13 (13) |
| **Clinical Manifestations** | | t(9;22)(q34;q22.1) | 1 (1) |
| Fever | 72 (72) | **Other cytogenetic abnormalities (N=94)** | |
| Lymphadenopathy | 25 (25) | 5q deletion | 1 (1.1) |
| Hepatosplenomegaly | 20 (20) | -7/del7q | 5 (5.3) |
| **Central nervous system infiltration** | | P53 deletion | 2 (2.1) |
| CNS 1 | 80 (80) | +8 | 16 (17) |
| TLP | 10 (10) | +21 | 8 (8.5) |
| CNS 3 | 10 (10) | 9q deletion | 12 (12.8) |
| **White blood cells count ($x10^9$/L)** | 39.5 (1-545) * | Complex Karyotype | 6 (6.4) |
| ≤100 | 78 (78) | **Genetic mutations (N, 70)** | |
| >100 | 22 (22) | FLT3\ITD mutation | 7/70 (10) |
| **Hb concentration (gm/dL)** | 8.0 (5-12) * | **Genetic Risk** | |
| < 7 | 39 (39) | Good Risk | 33 (33) |
| ≥ 7 | 61 (61) | Intermediate Risk | 46 (46) |

| | | | |
|---|---|---|---|
| **Platelet count (x10$^3$/mL)** | 33.0 (2-475) * | Poor Risk | 21 (21) |
| ≤ 10 | 10 (10) | **Response to induction 1** | |
| >10 | 90 (90) | CR | 71 (71) |
| **Bone marrow blast cells (%)** | 69 (15-97) * | Refractory | 8 (8) |
| **FAB classification** | | Not assessed (early death) | 21 (21) |
| M0 | 3 (3) | **MRD post induction 1 (N, 71)** | |
| M1 | 19 (19) | <0.1% | 14/71 (19.7) |
| M2 | 34 (34) | ≥ 0.01 | 16 /71 (22.5) |
| M4 | 30 (30) | Not done | 41/71 (57.7) |
| M5 | 11 (11) | **Risk stratification** | |
| M7 | 3 (3) | Standard | 48 (48) |
| **Cytogenetics (N, 100)** | | High | 31 (31) |
| Normal | 10 (10) | Not assessed (early death) | 21 (21) |
| Abnormal | 84 (84) | **HSCT** | 6 (6) |
| No mitosis | 6 (6) | **Outcome** | |
| **Modal chromosomal number (N=94)** | 46 (38-89)* | CR | 34 (34) |
| Diploid | 55 (58.5) | Refractory/Relapse | 28 (28) |
| Hypodiploid | 14 (14.9) | Treatment related mortality | 38 (38) |
| Hyperdiploid | 25 (26.6) | | |

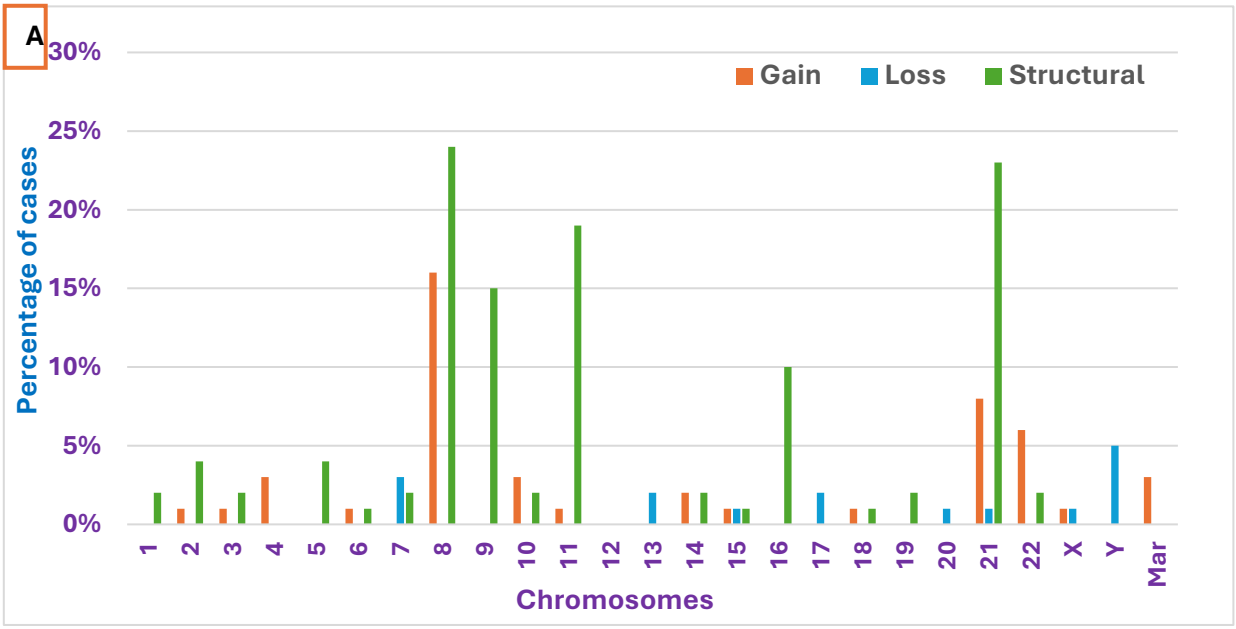
Note: Data are presented as number (percentage) unless otherwise indicated. * Shown as the median value with the range in between brackets. HB: haemoglobin, CR: complete remission, HSCT: Hematopoietic stem cell transplantation

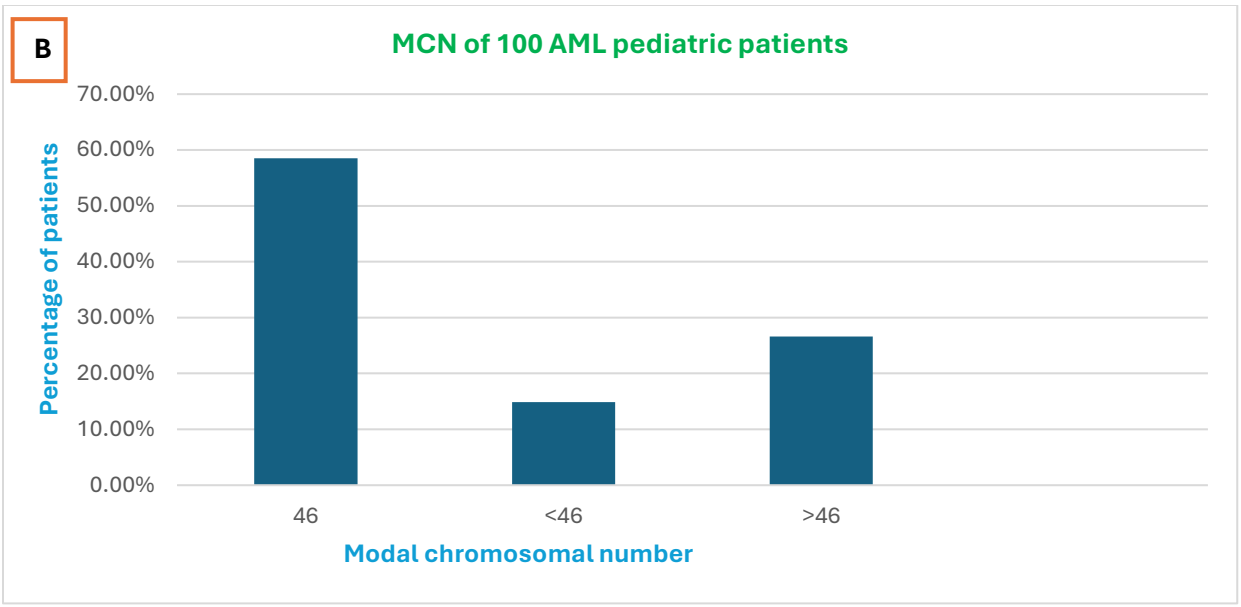
**TABLE 3 Results of association between study variables and OS and EFS in AML cases**

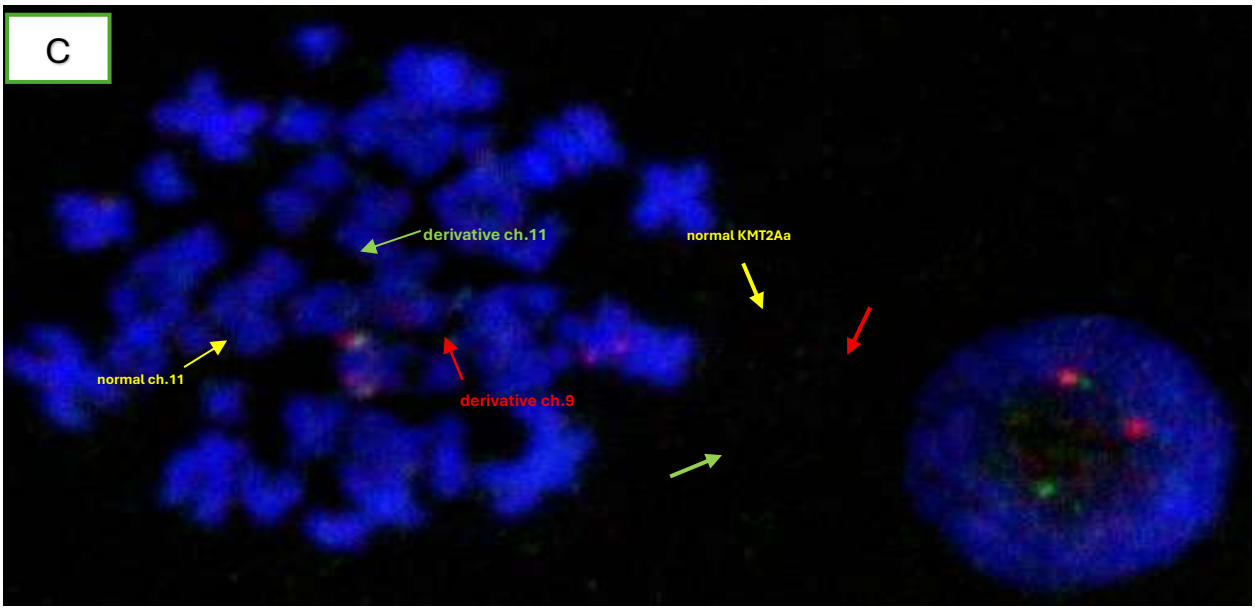
| **Variable** | | **Overall survival** | | **Event free survival** | |
|---|---|---|---|---|---|
| | | **Cum.OS at 1 year** | **P value** | **Cum.EFS at 1 year** | **P value** |
| **Age (years)** | <2 | 27.3 | 0.055 | 22.7 | 0.026 |
| | 2-10 | 61.5 | | 51.3 | |
| | >10 | 53.8 | | 48.7 | |
| **Gender** | Male | 54.8 | 0.65 | 46.8 | 0.561 |
| | Female | 44.7 | | 39.5 | |
| **WBC (x10$^9$/L)** | <100 | 53.8 | 0.031 | 47.4 | 0.021 |
| | >100 | 40.9 | | 31.8 | |
| **Genetic risk** | Good | 69.7 | 0.004 | 63.6 | 0.005 |
| | Intermediate | 45.7 | | 41.3 | |
| | Poor | 33.3 | | 19 | |
| **t(8:21)(q22;q22)** | Yes | 69.6 | 0.13 | 60.9 | 0.117 |
| | No | 45.5 | | 39 | |
| **Inv 16** | Yes | 70 | 0.081 | 70 | 0.058 |
| | No | 48.9 | | 41.1 | |
| **CBF** | t(8:21) | 69.6 | 0.032 | 60.9 | 0.020 |
| | Inv 16 | 70 | | 70 | |
| | No | 41.8 | | 34.3 | |
| **KMT2A aberrations** | Abnormal | 26.3 | 0.028 | 15.8 | 0.018 |
| | Normal | 56.8 | | 50.6 | |

| Variable | | Overall survival | | Event free survival | |
|---|---|---|---|---|---|
| | | Cum.OS at 1 year | P value | Cum.EFS at 1 year | P value |
| **KMT2A -r** | Yes | 23.1 | 0.089 | 15.4 | 0.049 |
| | No | 55.2 | | 48.3 | |
| **Ch 7 abn** | Yes | 20 | 0.064 | 0 | 0.108 |
| | No | 51.7 | | 44.9 | |
| **CNS status** | 1 | 55 | 0.069 | 50 | 0.107 |
| | TLP | 30 | | 20 | |
| | 3 | 30 | | 20 | |
| **Final risk** | SR | 81.2 | <0.001 | 72.9 | <0.001 |
| | HR | 38.7 | | 29 | |
| **Induction 1 response** | CR | 69 | <0.001 | 60.6 | <0.001 |
| | Refractory | 25 | | 12.5 | |
| | Early death | 0 | | 0 | |
| **BMT for HR** | Yes | 83.3 | 0.001 | 83.3 | 0.001 |
| | No | 28 | | 16 | |

**Figure 1: Cytogenetics results in pediatric cases with AML. (A); Distribution of chromosomal aberrations with gains, losses and structural abnormalities of each chromosome. (B); Distribution of Modal chromosomal number in all patients. (C); metaphase and interphase FISH of a patient with t(9;11) using KMT2A break apart probe, showing one fusion (yellow), one red and one green signals corresponding to one normal copy of KMT2A and the translocation of the second copy. (D); metaphase FISH of a case with t(10;11) using whole paint of chromosome 10 (Green) and chromosome 11 (red)**




B
MCN of 100 AML pediatric patients
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Percentage of patients
46
<46
>46
Modal chromosomal number


C
derivative ch.11
normal KMT2Aa
normal ch.11
derivative ch.9

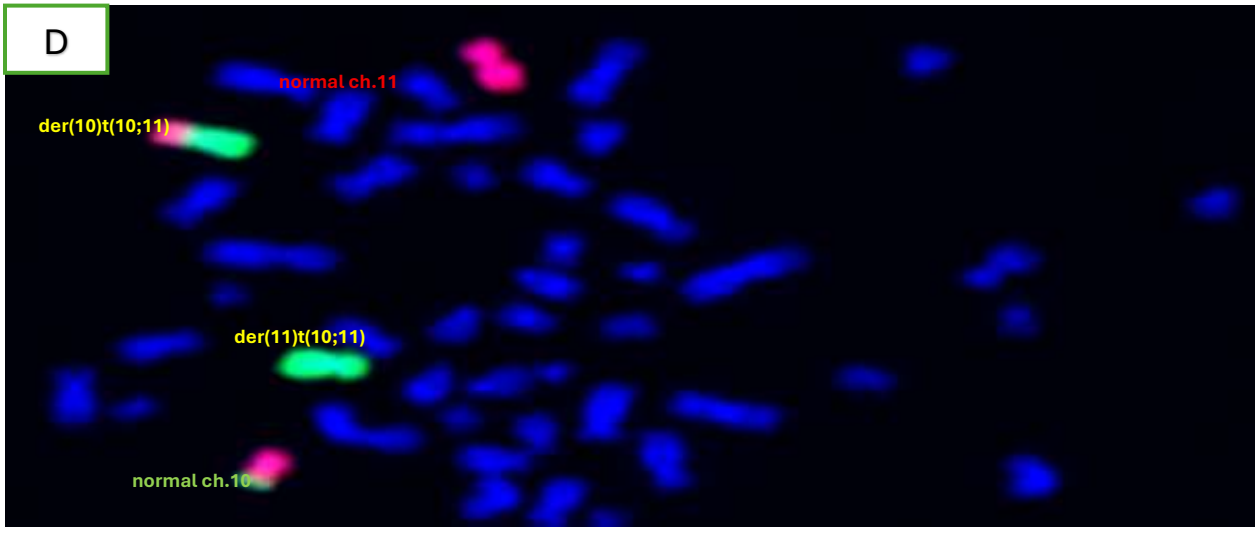

D
normal ch.11
der(10)t(10;11)
der(11)t(10;11)
normal ch.10

**Figure 2:** Final outcome of pediatric patients with AML

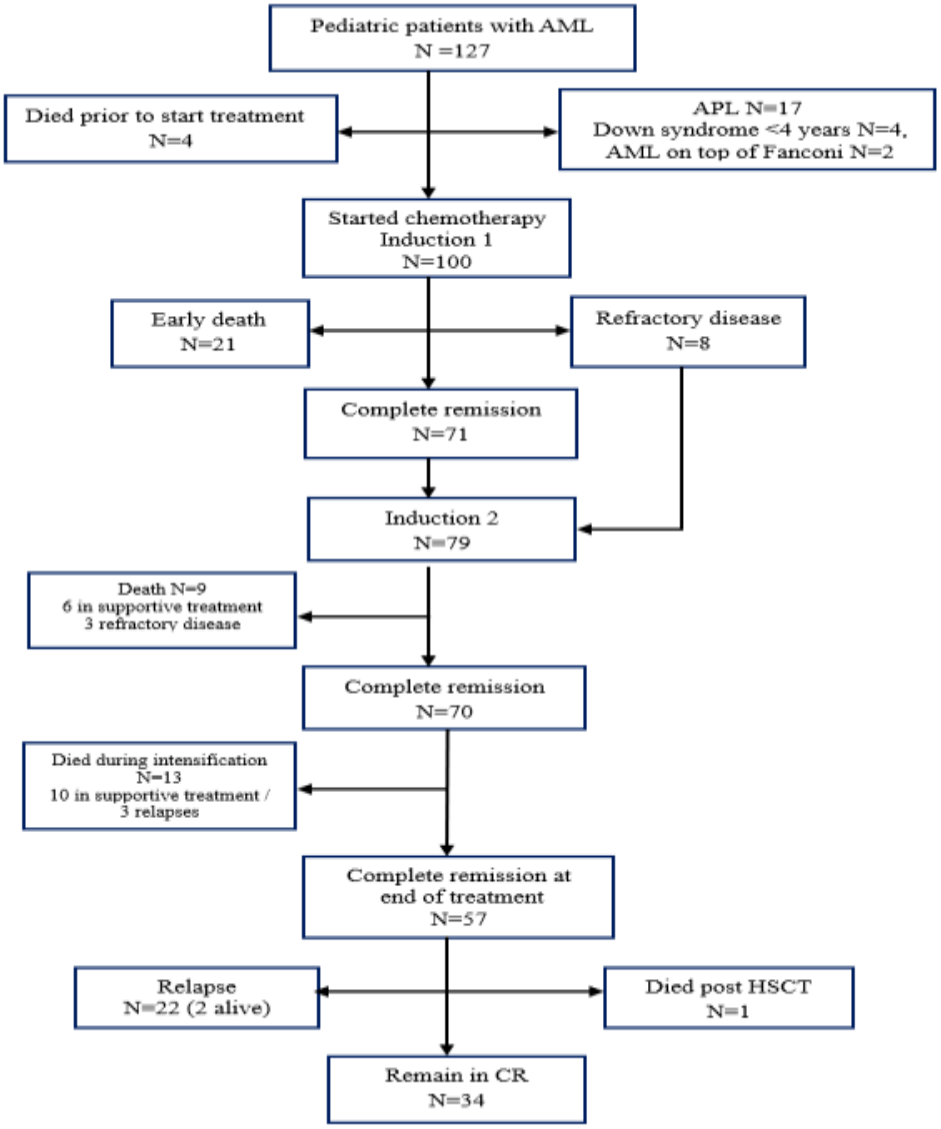
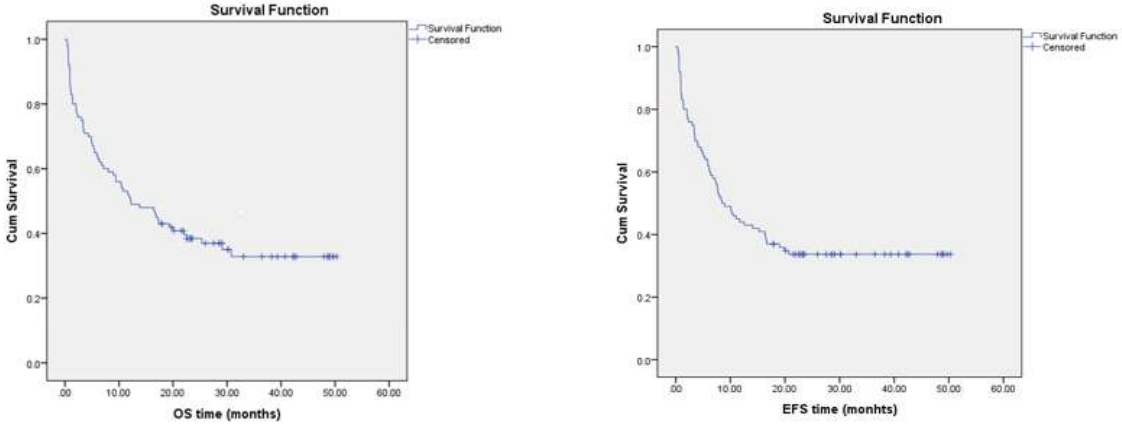
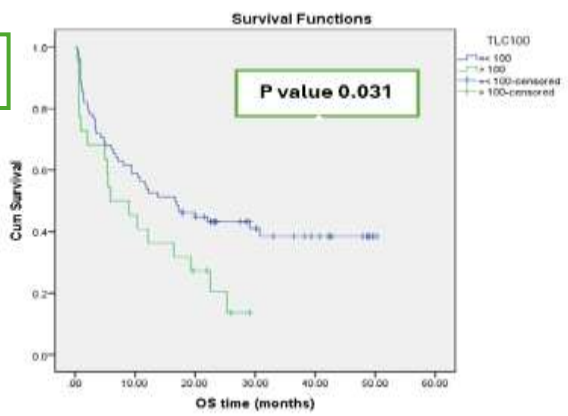


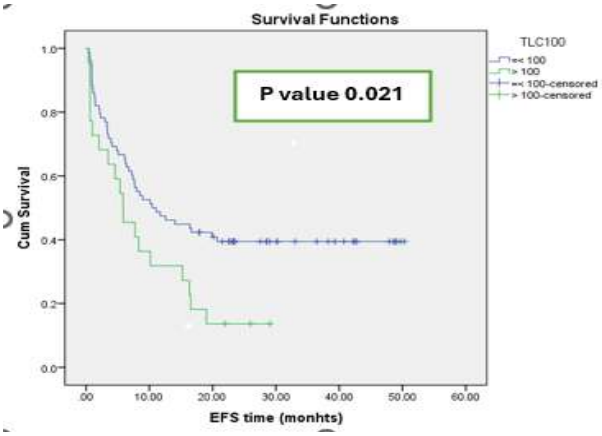

**Figure 3:** (A) Overall survival and Event free survival of whole group (B) OS and EFS according to TLC (C) OS and EFS according to genetic risk group (D) OS and EFS according to CBF leukemia (E) OS and EFS according to KMT2A abnormailties (F) OS and EFS according to final risk group

A

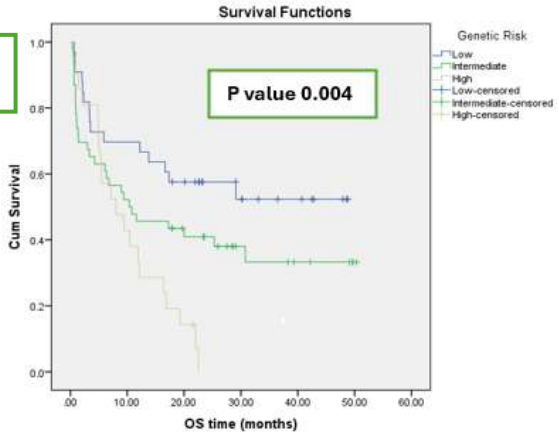


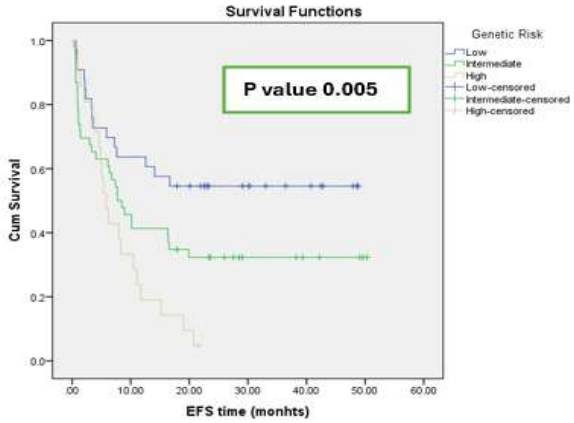
B


Survival Functions
TLC100
P value 0.031
Cum Survival
OS time (months)

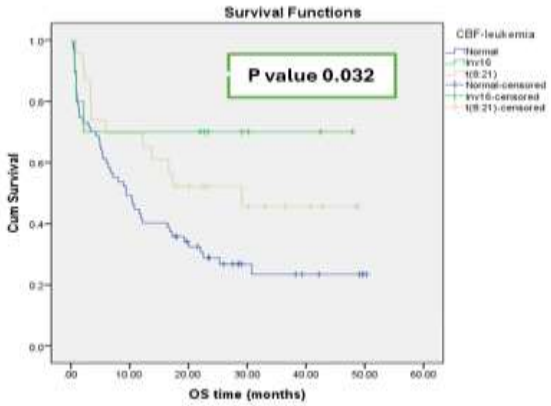

Survival Functions
TLC100
P value 0.021
Cum Survival
EFS time (monhts)

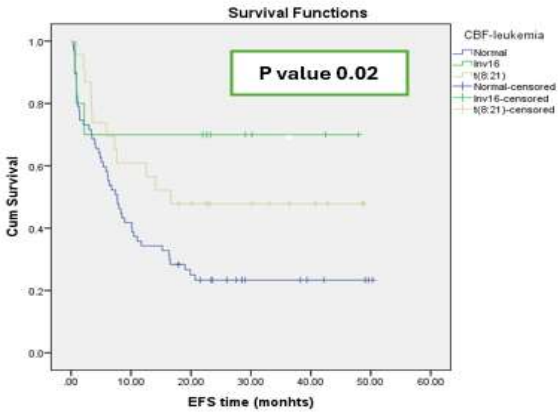
C


Survival Functions
Genetic Risk
P value 0.004
Cum Survival
OS time (months)

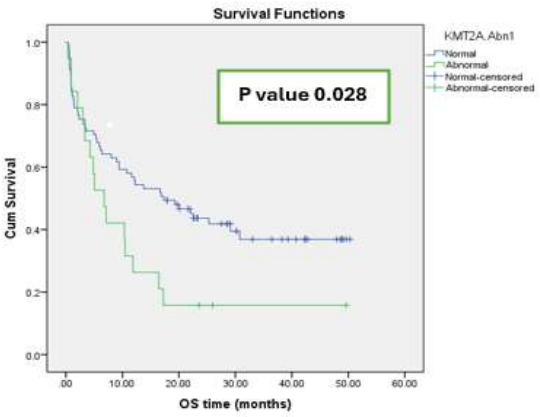

Survival Functions
Genetic Risk
P value 0.005
Cum Survival
EFS time (monhts)

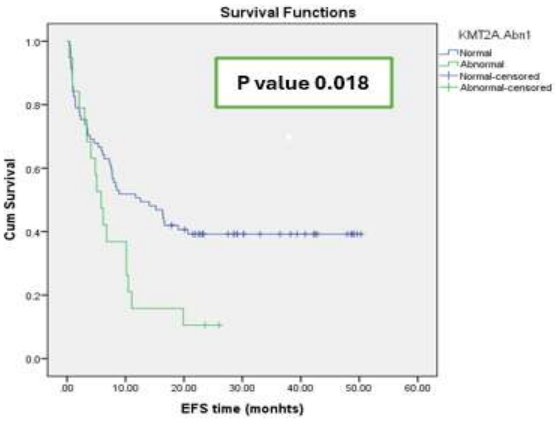
D


Survival Functions
CBF-leukemia
P value 0.032
Cum Survival
OS time (months)

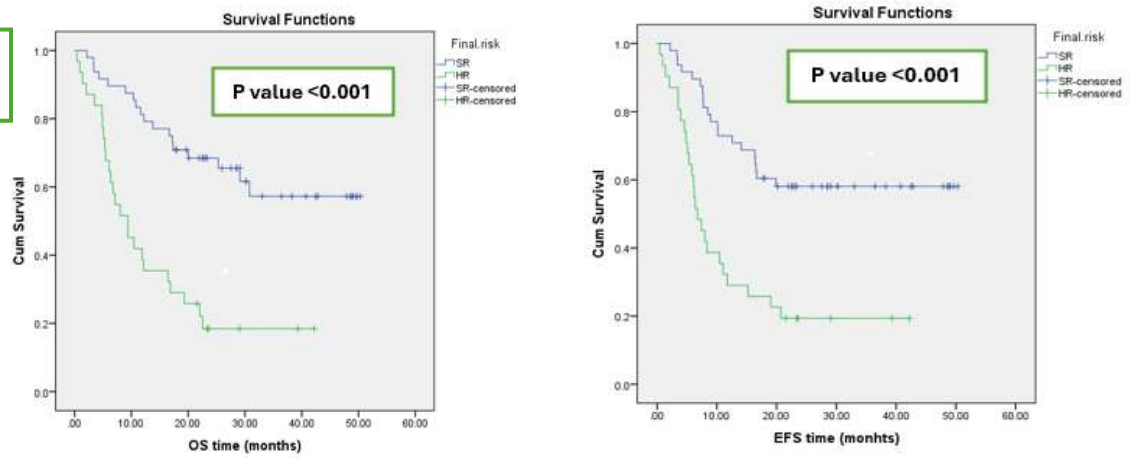

Survival Functions
CBF-leukemia
P value 0.02
Cum Survival
EFS time (monhts)

E


Survival Functions
KMT2A.Abn1
P value 0.028
Cum Survival
OS time (months)


Survival Functions
KMT2A.Abn1
P value 0.018
Cum Survival
EFS time (monhts)

F




**Supplementary tables:**

**Table S1: Cycles of chemotherapy:**

| Chemotherapy | Dose |
|---|---|
| **Induction course 1(MA):**<br>▪ Mitoxantrone<br>▪ Cytarabine | <br>12mg/m2 1 hour infusion days 1, 2, 3 ,4.<br>100mg/m2 1 hour infusion every 12 hours days 1-10 |
| **Induction course 2 (MA):**<br>**(For non-HR patients)**<br>▪ Mitoxantrone<br>▪ Cytarabine | <br><br>12mg/m2 1 hour infusion days 1, 2, 3.<br>100mg/m2 1 hour infusion every 12 hours days 1-8 |
| **Induction course 2 (FLA/mito)**<br>**(for HR patients):**<br>▪ Fludarabine<br>▪ Cytarabine<br>▪ Mitoxantrone | <br><br>30mg/m2 infusion over 30 minutes days 1-5<br>2g/m2 infusion over 4 hours on days 1-5<br>12 mg/m$^2$ 1 hour infusion days 3, 4, 5 |
| **Consolidation courses (HD Ara-C)**<br>**(for SR only):**<br>▪ Cytarabine | <br><br>3g/m2 infusion over 4 hours every 12 hours days 1, 3, 5 |
| **Consolidation courses (FLA±mito)**<br>**(for HR only):**<br>▪ Fludarabine<br>▪ Cytarabine<br>▪ Mitoxantrone | <br><br>30mg/m2 infusion over 30 minutes days 1-5<br>2g/m2 infusion over 4 hours on days 1-5<br>8-10 mg/m$^2$ 1 hour infusion days 3, 4, 5 |

**Table S2: Doses of triple intrathecal chemotherapy:**

| Age | Methotrexate | Cytarabine | Hydrocortisone |
|---|---|---|---|
| **<1year** | 5mg | 15mg | 5mg |
| **1-2** | 7.5mg | 20mg | 7.5mg |
| **2-3** | 10mg | 25mg | 10mg |
| **>3** | 12.5mg | 30mg | 12.5mg |