Minimal Residual Disease Assessment and Genetic Alterations in Childhood Acute Lymphoblastic Leukemia

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ABSTRACT

In pediatric acute lymphoblastic leukemia(ALL), we know that early treatment response and favorable genetic alterations are important in disease free survival and overall life expectancy. In this study we tried to find some associations regarding genetic alterations and flowcytometric feature, minimal residual disease (MRD), in survival of children with ALL. Overall, 92 ALL patients were detected at medical records. Sixty six of them completed induction treatment at our center. Initial leukocyte count, lymphoblast count on day 8, remission evaluation of bone marrow on days 15 and 33, karyotype and cytogenetic analysis were retrospectively evaluated. 83.2% of patients were B-ALL. Of 66 patients whose remission induction was completed, 40 were included in intermediate risk group. Numerical chromosomal abnormalities were detected in 20 patients; whereas structural chromosomal abnormality in 34. In patients with numeric and structural abnormality, 2 patients were dead in each group. Besides in 30 patients with no structural abnormality, 7 of them were dead at time of analysis. The 5-year event-free survival of 66 patients was 71.4% ($p< 0.001$) and overall survival was 87.5% ($p< 0.001$). Event-free and overall survival were significantly higher in patients with lower 15th and 33rd day MRD analysis ($p< 0.001$). There may be some discordance between MRD and genetic abnormalities in few cases; but better results can be obtained with MRD lower than 10^{-4} during induction. There are still factors that have been undetermined in prediction of prognosis in ALL. Targeted personalized treatments with detailed genetic and cellular analysis would be the future in leukemia.

Keywords: Acute lymphoblastic leukemia, Chromosomal abnormalities, Minimal residual disease, Survival

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children. In the last 30 years, disease-free survival and cure rates have improved markedly, by courtesy of advances in diagnosis, classification, and treatment. In many developed countries, 5-year life expectancy of childhood ALL cases is over 90%.[1] Genetic characterization plays an important role in diagnosis, classification, prognosis prediction and treatment decision.[2,3] Additionally, treatment response evaluation is performed by the help of minimal residual disease (MRD) analysis for achieving a strong prognostic factor in ALL.[4,5] In fact, better understanding the biology of the disease with increasing experience over years with genetic factors and cellular level analysis by flowcytometry will be an important aim to identify patients who receive unnecessary overtreatment or who develop relapse after inadequate treatment. By this way, this will reduce both morbidity and mortality in long-term follow-up. Here, we tried to evaluate genetic features, MRD analysis, risk groups and survival analysis in ALL patients diagnosed at our center and find an association between genetic alterations and MRD assessments in induction period.

PATIENT AND METHODS

In this study, data of patients younger than 18 year-old who were diagnosed with ALL between 2005 and 2021 in Department of Pediatric Hematology and Oncology at Baskent University were retrospectively analyzed. Age, gender, leukocyte count at first admission, steroid response regarding lymphoblast count by peripheral smear on day 8, remission evaluation and MRD analysis of bone marrow samples on day 15 and 33, karyotype analysis and cytogenetics of bone marrow samples at initial diagnosis, risk grouping, treatment regimen and time of last follow-up were reviewed for all patients. Patients 18 years old and older who were diagnosed with ALL and patients with leukemia other than ALL were excluded. In addition, those who did not complete their induction treatment at out center were also excluded from the study.

Majority of patients were treated with Berlin-Frankfurt-Munich (BFM) protocols (ALL-BFM 95, TRALL 2000, ALLIC-BFM 2002, ALLIC-BFM 2009). St. Jude total XIII protocol had been used in only 1 patient. In BFM protocols; cases aged between 1-5 years and leukocyte count < 20.000/mm3 are included in standard risk group (SRG). Cases under 1 year-old or > 6 years old are in intermediate risk group (IRG). Patients who have bone marrow flowcytometric MRD (FC-MRD) results after 15 days treatment between 0.1% and 10% are included in intermediate risk group. Those with lymphoblasts ≥ 1000/mm^3 on day 8 of treatment; or with FC-MRD ≥ 10% on day 15 and/or with ≥5% lymphoblasts in morphological examination in bone marrow samples on day 33 are all included in high risk group (HRG). Regardless of all these, patients with t(9;22), t(4;11) and hypodiploidy are included in high risk group.

St. Jude total XIII protocol is divided into standard and high risk groups. Patient aged 1-9 years with leukocyte count of < 50.000/mm^3 or DNA index of 1.16 and above is included in standard risk group. Central nervous system status 3 (5 or more leukocytes/microlitre with identifiable blast cells in an atraumatic sample or the presence of cranial nerve palsy), presence of testicular involvement, T-cell ALL, t(9;22), t(4;11), t(1;19) and associated B-cell ALL, presence of mixt lineage leukemia (MLL) gene rearrangement or near-haploidy and/or if there is 5% or more lymphoblasts in the bone marrow on the 15th day of remission induction, the case is included in high risk group.

For MRD analysis of the patients, bone marrow samples were taken into tubes containing ethylene diamine tetraacetic acid (EDTA) and delivered to the Hematology laboratory within 1 hour. After the samples were filtered, leukocyte counts were determined in a blood count device (CellDyneRuby, Abbott, IL, USA). If the leukocyte count was more than 10.000/mm^3, the samples were diluted with physiological saline. Then, 5 microliters of antibody and 100 microliters of bone marrow samples from each monoclonal antibody were added to the tubes and incubated for 15 minutes in a dark room at room temperature. At the end of the period, 1 ml of VersaLyse solution was added to the tubes to lyse the erythrocytes. For lysis, incubation was performed for 10 minutes in the dark. After those readings were performed on the device. For MRD determination, cocktails consisting of 9 colors were prepared in all analysis. Monoclonal antibody and erythrocyte lysis solutions were obtained from Beckman Coulter (Marseille, France). Analysis were performed using monoclonal antibodies consisting of CD45 KRO, CD34 PC7, CD19 PC5.5, CD10 PE, CD20 PB, CD38 A750, CD81 FITC, CD22 A700 and sIgM APC for B-ALL and CD45 KRO, CD3 PB, CD2 PC5.5, CD5 A750, CD1a APC, CD34 PC7, CD7 A700, CD4 FITC and CD8 PE for T-ALL.[6-8] Flow cytometric analysis were performed using a three-laser Navios device (3L10C; Beckman Coulter, Lismeehan, Ireland) and Kaluza v2.1 software. At least 1 million cells were read for each analysis. Debris was excluded before analysis. In patients who were first diagnosed at our center, LAIP (leukemia-associated immunophenotype) based MRD analysis were performed and for patients who were diagnosed at another center, the LAIP based DfN (different from normal) approach was used.

Twenty metaphase plaques were obtained for each patient, and karyotypes were reported according to the International System of Human Cytogenetic Nomenclature (ISCN) 2020. Numerical chromosomal aberrations were classified as follows; high hyperdiploidy (50 or more chromosomes), hyper-

Table 1. Demographic characteristics of patients who completed all treatment and 5-year follow-up

	B-ALL (n= 53)	**T-ALL** (n= 13)
Age (min-max) (med/month)	13 - 205 (77.5)	58 - 189 (122)
Gender	F: 19 / M: 34	F: 2 /M: 11
Leukocyte count at first admission (med/mm^3)	1600 - 218000 (20768)	2040- 465000 (127609)
Lymphoblast count < 1000/mm^3 in peripheral smear on day 8	50	11
15th day MRD 10-3 and below	37	8
15th day MRD 10-4 and below	17	2
33th day MRD 10-3 and below	51	11
33th day MRD 10-4 and below	31	7
SRG	16	-
IRG	31	9
HRG	6	4
EFS	70.4%	75%
OS	90%	75%

MRD= minimal residual disease, SRG= standard risk group, IRG= intermediate risk group, HRG= high risk group, EFS= event free survival, OS= overall survival

diploidy (47-49 chromosomes), high hypodiploidy (40-43 chromosomes), low hypodiploidy (32-39 chromosomes), near haploid (24-31 chromosomes). The presence of 3 or more chromosomal abnormalities that could not be included in these groups was defined as complex karyotype. Structural chromosomal anomalies were grouped as t(9;22), t(4;11), t(1;19), t(8;14) and other anomalies that did not cause translocation but caused various point changes.

This study was approved by the ethics committee of Başkent University (Project no: KA21/456, Date: 23.11.2021).

Statistical Analysis

Statistical analysis were carried out using the SPSS 22.0. Categorical measurements were summarized as frequency and percentages, and continuous measurements as mean, minimum-maximum, and median. Kaplan-Meier method was used for survival curves and Long-rank test was used to calculate survival differences between demographic groups. Statistical significance level was taken as 0.05 in all tests.

RESULTS

A total of 92 patients were diagnosed with ALL. 57.6% of them were male. Mean age at diagnosis 4.5 years (0.5-17 years) for B-ALL and 10.1 years (4.8-15 years) for T-ALL. Mean total leukocyte count at first diagnosis was 29.214/mm^3 of patients with B-ALL (min: 1590/mm^3 - max: 276,000/mm^3) and it was 76.800/mm^3 in T-ALL patients (min: 2040/mm^3 - max: 465.000/mm^3). According to flowcytometric analysis, 83.2% patients were classified as B-cell ALL and 16.8% as T-cell ALL.

Twenty six of 92 patients were lost to follow up. Remission induction could be completed in 66 patients. Five patients were considered as steroid resistant according to the peripheral smear on day 8 and 3 of them were B-ALL. There were 7 patients with ≥ 10% FCMRD of bone marrow on day 15 and only 1 patient with ≥ 5% on day 33. Most patients were classified in IRG (Table 1).

The karyotype analysis at first admission were successful for 60 patients. For other patients, adequate metaphase plaques could not be obtained or the samples were clotted. In successful cultures, normal karyotype was detected in 40 patients (43.5%) (34 B-ALL vs 6 T-ALL). Numerical karyotype anomalies were detected in 20 patients (18.4%) (17 B-ALL vs 3 T-ALL). Table 2 shows the details of

Table 2. Numerical chromosomal abnormalities

1	B ALL	47,XY,+der(21)(18)/46XY(2)	Sole abnormality	A
2	B ALL	53-56,XXY,+4,+6,+10,+13,+14,+15,+17,+18,+19,+20,+21,+mar[cp3]/46,XY[17]	Hyperdiploidy	A
3	B ALL	45-48,XY,-1[3],+8[3], t(9;22)(q34,q11.2)[2],+21[3],+mar[2][cp10]	Komplex karyotype	A
4	B ALL	35,XX,-2,-3,-7,-9,der(10),-12,-13,-15,-16,-17,-20[10]/70,XXXX, +1,+1,+der(3),+5,+5+6,der(7),+8,+der(10),+11,+11,-12,+13,+14,+15,+16,+17, +18,+19,+19,+21,+21,+22,+22,+mar[3]/46,XX[7]	Hypodiploidy/ Hyperdiploidy	A
5	B ALL	54-55,XXX,+4,-7,+9,+14,der(17q),+21,+21,+4mar[cp8]/46,XX[2]	Hyperdiploidy	A
6	B ALL	62-63,XXX,der(1)[5],+der(2),+4,+der(5),+6,+8,+10,+11,+12,+14,+15,+17,+18,+18, +19,+20,+21,+21,+22,+mar1[7],+mar2[3]mc[cp20]	Hyperdiploidy	A
7	B ALL	46-54,XX,+X,+8,+9,+10,-17,+21[cp5]/46,XX[1]	Hypodiploidy/Hyperdiploidy	A
8	T ALL	62<3n>,XY,+Y,+3,+4,+4,+6,+8,+10,+10,+11X4,+12X2,+13X2,+15X2,+17,-19,-21 (near triploidic clone)	Hyperdiploidy	A
9	B ALL	55<2n>,XX,+X,+der(2),+4,+8,+10,+14,+17,+18,+20,+21 (Near triploidic clone)	Hyperdiploidy	A
10	B ALL	45,XY,der(1)del(1q),+2,-5,-12,der(12)[1]/46,XY[19]	Komplex karyotype	A
11	B ALL	45-46,XX,der(2),+der(4),-22[18]/46XX[2]	Komplex karyotype	A
12	B ALL	46,XY,t(12;21)(p13;q22),t(15;19)(q11;q11)c	Sole abnormality in addition to the constitutional translocation	A
13	T ALL	46,XY,der(1)del?(1p),del(9)(p24),inv(14)[6]/46XY,del(9)(p24)[14]	Komplex karyotype	A
14	B ALL	46,XY,del(3p),der(13)t(1;13)	Komplex karyotype	A
15	B ALL	47,XY,+4,+10,+11,+17(cp3)/46,XY(17)	Hyperdiploidy	A
16	B ALL	46-47,XX, trc(11;17;19)	Sole abnormality	A
17	T ALL	47,XY,+mar	Sole abnormality	E
18	B ALL	56,XXX,+4,+del(6q),+8,+9,+10,+15,+17,+18,+21	Hyperdiploidy	E
19	B ALL	55,XY,der(1),+4,+6,+8,+9,+10,der(11),+14,+17,+18,+19,+21,+22[cp4]/46,XY[16]	Hyperdiploidy	A
20	B ALL	58,XXX, +4,+6,-7,+8,+10,+13,+14,+17,+18,+20,+21	Hyperdiploidy	A

A= Alive, E= Exitus

the numerical chromosomal anomalies. The most frequently detected numerical abnormality in patients with B-ALL was hyperdiploidy (n= 8). Most patients with hyperdiploidy were in IRG. Two patients died in this group of numeric abnormalities, one of which was a patient with B-ALL and hyperdiploidy.

Structural abnormalities with fluorescence in situ hybridization (FISH) analysis were detected in 45 patients (48.9%). The most frequently detected translocation was t(12;21) (n= 10, 15.2%), followed by t(9;22) (n= 3, 3.3%) (Table 3) and there were 23 patients with chromosomal structural problems at different points that did not cause translocations. If we look at the risk groups, t(12;21) was most frequently detected in IRG, while 1 patient with t(12;21) was included in HRG because of not achieving remission on days 8 and 15. Two cases were dead with structural abnormality other than known prognostic translocations.

Of 20 patients with numerical chromosomal anomalies, t(12;21) and t(9;22) were detected in 3 patients; various structural anomalies were detected in 14 of the rest. FISH analysis of one patient was normal, and no available results could be obtained in 2 patients. The major features and outcome of the patients with numerical and structural chromosomal abnormalities had been shown in Tables 2 and 3.

The 5-year event-free survival (EFS) was 71.4% and overall survival (OS) was 87.5% for 66 patients with long term follow-up at our center. In B-ALL patients (n= 53), EFS was 70.4% and OS was 90% ($p< 0.001$), while EFS and OS were 75% ($p< 0.001$) in T-ALL patients (n= 13). According to risk groups; the 5-year EFS was 87.4% in SRG (n= 16), 65% in MRG (n= 40), and 66.7% in HRG (n= 10) ($p< 0.001$). In case of overall survival; they were 94.1% for SRG, 91.2% for MRG, and 66.7% for HRG in 5 years ($p< 0.001$).

Table 3. Structural chromosomal abnormalities

	Total	Remission	Relapse	Exitus
Normal karyotype	30	23	8	7
t(12;21)	10	10	3	0
t(9;22)	3	3	0	0
Other translocations	2	1	0	1
Other abnormalities	19	18	1	1
NA	2	2	1	0

In MRD assessment on day 15, EFS was 77.8% (p< 0.001) and OS was 84.9% (p< 0.001) in patients with a value of $1x10^{-3}$ and below (n= 45), and EFS was 58.4% and OS was 62.6% in patients with a value above $1x10^{-3}$ (n= 17). In MRD assessment on day 33, five-year EFS and OS were 76.3% (p< 0.001) and 93.5% (p< 0.001) respectively in patients with a MRD value of $1X10^{-3}$ and below (n= 62). In 4 patients, the prognosis was poor with a MRD value above $1x10^{-3}$ (Table-1).

The 5-year EFS was 61.8% in 30 patients without any cytogenetic abnormality at initial diagnosis, and 53.3% in 10 patients with the most frequent structural abnormality of t(12;21) (p< 0.001).

DISCUSSION

In BFM-based protocols, risk classification is based on age, initial leukocyte count, prednisone response on day 8, treatment response evaluations on day 15 and day 33 in bone marrow samples. Independent of these, presence of chromosomal specific abnormalities studied from bone marrow samples at initial diagnosis is also an important prognostic factor. According to the previous data, presence of t(9;22), t(4;11) or hypodiplody (chromosome count ≤ 44) at first diagnosis are features of high risk disease regardless of any other criteria and these patients would be treated with more intensive chemotherapy regimens. The prognosis of these patients is worse than those with other chromosomal abnormalities and some of these patients may even go as far as allogeneic stem cell transplantation.[9,10] Additionally, although not included in the risk grouping features, hyperdiploidy is a numerical chromosomal abnormality known to have positive affect on prognosis independent of other criteria.[11] In the current study majority of the cases were B-ALL and most of them were at IRG. Cases with hyperdiploidy had a favorable outcome 90%. Only 15% of the cases were considered in HRG according to the above mentioned criteria which is comparable with 21% of BFM group results.[12] In five patients of HRG group, 3 of them had t(9;22) and they had no numeric abnormalities. In two patients in HRG group there were sole abnormalities as numeric abnormalities, and an additional unknown structural abnormality in one of them (47,XY,+der(21)(18)/46XY(2) vs 47,XY,+mar). Except t(9;22) with a therapeutic targeted treatment option, it is not clear that the mentioned abnormalities did have any contribution to these high risk patients with unfavorable treatment response.

An important point is that many groups that follow up and treat childhood leukemia pay attention to MRD-based remission assessment.[4,8] However, in developing countries, there may be difficulties in accessing MRD analysis even on day 15. Additionally, it should not be forgotten that MRD evaluation is a method that depends on the person and requires experience.[13] In our data, after starting MRD analysis, we determined that we had reached more accurate treatment response of the bone marrow by morphology and flow cytometry on day 15 and 33 of induction treatment resulting in a more predictable life expectancy similar with the literature.[14-16] However, we also encountered some special situations. For example, in two patients with T-ALL with lymphoblast percentage below 5% by morphology on day 33 and MRD value above $1x10^{-3}$, remission could not be achieved in peripheral smear on day 8 and in MRD analysis on day 15. The third patient, who did not achieve remission on day 33 was diagnosed with B-ALL,

and initial treatment response evaluations on days 8 and 15 were in normal range. In another case with B-ALL, who had poor response to treatment by day 8 and 15; died on the 26th day of induction after diagnosis due to multiorgan failure and could not be evaluated on day 33. The normal bone marrow cytogenetic analysis of these 2 patients with a diagnosis of B-ALL had been associated with a poor response to chemotherapeutics and a worse prognosis compared to patients with unfavorable genetic alterations and this would be explained with other prognostic factors some of which are still unknown in childhood leukemia.

Relapse was observed in 8 of the 45 patients whose MRD analysis had values of 0.1% and below on day 15. Among these patients who developed relapse disease, 7 had isolated medullary relapse and B-ALL, only one was T-ALL patient with isolated central nervous system relapse. The numerical chromosomal anomaly was not detected in any of these relapsed patients at their initial diagnosis. A structural chromosomal anomaly was detected in only 2 patients. One of them was a patient who also had a positive diepoxybutane (DEB) test and was diagnosed with Nijmegen Breakage syndrome. The other was a patient with B-ALL who didn't have hyperdiploidy but had structural anomalies at various points. Values of 0.01% and below which are considered true negative MRD values[7,8], were detected in 19 patients, but despite all relapse developed in 3 of them.

A total of 62 patients had MRD analysis of 0.1% or less on day 33, and 38 of them had MRD results of 0.01% or less which can be considered as an adequate response. However, among these 13 patients developed relapse and 8 of them had a true negative MRD value. Approximately 20% of our cases with true negative values of MRD experienced relapse. Although it may be seen as an important feature to obtain a MRD result near zero and/or true negative value during induction treatment; disease relapse can occur independent of this evaluation. In such cases, presence of probable unidentified genetic disorders might be associated with poor prognosis and these patients should be examined to explain the cause of relapse. Under our circumstances, we were unable to detect any known chromosomal abnormality that could explain this in our patients both at first diagnosis and at the time of relapse. By this study, we consider that other variables should be considered in estimation of relapse. It may be wise to start MRD evaluation on day 78 of treatment and include it in study protocols of childhood leukemia. There are some clinical trials supporting this idea in the literature.[7,17]

Our study have some limitations with retrospective design. Our sample size was small due to the lost of follow-up who were diagnosed at our center and could not complete induction treatment because of socioeconomic problems. Therefore, the relative number of patients completing the study is an important limitation. Additionally, karyotype analysis from bone marrow samples could not be performed in all patients due to insufficient metaphase plaque or clotted samples.

In conclusion, although good points have been reached in terms of life expectancy over the years in childhood acute lymphoblastic leukemia, it is thought that there are factors other than MRD analysis and cytogenetic and molecular profiling of the cases that we have not yet identified or defined in prognosis and relapse estimations. In addition to the MRD analysis on days 15 and 33, it may be useful to perform an MRD analysis on day 78. Additionally, we observed in our daily practice, different responses can be obtained with variable sensitivity and side effects. Besides determination of molecular and cytogenetic features of malignant cells, studies exploring pharmacogenomic and host immunological properties might be examined in future research.

REFERENCES

1. Pui CH, Yang JJ, Hunger SP, et al. Childhood acute lymphoblastic leukemia: progress through collaboration. J Clin Oncol 33: 2938-2948, 2015.
2. Ceppi F, Cazzaniga G, Colombini A, et al. Risk factors for relapse in childhood acute lymphoblastic leukemia: prediction and prevention. Expert Rev Hematol 8: 57-70, 2015.
3. Taylor J, Xiao W, Abdel-Wahab O. Diagnosis and classification of hematologic malignancies on the basis of genetics. Blood 130: 410-423, 2017.
4. O'Connor D, Enshaei A, Bartram J, et al. Genotype-specific minimal residual disease interpretation improves stratification in pediatric acute lymphoblastic leukemia. J Clin Oncol 36: 34-43, 2017.

5. Bartram J, Wade R, Vora A, et al. Excellent outcome of minimal residual disease-defined low-risk patients is sustained with more than 10 years follow-up: Results of UK paediatric acute lymphoblastic leukemia trials 1997-2003. Arch Dis Child 101: 449-454, 2016.

6. Wood BL. Principles of minimal residual disease detection for hematopoietic neoplasms by flowcytometry. Cytometry B Clin Cytom 90: 47-53, 2016.

7. Theunissen P, Mejstrikova E, Sedek L, et al. Standardized flowcytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. Blood 129: 347-357, 2017.

8. Modvig S, Hallböök H, Madsen HO, et al. Value of flowcytometry for MRD-based relapse prediction in B-cell precursor ALL in a multicenter setting. Leukemia 35: 1894-1906, 2021.

9. Safavi S, Paulsson K. Near-haploid and low-hypodiploid acute lymphoblastic leukemia: two distinct subtypes with consistently poor prognosis. Blood 129: 420-23, 2017.

10. Carroll AJ, Shago M, Mikhail FM, et al. Masked hypodiploidy: hypodiploid acute lymphoblastic leukemia (ALL) mimicking hyperdiploid ALL in children: A report from the Children's Oncology Group. Cancer Genet 238: 62-68, 2019.

11. Enshaei A, Vora A, Harrison C, et al. Defining low-risk high hyperdiploidy in patients with paediatric acute lymphoblastic leukemia: a retrospective analysis of data from the UKALL97/99 and UKALL2003 clinical trials. Lancet Haematol 8: e828-e839, 2021.

12. Radu LE, Colita A, Pasca S, et al. Day 15 and day 33 minimal residual disease assessment for acute lymphoblastic leukemia patients treated according to the BFM ALLIC 2009 protocol: single-center experience of 133 patients. Front Oncol 10: 923, 2020.

13. Lhermitte L, Barreau S, Morf D, et al. Automated identification of leukocyte subsets improves standardization of data base-guided expert-supervised diagnostic orientation in acute leukemia: a Euro Flow study. Mod Pathol 34: 59-69, 2021.

14. Schwab CJ, Chilton L, Morrison H, et al. Genes commonly deleted in childhood B-cell precursor acute lymphoblastic leukemia: association with cytogenetics and clinical features. Haematologica 98: 1081-1088, 2013.

15. Campana D. Minimal residual disease monitoring in childhood acute lymphoblastic leukemia. Curr Opin Hematol 19: 313-318, 2012.

16. Popov A, Henze G, Roumiantseva J, et al. A simple algorithm with one flow cytometric MRD measurement identifies more than 40% of children with ALL who can be cured with low-intensity therapy. The ALL-MB 2008 trial results. Leukemia 36: 1382-1385, 2022.

17. Petit A, Trinquand A, Chevret S, et al. Oncogenetic mutations combined with MRD improved outcome prediction in pediatric T-cell acute lymphoblastic leukemia. Blood 131: 289-300, 2018.

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