European Review for Medical and Pharmacological Sciences 2018; 22: 6020-6029

The therapeutic efficacy of pediatric ALL patients with MLL gene rearrangement treated with CCLG-ALL2008 protocol

Y.-N. SUN, Y.-X. HU, L. GAO, P.-F. XIAO, J. LU, S.-Y. WU, M. WANG, X.-J. SHAO, C.-Y. ZHOU, J. LING, J.-Q. LI, J. PAN, J. GAO, S.-Y. HU

Department of Hematology, Children's Hospital of Soochow University, Suzhou, Jiangsu, China

Abstract. – OBJECTIVE: In this study, we retrospectively evaluated the therapeutic efficacy of China Children Leukemia Group-ALL2008 (CCLG-ALL 2008) protocol in pediatric patients with mixed-lineage leukaemia (MLL) gene rearrangement of acute lymphoblastic leukemia (ALL) to identify the prognostic factors.

PATIENTS AND METHODS: Six hundred and thirty-four patients with ALL were enrolled in this study between June 2008 and Dec 2014. High-risk group (HR) consisted of 217 cases, of which 28 cases were MLL related positive (first group), 22 cases were BCR/ABL positive (second group), and 167 cases were negative with MLL related or BCR/ABL (third group). The therapeutic efficacy was evaluated at the time points of day 8 (TP1), day 15 (TP2), day 33 (TP3) and 12th week (TP4) with the protocol, respectively. Overall-survival (OS) and relapse-free-survival (RFS) and treatment-related mortality (TRD) were analyzed as well.

RESULTS: The first group accounted for 4.4% of all patients. Compared with the second and third group, the first group had more cases younger than 2 years, with initial leukocytes ≥50×109/L, and poor response on TP2. Moreover, patients older than 2 years old had a good 5 years OS (84% ± 9% *vs.* 37% ± 20%, $p<0.05$) and RFS (84% ± 9% *vs.* 29% ± 17%, $p<0.05$). There were no significant differences in the recurrence rate, TRD, 5 years OS and RFS among three groups. For the first group, compared with good response to prednisone, patients with poor response to prednisone had a poor 5 years RFS (41% ± 17% *vs.* 81% ± 10%, $p<0.05$). Multivariate Cox regression analysis identified that RFS and OS were influenced by such factors as age, MLL fusion partners, and prednisone response ($p<0.05$).

CONCLUSIONS: Such factors as younger age than 2 years old, MLL/AF4 fusion gene, poor response to prednisone, or no complete remission (CR) on TP3 were poor prognostic parameters in predicting the outcome in childhood ALL with MLL gene rearrangement treated with CCLG-ALL 2008 protocol.

Key Words

Acute lymphoblastic leukemia, Mixed lineage leukemia gene, Prognostic factors, Treatment.

Introduction

Mixed Lineage Leukemia (MLL) gene is a histone methyltransferase that plays a role in the epigenetic regulation of transcription, and the functional version of this protein is critical to embryonic development and hematopoietic formation[1]. The MLL locus, which maps to 11q23, has been shown by conventional and molecular cytogenetic analysis to be involved in rearrangements with up to 100 genetic loci, through chromosomal translocations, internal gene duplications, chromosome 11q deletions or inversions, and MLL gene insertions into other chromosomes or *vice versa*[2]. MLL rearrangements are found in>70% of infant leukaemias, whether the immunophenotype is more consistent with ALL or AML, but are less frequent in leukemias from older children, MLL translocations are also found in therapy-related leukemias (t-leukemias) that develop in patients previously treated with topoisomerase II inhibitors for other malignancies[3]. The presence of distinct MLL rearrangements is a dismal independent prognostic factor, while very few MLL rearrangements display either a good or intermediate outcome[4]. Infants with MLL-r ALL bear dismal outcome with published event-free survival (EFS) rate of no more than 40 % even when treated with intensive chemotherapy with or without hematopoietic stem cell transplantation (HSCT). In contrast, MLL-r in children 1 year or older is rare, and their outcome and optimal treat-

Corresponding Author: Shao-Yan Hu, MD; e-mail: hu_shaoyan@sina.com

ment options remain controversial [5]. Since 2008, China Children Leukemia Group-ALL 2008 (CCLG-ALL 2008) protocol has been designed and carried out in China for more than 7 years[6]. However, no reports yet so far assess its efficacy on pediatric ALL patients with MLL rearrangement. In this study, we analyzed a series of patients enrolled in CCLG-ALL 2008 protocol to compare the outcome between patients with positive and negative MLL rearrangement in order to assess clinical features including response to treatment, overall survival (OS), and relapse-free survival (RFS). In the current work, we sought to identify additional prognostic factors that may be helpful in selecting subsets of patients who would benefit most from complementary therapy.

Patients and Methods

Patients

During March 2008 to December 2014, 634 patients were enrolled in this protocol, of which 217 cases were categorized in high-risk (HR) group according to risk evaluation system of CCLG-ALL2008 protocol as indicated elsewhere. Briefly, the criteria of HR includes BCR/ABL+ led by t(9;22), MLL fusion gene, the count of peripheral blasts^3 1000/μl after 7 days' treatment of prednisone (no response to steroid), the bone marrow graded as M1(blasts<5%) after induction treatment for 15 days, non-remission (blasts>5%) in the bone marrow after 33 days of induction treatment, and MRD^3 10^{-2} at day 33 or MRD^3 10^{-3} by 12 weeks after treatment. Of these 634 evaluated patients, 28 (4.4%) with positive MLL rearrangement were treated with CCLG-ALL2008 protocol for high-risk (HR) group, including 3 infants (42.9%, 3/7), 15 male and 13 female cases, with a median age of 78 months (6 to 177 months), The detection of MLL rearrangements was mainly based on reverse transcription polymerase chain reaction RT-PCR or MLL split-signal fluorescence in situ hybridization analysis (FISH)[7]. 27 cases were detected by RT-PCR, 1 case was only detected by FISH, the forms were shown in Table I All the patients were followed up to June 2015, with a median follow-up time of 21 months (from 7 years and 2 months to 6 months). The clinical characteristics, including age, gender, initial leukocyte count, the leukemia cell lineage, the partner of MLL in the fusion gene, were analyzed and the clinical outcomes treated by CCLG-2008 protocol between patients with positive MLL and negative were compared. The therapeutic efficacy was estimated at the time points of day 8, day 15, day 33 and 12th week after treatment, respectively. Relapse rate, treatment-related mortality (TRD) and 5 years OS and RFS were also calculated within groups of MLL positive and negative patients. The study was approved by the Institutional Ethics Committee. Written informed consent was obtained from the parents or guardians of the patients or from the patients themselves, depending on the age and conceptual ability of the patients.

Treatment

All the patients of HR group received CCLG-ALL 2008 protocol for HR group, including 7-day prednisone induction followed by subsequent five phases: remission induction (VDLD), early reinforcement (CAM), consolidation therapy, delayed reinforcement (DIa & DIb) and maintenance treatment. Female: 2 years, male: 2.5 years, intrathecals 23 times[8].

Statistical Analysis

One-way ANOVA including post-hoc Tukey multiple comparison tests were used to study the difference. Difference between subgroups were compared and tested with Fisher's Exact Test for ordinate variables. $p<0.05$ was considered as of statistical significance. 5 years OS and RFS was estimated with Life table method. Survival curves were obtained using the Kaplan-Meier method and differences in survival were tested using the Log-Rank test. $p<0.05$ was regarded as indicate statistical significance. Duration of OS was defined as time from diagnosis until death from any cause; patients remaining alive were censored at the date of last contact. RFS was calculated based on the date of diagnosis to occurrence of relapse after achieving a complete remission (CR). Multivariate Cox regression modeling was done for OS and RFS using a forward-selection stepwise modeling process. Statistical analysis was done using SPSS 21.0 version (SPSS Inc., Chicago, IL, USA).

Results

Clinical Characteristics of MLL+ Group

The general clinical characteristics of these 28 MLL+ patients were shown in Table II. We concluded that 23 cases were B-ALL, 1 was T, B-ALL, and 4 cases were T-ALL in this cohort of 28 MLL+ patients.

Table I. Gene Partners of 27 positive MLL rearrangements.

Gene Partners	MLL-AF4	dup MLL	MLL-AF9	MLL-AF10	MLL-ENL	Overall
No.	9	6	6	2	4	27

Comparisons of Clinical Characteristics Between MLL+ Group and Control Groups

The frequency of MLL gene rearrangement was 4.4% (28/634), accounting for 12.9% (28/217) in the HR group. Compared with MLL-HR group, the number of patients of age younger than 2 years or initial leukocyte count $\geq 50\times10^9$/L was significantly increased ($p<0.05$), while no difference was found in gender and immunophenotype between these two groups ($p>0.05$). Compared with MLL-BCR/ABL- group, the number of patients of age younger than 2 years or initial leukocyte count $\geq 50\times10^9$/L also was significantly increased ($p<.05$). Furthermore, no difference in gender, age, initial leukocyte count, and immunophenotype was found between MLL+ group and MLL-BCR/ABL+ group ($p>0.05$) (Table II).

Treatment Efficacy in MLL+ Group

Of these 28 MLL+ patients, 10 cases were not sensitive to prednisone judged by more than 1000/μl of immature cells in peripheral blood on day 8 from the induction, while 18 cases were sensitive to prednisone, accounting for 35.7% and 64.3%, respectively. On day15 of the induction, 16 cases could be graded as M1 in which marrow blast cells were less than 5%, and 12 cases were non-M1. The complete remission rate (CR) was 89.3% (25/28) by bone marrow examination on day 33 from the induction. In addition, MRD was detected in 23 cases on day 33 of the induction, of which the cases with MRD $\geq 1\times10^{-2}$ accounted for 17.4% (4/23), and cases with MRD$<1\times10^{-2}$ accounted for 82.6% (19/23). Moreover, MRD detected in 21 cases in the 12th week of the induction showed the MRD $\geq 1\times10^{-3}$ in 5 cases (23.8%), and MRD$< 1\times10^{-3}$ in 16 cases (76.2%) (Table III).

Comparisons of Treatment Efficacy Between MLL+ Group And Control Groups

Compared with MLL-HR group, the number of cases of non-M1 on day15 was significantly higher ($p<0.05$) in MLL+ group; while the number of cases in response to prednisone, blast cell on day 33, MRD on day 33 and 12th week did not differ between these two groups ($p>0.05$). The number of cases with CR on day 33 in MLL+ group was significantly higher than that in MLL-BCR/ABL+ group ($p<0.05$). However, the number of cases with MRD $\geq 1\times10^{-2}$ on day 33 in BCR/ABL+ group was significantly higher than that in MLL+ group ($p<0.05$), while no differences in the response rate to prednisone, the percentage of blast cell in bone marrow on day 15 and the MRD

Table II. Clinical characteristics of MLL+ group and HR group with MLL.

Group/Characteristics	MLL+HR (n)	MLL–HR (n)	MLL-BCR/ABL+(n)	MLL-BCR/ABL–(n)
Gender				
Male	15	128	17	111
Female	13	61	5	56
Age				
< 2 Years old	7	13	1	12
≥ 2 Years old	21	176	21	155
		p=0.007		p=0.009
Initial leukocytes				
$\geq 50\times10^9$/L	14	49	6	43
$< 50\times10^9$/L	14	140	16	124
		p=0.013		p=0.013
Immunophenotype				
B	23	151	22	129
T	4	37	0	37

Table III. Treatment efficiency of MLL+ group and HR group with MLL–.

Treatment Efficiency/ Group	MLL+HR (n)	MLL–HR (n)	MLL-BCR/ABL+(n)	MLL-BCR/ABL–(n)
Response to prednisone				
Nonsensitive	10	91	6	85
Sensitive	18	96	15	81
Blast cell on day 15				
≥ 5%	12	142	15	127
< 5%	16	47	7	40
		p=0.001		p=0.001
Blast cell on day 33				
≥ 5% (NR)	3	40	9	31
< 5% (CR)	25	148	12	136
			p=0.017	
MRD on day 33				
$\geq 1\times10^{-2}$	4	42	11	31
$<1\times10^{-2}$	19	90	7	83
			p=0.008	
MRD on 12th week				
$\geq 1\times10^{-3}$	5	37	5	32
$<1\times10^{-3}$	16	70	8	62

on day 12th week have been found between these two groups (p>0.05). With regard to non-M1 status on day 15, the number of patients of MLL+ group was significantly higher than that in MLL-BCR/ ABL- group ($p\leq0.05$),while the response to prednisone and the percentage of blast cell on day 33, and the MRD on day 33 and 12th week did not differ between these two groups (p>0.05) (Table III).

Prognosis

Among these 28 patients with MLL gene rearrangement, 6 cases relapsed after complete remission with a relapse rate of 21.4% (6/28). One case failed to achieve complete remission after the treatment course. All the 7 cases eventually died with a mortality rate of 25%. One case was lost after ten months following-up from diagnosis. Compared with HR group of MLL-, MLL+ group didn't show any statistical difference in the recurrence rate and TRD (p>0.05) (Table IV).

Survival of MLL+ and HR Group With MLL-

Compared with MLL- Group, MLL-BCR/ ABL+ group, and MLL-BCR/ABL- group respectively, MLL+ group didn't show any statistical difference in 5 years OS or 5 years RFS. The 5 years OS of MLL+ group, MLL- HR Group, MLL-BCR/ABL+ group and MLL-BCR/ABL-HR group were 72% ± 9%, 67% ± 5%, 54% ± 13% and 69% ± 6%, respectively. 5 years of RFS of these four groups were 68% ± 9%, 53% ± 7%, 54% ± 13%, 54% ± 7%, respectively (Figure 1a, Figure 1b).

Influence of Clinical Characteristics or Treatment Efficiency on OS and RFS

As shown in Figure 2a and Figure 2b, the age affected in MLL+ group. Patients younger than 2 years old had a poor 5 years OS than those older than 2 years (37% ± 20% *vs.* 84% ± 9%, p=0.028). They also had a poor 5 years RFS than those older than 2 years cases (29% ± 17% *vs.* 84% ± 9%, p=0.007). 5 years OS of groups with positive and negative MLL/AF4 gene rearrangement were 53 ± 17 % and 81 ± 10 %, respectively, with no statistical significance (p=0.076), while the 5 years RFS between these two groups was also no

Table IV. Relapse rate and mortality of MLL+ and HR group with MLL–.

Group	Relapse rate (%)	Mortality (%)
MLL+	21.4 (6/28)	25.0 (7/28)
MLL–	23.8 (45/189)	22.8 (43/189)
MLL-BCR/ABL+	22.7 (5/22)	36.4 (8/22)
MLL-BCR/ABL–	24.0 (40/167)	21.0 (35/167)

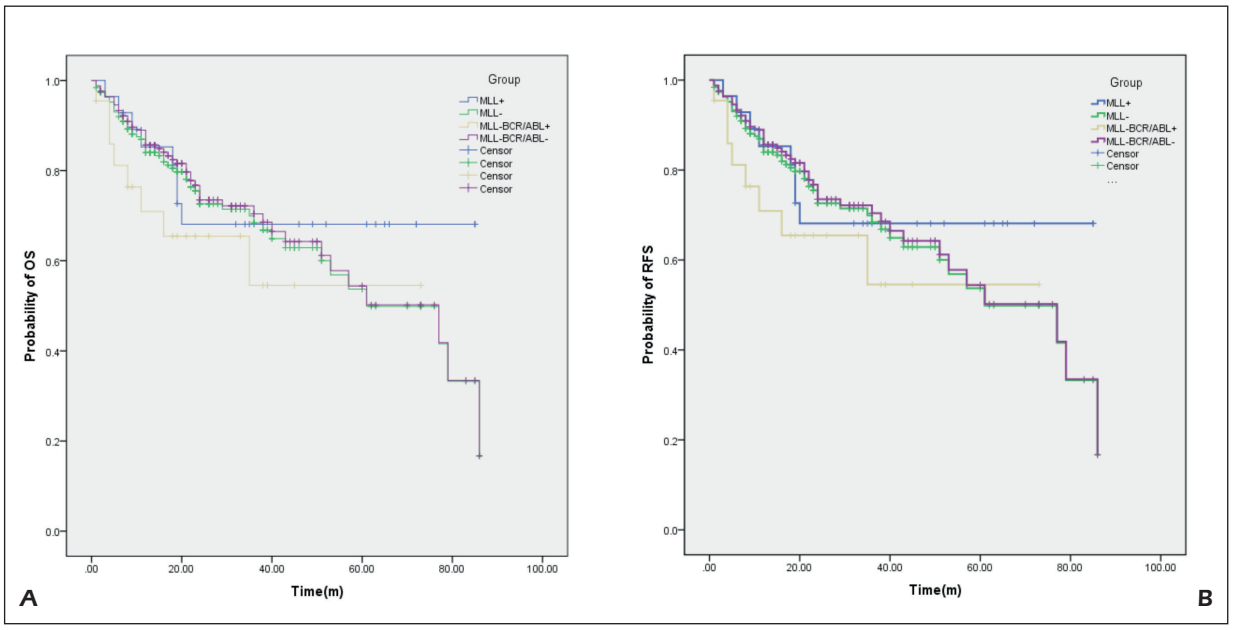

Figure 1. Survival of MLL+ group and control groups.

statistical significance (53% ± 17% *vs.* 75 ± 11%) (p=0.143). Besides, the difference of 5 years OS between groups insensitive and sensitive to prednisone was also no statistical significance (55% ± 17% *vs.* 81% ± 10%) (p=0.091) (Figure 3a). The difference in 5 years RFS between groups insensitive and sensitive to prednisone was statistically significant (41 ± 17 % *vs.* 81 ± 10%, respectively p=0.025) (Figure 3b). 5 years OS of the NR group was significantly lower than that of CR group (33% ± 27% *vs.* 77% ± 9%, p=0.03) (Figure 4a). 5 years RFS of the NR group was also significantly lower than that of CR group (33% ± 27% *vs.* 72% ± 10%, p=0.045) (Figure 4b). Additional factors such as gender, initial leukocyte count, immunophenotype, MRD on day 33 and 12th week didn't

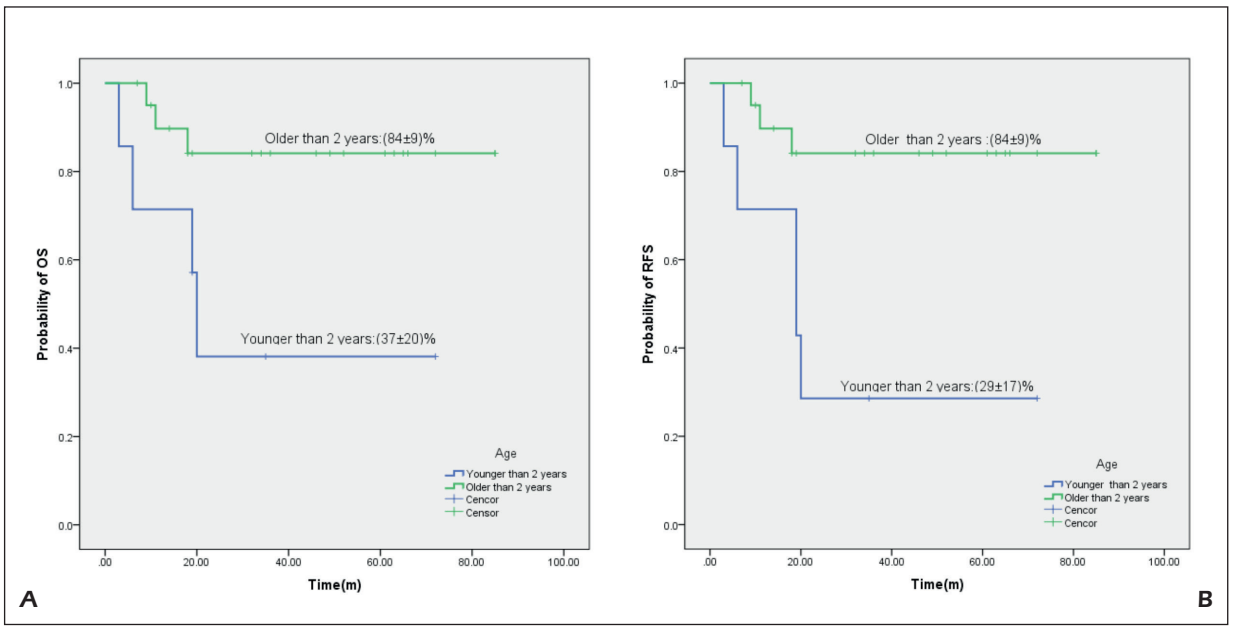

Figure 2. Survival of younger and older than 2 years groups.

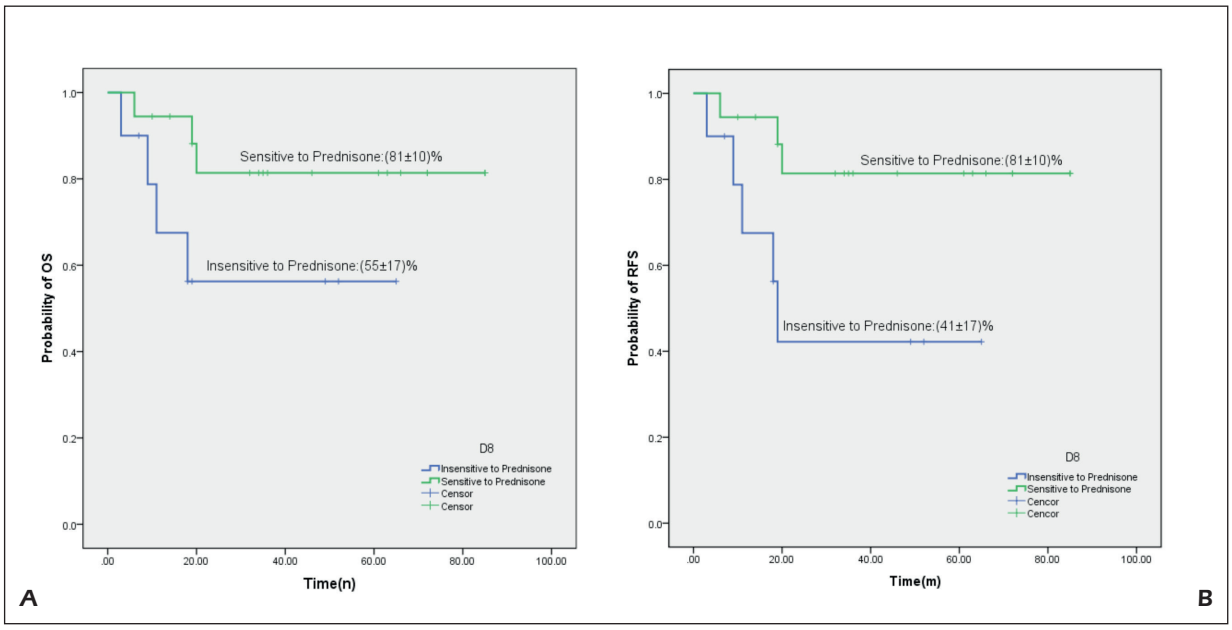

Figure 3. Survival of insensitive and sensitive to prednisone groups.

exert statistical influence on OS or RFS for ALL patients with MLL gene rearrangement treated by CCLG-ALL 2008 protocol (p>0.05). Multivariate Cox regression analysis found that age or prednisone response on day 8 had impact on OS (p<0.05) (Table V), while age, MLL fusion partners, or prednisone response on day 8 had impact on RFS (p<0.05) (Table VI).

Discussion

The rearrangements involving the mixed lineage leukemia (MLL) gene on chromosome 11q23 has been widely believed to be a pivotal prognostic factor of acute leukemia. In the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues published in 2008, the B-LBL/

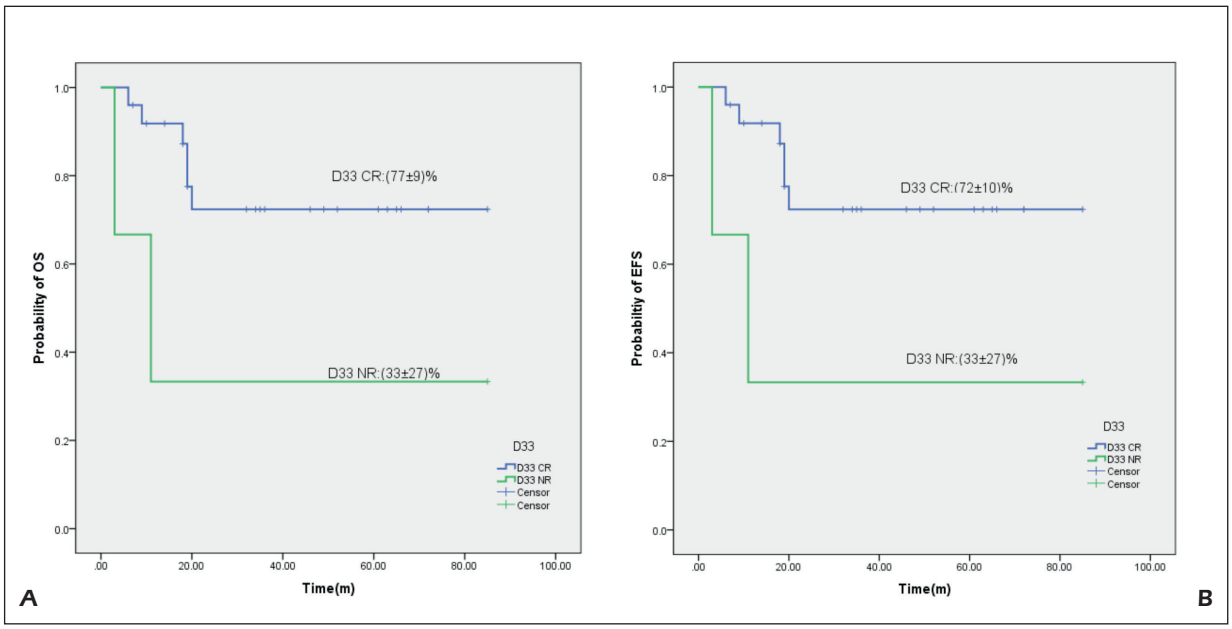

Figure 4. Survival of D33 CR and D33 NR groups.

Table V. Clinical characteristics of MLL+ group and HR group with MLL.

Factor	HR	95% CI		p
Gender	9.834	0.267	362.262	0.214
Age	0.006	0.000	0.797	0.040
Initial leukocytes	3.884	0.068	221.424	0.511
Immunophenotype	27.532	0.162	4678.330	0.206
Gene	0.003	0.000	1.049	0.052
Prednisone Response	0.013	0.000	0.702	0.033
D15 BM	7.282	0.271	195.619	0.237
D33 BM	0.065	0.000	66.696	0.440

ALL with MLL gene rearrangement had been defined as a new molecular subtype with high risk and poor prognosis[9]. Translocations involving the chromosome 11q23 gene MLL are noted in over 70% of infant leukemia, 2-5% of childhood ALL, and 5-10% of childhood AML[10]. We have found and treated 28 out of 634 evaluated patients (4.4%, 28/634) with positive MLL rearrangement, including 3 infants cases (42.9%, 3/7) with the CCLG-ALL2008 protocol for high-risk (HR) group, which is consistent with the literature.

The ALL patients with 11q23/MLL rearrangement had been identified with unique clinical, hematological and prognostic features, such as high initial leucocyte count, hepatomegaly, splenomegaly. While the overall survival of acute leukemias in pediatric patients reaches about 90%, MLL-r leukemia patients still display poor survival rates. Despite their dismal clinical behavior, our knowledge about the pathological disease mechanism(s) exerted by some fusion proteins from distinct MLL-rearranged leukemias is quite good but does not yet translate into therapeutic success[11]. In our cohort study of 28 MLL+ patients, we have 23 cases of B-ALL, 1 case of T, B-ALL, and 4 cases of T-ALL. Moorman et al[12] also reported that most MLL patients had a common or pre-B-cell immunophenotype (18 of 29, 62%).

We found in our study that the number of MLL+ cases with non-M1 on day15 was significantly higher than that with MLL- group ($p<0.05$). With regard to non-M1 status on day 15, the number of patients of MLL+ group was significantly higher than that in MLL-BCR/ABL- group ($p<0.05$). These results demonstrated a poor response to early treatment in our Childhood ALL with MLL gene rearrangement treated with CCLG-ALL2008 protocol for HR group, and suggested a poor prognosis. For our 28 patients with MLL gene rearrangement, the relapse rate was 21.4%, the mortality was 25% and the 5 years OS of MLL+ group was 72% ± 9%, the 5 years of RFS was 68% ± 9%. Recently, Tokyo Children's Cancer Study Group reported[5] that the 5-year EFS and OS rate were 60.0% ± 9.7 % and 64.0% ± 9.6%, respectively. Compared to HR group of MLL-, MLL+ group did not show any statistical difference in the recurrence rate, TRD, and 5 years OS or 5 years RFS.

Although there was no difference between the MLL+ group and control groups for 5 years OS or 5 years RFS, some atypical clinical features and treatment response of these patients with MLL gene rearrangement treated by CCLG-ALL2008 protocol were associated with some distinct outcomes. Our study showed that the age did affect

Table VI. Multivariate COX regression analysis on RFS.

Factor	HR	95% CI		p
Gender	13.596	0.343	538.439	0.164
Age	0.003	0.000	0.452	0.023
Initial leukocytes	6.693	0.115	389.846	0.359
Immunophenotype	33.629	0.206	5494.276	0.176
Gene	0.002	0.000	0.835	0.044
Prednisone Response	0.006	0.000	0.330	0.013
D15 BM	7.631	0.288	202.350	0.224
D33 BM	0.33	0.000	61.501	0.374

in MLL+ group, patients younger than 2 years old had a poor 5 years OS and a poor 5 years RFS than those older than 2 years cases (37% ± 20% *vs.* 84% ± 9%, $p<0.05$; and 29% ±17% *vs.* 84% ± 9%, $p<0.05$, respectively). The cases of MLL-r AML in infants do not generally have worse outcomes than their non- MLL-r AML counterparts . Pediatric patients greater than 1 year of age with MLL-r ALL are better than infants, although not as well as their non-MLL-r counterparts. Most recent data estimate a 5-year EFS of ~60% compared to ~92% in pediatric ALL overall[13]. The presence of an MLL translocation was associated with a higher risk of relapse in patients younger than 4 years, but not in patients of 4 years or older[12]. Study on 497 cases with MLL rearrangement from 11 collaborative groups indicated that age is a significant prognostic factor, cases less than 1-year-old had poor prognosis[14]. Pui et al[14] reported that 5 years EFS of ALL with MLL-AF4 gene in infant, in children of 1-9 years old, and in children older than 10 years was 19%, 43%, 39%, respectively. For ALL with MLL-AF9 gene in infant and in children of 1-9 years old, the 5 years EFS was 38%, 50%, respectively; while 5 years EFS of ALL with MLL/ENL gene in infant, in children of 1-9 years old, and in children older than 10 years was 26%, 67%, 60%, respectively[14]. MLL/AF4 is the most common type of MLL translocation in children. Despite recent improvements in the overall treatment outcome for ALL patients, MLL-AF4 fusion is still connected with a dismal prognosis in infants (especially those younger than 6 months) and adults [15]. But a report from the Tokyo Children's Cancer Study Group showed that with intensive chemotherapy and allogenetic HSCT, a favorable outcome of children (≥1 year old) with MLL-AF4-positive ALL could be achieved[5]. Balgobind et al[16] retrospectively collected outcome data of 756 children with 11q23- or MLL-rearranged AML from 11 collaborative groups to identify differences in outcome based on translocation partners. They found that cases with t (6; 11) had the worst prognosis, followed by cases with t (4; 11) and t (10; 11). Patients with t(1; 11) and t(9; 11) had longer survival. In our study, the 5 years OS of groups with positive and negative MLL/AF4 gene rearrangement were 53 ± 17% and 81 ± 10%, respectively, with no statistical significance ($p=0.076$), while the 5 years RFS between these two groups was also no statistical significance (53% ± 17% *vs.* 75 ± 11%) ($p=0.057$). But m ultivariate COX regression analysis found that MLL fusion partners had impacted on RFS ($p<0.05$).

The important prognostic significance of response to prednisone and the bone marrow status during and after induced remission had been widely recognized as a consensus. Our results of multivariate COX regression analysis indicated that prednisone response on day 8 had impact on OS or RFS ($p<0.05$). The difference in 5 years RFS between groups insensitive and sensitive to prednisone was statistically significant ($p<0.05$). In infant MLL-r B-ALL, a poor response to prednisone (≥1000 blasts/mL in peripheral blood on day 8) is also an independent negative prognostic factor[17]. Infants with t (4;11) ALL had an especially dismal prognosis when their disease was characterized by a poor early response to prednisone. A poor prednisone response also appeared to confer a worse outcome for older children with t (4;11) ALL[18]. Numerous studies had confirmed that MRD quantitative detection had decisive significance in evaluating the prognosis of ALL. However, our study on MRD on day 33 and 12th week showed no statistical impact on OS or RFS ($p>0.05$) in 28 cases with MLL gene rearrangements. It became also clear from recent studies that the follow-up of patients during treatment and therapy adjustment based on minimal residual disease (MRD) monitoring has a very strong impact on outcome[19]. Although our results found a better overall therapeutic efficacy of childhood ALL with MLL gene rearrangement, the patients younger than 2 years of age, with insensitivity to prednisone, with MLL/AF4 gene rearrangement, or NR on D33 still had poor outcomes. The age, fusion partners (MLL/AF4), and response to treatment might be the major factors of treatment effect of CCLG-ALL2008 protocol for high-risk (HR) group.

Conclusions

The traditional chemotherapy had been shown insufficient to improve the prognosis of patients with MLL gene rearrangement. The role of hematopoietic stem cell transplantation (HSCT) in the treatment of in childhood ALL with MLL gene rearrangement remained controversial. In Japan, a total of 132 cases of allogeneic HSCT for infant ALL with MLL gene rearrangements were analyzed demonstrating a 5-year OS rate of 67.4% ± 4.5% after transplantation[20]. But Mann et al[21] found that the advantage of HSCT was restricted to subgroup with 2 additional unfavorable prognostic features: age less than 6 months

and either poor response to steroids at day 8 or leukocytes more than or equal to 300 g/L. Recently, a study reported that compared with other nucleoside analogues, clofarabine effectively targeted primary MLL-rearranged infant ALL cells at the lowest concentrations *in vitro*[22]. We hope that the pediatric ALL patients with MLL gene rearrangement, especially some refractory children, can reach a similar prognosis as general ALL children in the near future, and acquire a better quality of life.

Acknowledgements

The authors would like to thank the grants from National Natural Science Foundation of China (No. 81370627, and No. 81770193), Jiangsu Province Projects (BRA2017541, BE2017659, and CXTDA2017014), Suzhou Projects (SZZX201504, SZS201615, LCZX201507, and SYS201643), Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Conflict of Interests

The authors declare that they have no conflict of interests.

References

1) Wright RL, Vaughan AT. A systematic description of MLL fusion gene formation. Crit Rev Oncol Hematol 2014; 91: 283-291.
2) Cerveira N, Lisboa S, Correia C, Bizarro S, Santos J, Torres L, Vieira J, Barros-Silva JD, Pereira D, Moreira C, Meyer C, Oliva T, Moreira I, Martins Â, Viterbo L,Costa V, Marschalek R, Pinto A, Mariz JM, Teixeira MR. Genetic and clinical characterization of 45 acute leukemia patients with MLL gene rearrangements from a single institution. Mol Oncol 2012; 6: 553-564.
3) Krivtsov AV, Armstrong SA. MLL translocations, histone modifications and leukaemia stem-cell development. Nat Rev Cancer 2007; 7: 823-833.
4) Meyer C, Hofmann J, Burmeister T, Gröger D, Park TS, Emerenciano M, Pombo de Oliveira M, Renneville A, Villarese P, Macintyre E, Cavé H, Clappier E,Mass-Malo K, Zuna J, Trka J, De Braekeleer E, De Braekeleer M, Oh SH, Tsaur G, Fechina L, van der Velden VH, van Dongen JJ, Delabesse E, Binato R, Silva ML, Kustanovich A, Aleinikova O, Harris MH, Lund-Aho T, Juvonen V, Heidenreich O, Vormoor J, Choi WW, Jarosova M, Kolenova A, Bueno C, Menendez P,Wehner S, Eckert C, Talmant P, Tondeur S, Lippert E, Launay E, Henry C, Ballerini P, Lapillone H, Callanan MB, Cayuela JM, Herbaux C, Cazzaniga G, Kakadiya PM, Bohlander S, Ahlmann M, Choi JR, Gameiro P, Lee DS, Krauter J, Cornillet-Lefebvre P, Te Kronnie G, Schäfer BW, Kubetzko S, Alonso CN, zur Stadt U, Sutton R, Venn NC, Izraeli S, Trakhtenbrot L, Madsen HO, Archer P, Hancock J, Cerveira N, Teixeira MR, Lo Nigro L, Möricke A, Stanulla M, Schrappe M, Sedék L, Szczepański T, Zwaan CM, Coenen EA, van den Heuvel-Eibrink MM, Strehl S, Dworzak M, Panzer-Grümayer R, Dingermann T, Klingebiel T, Marschalek R. The MLL recombinome of acute leukemias in 2013. Leukemia 2013; 27: 2165-2176.
5) Tomizawa D, Kato M, Takahashi H, Fujimura J, Inukai T, Fukushima T, Kiyokawa N, Koh K, Manabe A, Ohara A. Favorable outcome in non-infant children with MLL-AF4-positive acute lymphoblastic leukemia: a report from the Tokyo Children's Cancer Study Group. Int J Hematol 2015; 102: 602-610.
6) Hu YX, Lu J, He HL, Wang Y, Li JQ, Xiao PF, Li J, Lv H, Sun YN, Fan JJ, Chai YH, Hu SY. A prospective evaluation of minimal residual disease as risk stratification for CCLG-ALL-2008 treatment protocol in pediatric B precursor acute lymphoblastic leukemia. Eur Rev Med Pharmacol Sci 2016; 20: 1680-1690.
7) Iqbal Z Akhtar T, Awan T, Aleem A, Sabir N, Rasool M, Absar M, Akram AM, Shammas MA, Shah IH, Khalid M, Taj AS, Jameel A, Alanazi A, Gill AT, Hashmi JA, Hussain A, Sabar MF, Khalid AM, Qazi MH, Karim S, Siddiqi MH, Mahmood A, Iqbal M, Saeed A, Irfan MI. High Frequency and poor prognosis of late childhood BCR-ABL-positive and MLL-AF4-positive ALL define the need for advanced molecular diagnostics and improved therapeutic strategies in pediatric B-ALL in Pakistan. Mol Diagn Ther 2015; 19: 277-287.
8) Lu J, Ashwani N, Zhang M, He H, Lu J, Wang Y, Zhao W, Cao L, Ji Z, He Y, Hunag Y, Chen R, Hu S. Children diagnosed as mixed-phenotype acute leukemia didn't benefit from the CCLG-2008 protocol, retrospective analysis from single center. Indian J Hematol Blood Transfus 2015; 31: 32-37.
9) Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW. WHO classification of tumours of haematopoietic and lymphoid tissues. Lycn France: IARC Press; 2008: 152.
10) Teachey DT, Hunger SP. Predicting relapse risk in childhood acute lymphoblastic. Br J Haematol 2013; 162: 606-620.
11) Steinhilber D, Marschalek R. How to effectively treat acute leukemia patients bearing MLL-rearrangements? Biochem Pharmacol 2018; 147: 183-190.
12) Moorman AV, Ensor HM, Richards SM, Chilton L, Schwab C, Kinsey SE, Vora A, Mitchell CD, Harrison CJ. Prognostic effect of chromosomal abnormalities in childhood B-cell precursor acute lymphoblastic leukaemia: results from the UK Medical Research Council ALL97/99 randomised trial. Lancet Oncol 2010; 11: 429-438.
13) Winters AC, Bernt KM. MLL-Rearranged Leukemias - An update on science and clinical approaches. Front Pediatr 2017; 4: 1-21.
14) Pui CH, Gaynon PS, Boyett JM, Chessells JM, Baruchel A, Kamps W, Silverman LB, Biondi A, Harms DO, Vilmer E, Schrappe M, Camitta B. Outcome of treatment in childhood acute lymphoblastic leukaemia with rearrangements of the 11q23 chromosomal region. Lancet 2002; 359: 1909-1915.
15) Pui CH, Campana D. Age-related differences in leukemia biology and prognosis: the paradigm of MLL-AF4-positive acute lymphoblastic leukemia. Leukemia 2007; 21: 593-594.

16) Balgobind BV, Raimondi SC, Harbott J, Zimmermann M, Alonzo TA, Auvrignon A, Beverloo HB, Chang M, Creutzig U, Dworzak MN, Forestier E, Gibson B, Hasle H, Harrison CJ, Heerema NA, Kaspers GJ, Leszl A, Litvinko N, Nigro LL, Morimoto A, Perot C, Pieters R, Reinhardt D, Rubnitz JE, Smith FO, Stary J,Stasevich I, Strehl S, Taga T, Tomizawa D, Webb D, Zemanova Z, Zwaan CM, van den Heuvel-Eibrink MM. Novel prognostic subgroups in childhood 1lq23/MLL-rearranged acute myeloid leukemia: results of an international retrospective study. Blood 2009; 114: 2489-2496.
17) Sanjuan-Pla A, Bueno C, Prieto C, Acha P, Stam RW, Marschalek R, Menéndez P. Revisiting the biology of infant t(4;11)/MLL-AF4+ B-cell acute lymphoblastic leukemia. Blood 2015; 126: 2676-2685.
18) Pui CH, Chessells JM, Camitta B, Baruchel A, Biondi A, Boyett JM, Carroll A, Eden OB, Evans WE, Gadner H, Harbott J, Harms DO, Harrison CJ, Harrison PL, Heerema N, Janka-Schaub G, Kamps W, Masera G, Pullen J, Raimondi SC, Richards S, Riehm H, Sallan S, Sather H, Shuster J, Silverman LB, Valsecchi MG, Vilmer E, Zhou Y, Gaynon PS, Schrappe M. Clinical heterogeneity in childhood acute lymphoblastic leukemia with 11q23 rearrangements. Leukemia 2003; 17: 700-706.
19) Meyer C, Burmeister T, Gröger D, Tsaur G, Fechina L, Renneville A, Sutton R, Venn NC, Emerenciano M, Pombo-de-Oliveira MS, Barbieri Blunck C, Almeida Lopes B, Zuna J, Trka J, Ballerini P, Lapillonne H, De Braekeleer M, Cazzaniga G, Corral Abascal L, van der Velden VHJ, Delabesse E, Park TS, Oh SH, Silva MLM, Lund-Aho T, Juvonen V, Moore AS, Heidenreich O, Vormoor J, Zerkalenkova E, Olshanskaya Y, Bueno C, Menendez P, Teigler-Schlegel A, Zur Stadt U, Lentes J, Göhring G, Kustanovich A, Aleinikova O, Schäfer BW, Kubetzko S, Madsen HO, Gruhn B, Duarte X, Gameiro P, Lippert E, Bidet A, Cayuela JM, Clappier E, Alonso CN, Zwaan CM, van den Heuvel-Eibrink MM, Izraeli S, Trakhtenbrot L, Archer P, Hancock J, Möricke A, Alten J, Schrappe M, Stanulla M, Strehl S, Attarbaschi A, Dworzak M, Haas OA, Panzer-Grümayer R, Sedék L, Szczepański T, Caye A, Suarez L, Cavé H, Marschalek R. The MLL recombinome of acute leukemias in 2017. Leukemia 2018; 32: 273-284.
20) Kato M, Hasegawa D, Koh K, Kato K, Takita J, Inagaki J, Yabe H, Goto H, Adachi S, Hayakawa A, Takeshita Y, Sawada A, Atsuta Y, Kato K. Allogeneic haematopoietic stem cell transplantation for infant acute lymphoblastic leukaemia with KMT2A (MLL) rearrangements: a retrospective study from the paediatric acute lymphoblastic leukaemia working group of the Japan Society for Haematopoietic Cell Transplantation. Br J Haematol 2015; 168: 564-570.
21) Mann G, Attarbaschi A, Schrappe M, De Lorenzo P, Peters C, Hann I, De Rossi G, Felice M, Lausen B, Leblanc T, Szczepanski T, Ferster A, Janka-Schaub G,Rubnitz J, Silverman LB, Stary J, Campbell M, Li CK, Suppiah R, Biondi A, Vora A, Valsecchi MG, Pieters R; Interfant-99 Study Group. Improved outcome with hematopoietic stem cell transplantation in a poor prognostic subgroup of infants with mixed-lineage-leukemia (MLL)-rearranged acute lymphoblastic leukemia: results from the Interfant-99 Study. Blood 2010; 116: 2644-2650.
22) Stumpel DJ, Schneider P, Pieters R, Stam RW. The potential of clofarabine in MLL-rearranged infant acute lymphoblastic leukaemia. Eur J Cancer 2015; 51: 2008-2021.