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Pediatric-inspired regimen for adolescent and adult patients with Philadelphia chromosome-negative acute lymphoblastic leukemia: a prospective study from China

Running title: Pediatric-inspired regimen for patients with Ph$^-$ ALL

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The study was approved by the ethics committee of institute of hematology and blood diseases hospital.

Patient consent statement:

Informed consents were obtained for this study and the publication of the manuscript.

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Author contribution statement:

Y-C.M. and J-X. W. contributed to the study design. X-Y.G. wrote the initial draft of the manuscript. All authors analyzed data and approved the final version of the manuscript.

Keywords: acute lymphoblastic leukemia, Philadelphia chromosome-negative, pediatric-inspired regimen, minimal residual disease, prognosis

Abstract

Several international centers have used and reported pediatric-inspired regimens for adolescent and adult patients with Philadelphia chromosome-negative acute lymphoblastic leukemia (Ph^- ALL). However, there is a lack of prospective data on the Chinese population. Herein, we performed a prospective study with a pediatric-inspired regimen (IH-2014 regimen) in treating adolescent and adult Ph^- ALL patients in our center. From 2014 to 2021, a total of 415 patients aged between 14 and 65 years (median age, 27) were included in this study. After a median follow-up of 40.8 months, the 5-year overall survival, disease-free survival, and event-free survival rates were 53.8%, 51.1% and 45.0%, respectively. The regimen was generally well tolerated and safe, and the overall chemotherapy-related mortality was 3.6%. Age ≥ 40 years and persistent detectable minimal residual disease (MRD) post-induction were independent prognostic factors. Traditional risk factors for adult patients combined with MRD post-induction exhibit predictive significance for survival and relapse, which is helpful in the selection of subsequent treatment. Patients with high risk factors who can achieve deep MRD response after induction do not derive benefit from allogeneic hematopoietic stem cell transplantation.

Introduction

Using multidrug combination chemotherapy to treat pediatric Philadelphia chromosome-negative acute lymphoblastic leukemia (Ph^-ALL) is a great success in modern oncology, with 5-year OS rate exceeding 80%.[1] In contrast to children, the clinical outcomes of adult patients with Ph^- ALL by applying adult chemotherapy regimens are dismal, more than 50% of patients will experience relapse.[2-4] Based on pediatric experience, several retrospective studies have found that adolescents and young adults (AYAs) patients with Ph^- ALL derive more survival benefits from pediatric-inspired regimens,[5-7] and these findings have been further confirmed by large prospective studies.[8] The development of adolescent and adult ALL treatment in the Chinese mainland lags behind that of developed countries. Moreover, there is a lack of prospective data on the efficacy and safety of pediatric-inspired regimens in

adolescent and adult patients with Ph- ALL in China.[9,10] Herein, we present our single-center prospective data on a pediatric-inspired regimen (IH-2014 regimen) for treating patients aged 14–65 years with Ph- ALL. The primary objective of the present study was to assess the efficacy and safety of a pediatric-inspired regimen and to explore the prognostic significance of minimal residual disease (MRD) combined with traditional risk factors.

Methods

Patients

In total, 415 consecutive patients (aged ≥ 14 years and ≤ 65 years) with newly diagnosed Ph- ALL were enrolled and treated from April 2014 to December 2021. A full description of genetic and molecular diagnostic methods is available in the Supplementary Material. The list of fusion genes and Ph-like related genes were shown in Supplementary Table 1 and 2. Before sample collection and treatment, every patient signed a consent form. This study was approved by the institutional ethics committee (Blood Diseases Hospital Ethics Committee) and was registered at Chinese Clinical Trial Registry Website with the registration number of ChiCTR-OOC-15006328.

Treatments

The treatment of patients followed a revised edition of the Children's Oncology Group protocol (CCG-1961).[11] Supplementary Table 3 and 4 show the specific protocol details (age ≤ 55 and age > 55 years, respectively). Additional details about treatments can be found in the Supplementary Material.

Risk stratification

Patients with Ph- ALL who presented with one or more of the following conditions were categorized as high risk (HR): age ≥ 40 years, white blood cell count ≥ 30×10^9/L for B-cell ALL or ≥ 100×10^9/L for T-cell ALL, hypodiploid ALL, mature T-ALL or early T-ALL, ALL with t(v;11q23) or *MLL* rearrangements/*KMT2A* rearrangements, and ALL with t(1;19)/*TCF3-PBX1* or with complex karyotype (≥ 5 unrelated clonal abnormalities). Patients without these high-risk factors were stratified into the standard risk (SR) group.[12]

Hematopoietic stem cell transplantation (HSCT)

Allogeneic HSCT (allo-HSCT) was recommended to HR patients with a donor (related or unrelated) or SR patients with persistent MRD at the end of induction. Autologous HSCT (auto-HSCT) was recommended to SR patients unable to tolerate multiple courses of intensive chemotherapy and had early achievement of MRD negativity or HR patients with early achievement of MRD negativity but without a suitable donor. The transplantation procedures were not completely uniform due to patients receiving transplantation in different transplant centers in China.

Response evaluation and MRD definitions

The response evaluation criteria and MRD assessment are shown in the Supplementary Material. MRD was measured using bone marrow (BM) aspirates with eight-color multiparametric FCM on day 14 of induction, at end of induction (EOI, days 29–42) , post the first consolidation (post-C1), and throughout the treatment period. MRD levels of $< 0.01\%$ and $\geq 0.01\%$ was considered as negative and positive, respectively.[13] Complete remission (CR) patients who were MRD-positive were further stratified into three groups according to MRD level: MRD low positive (MRD-lp), $\geq 0.01\%$ to $< 0.1\%$; MRD high positive (MRD-hp), $\geq 0.1\%$ to $< 1\%$; and MRD very high positive (MRD-vhp), $\geq 1\%$.

Statistical analysis

The primary end points of the study were overall survival (OS) and event-free survival (EFS). The analysis of the data was conducted using GraphPad Prism 8 software and R statistical software (version 3.0). The survival rates were calculated using the Kaplan–Meier method and survival curves. To compare patients who received an allo-HSCT with those who did not, a separate landmark analysis was performed at 6 months (median time to allo-HSCT) after enrollment. The multivariate Cox proportional hazards regression model analysis included variables from the univariate analysis that had *P*-values of ≤ 0.2. Cumulative incidence of relapse (CIR) and treatment-related mortality (TRM) were calculated via a competing risk analysis with the Gray's test.

Results

In total, 415 patients were registered, and Figure 1 shows the patient flow chart. The participants' median age was 27 (range: 14–65 years) years, and males accounted for 59.3% of all participants. Further, 312 patients presented with B-precursor ALL and 103 with T-cell ALL. The patients' demographic and baseline clinical characteristics are displayed in Table 1. Multiple molecular method were available to screen Ph-like ALL since 2017 and a total of 24 patients were identified with the confirmed diagnosis of Ph-like ALL. The genomic alterations in patients with Ph-like ALL are listed in Supplementary Table 5.

Remission induction

All registered patients received induction therapy. In total, 12 patients died during induction therapy, and the induction-related mortality (IRM) was 2.89%. Two patients were discharged after induction therapy in stable condition without further efficacy evaluation at our center. Among 401 efficacy-evaluable patients, 355 (85.5%) achieved CR after induction chemotherapy. Of 47 patients with IF, 29 (61.7%) achieved CR after the first salvage chemotherapy. The overall CR rate after two courses of chemotherapy was 92.5%.

OS, EFS, and DFS

Patients who survived were followed-up until September 30, 2022. With a median follow-up time of 40.8 months, the median OS of the cohort has not yet been reached . The median EFS of all patients and the DFS of patients who achieved CR within two courses of chemotherapy were 25.6 months and not reached, respectively. The 5-year OS, EFS, and DFS rates were 53.8% (95% CI: 48.1%–59.5%), 45.0% (95% CI: 31.3%–50.3%), and 51.1% (95% CI: 45.4%–56.8%), respectively (Figure 2A). Table 2 shows the EFS and OS of the overall cohort and subgroups.

HSCT

During the study period, a total of 241 patients underwent HSCT. Most of the patients (92.9%, n = 224) underwent allo-HSCT (184 in CR1, 40 in CR2 or beyond), and 17 (7.1%) patients underwent auto-HSCT in CR1. Among the 224 patients who underwent allo-HSCT, 75 were in the SR group and 149 were in the HR

group. Among the 17 patients who underwent auto-HSCT, 8 were in the SR group and 9 were in the HR group. The 5-year OS rates of patients receiving allo-HSCT and auto-HSCT were 63.8% (95% CI: 52.9%–70.1%) and 70.1% (95% CI: 41.3%–98.9%), respectively (P = 0.322) (Figure 2B). It should note that patient characteristics were not comparable between the two groups.

Of the 42 patients diagnosed as ETP-ALL, 22 patients underwent allo-HSCT in CR1, the remaining 20 patients did not undergo allo-HSCT. In a landmark analysis of including only patients alive at 6 months (median time to allo-HSCT), the 5-year OS rates of patients with ETP-ALL who received allo-HSCT (n = 22) and those who did not (n = 13) were 43.5% (95% CI: 16.1%–70.9%) and 6% (95% CI: 0%–26.3%), respectively (P = 0.016) (Figure 2C). Of the 24 patients diagnosed as Ph-like ALL, 20 and 2 patients underwent allo-HSCT in CR1 and CR2, respectively, and the remaining 2 patients did not undergo allo-HSCT. Much higher 5-year OS was seen in Ph-like ALL patients who received allo-HSCT compared with no allo-HSCT (77.5% vs 0%, P < 0.001) (Figure 2D).

Prognostic value of MRD

There were 386, 389, and 346 BM samples available for MRD evaluation on day 14 of induction, at EOI, and post-C1, respectively. Figure 3A presents the distribution of MRD status over time. Figure 3B depicts the compositions of MRD levels at EOI stratified according to disease types. Notably, Ph-like ALL and ETP-T-ALL were associated with extremely lower achievements of MRD negativity at EOI compared with other types.

The 5-year OS rates of patients with MRD negativity (n = 193), MRD-lp (n = 52), MRD-hp (n = 55), and MRD-vhp (n = 89) at EOI were 68.0% (95% CI: 60.2%–75.8%), 44.8% (95% CI: 28.9%–60.7%), 52.4% (95% CI: 34.2%–70.6%), and 36.7% (95% CI: 24.2%–49.2%), respectively (P < 0.001) (Figure 4A). If patients were censored at the time of HSCT, the 5-year OS rates of these patients were 74.9% (95% CI: 65.1%–84.7%), 39.7% (95% CI: 18.7%–60.7%), 42.3% (95% CI: 11.7%–72.9%) and 0%, respectively (P < 0.001) (Figure 4B). Patients with a negative MRD at EOI had a more favorable OS than those with a positive MRD at

EOI. There was no statistically significant difference in terms of survival time between patients with MRD-lp and MRD-hp. Further, patients with MRD-vhp had the worst survival.

The relapse probabilities had a similar pattern. Patients with MRD negativity at EOI had a lower 5-year CIR than those with MRD positivity at any levels (31.9% vs. 47.4%, 43.8%, and 49.1%). Compared to MRD-lp and MRD-hp patients, patients with MRD-vhp experienced a significantly higher rate of early relapse. However, with a longer follow-up, the 5-year CIR did not differ significantly among patients with a positive MRD in any of the three groups, with a rate of 47.4%, 43.8%, and 49.1% in patients with MRD-lp, MRD-hp, and MRD-vhp, respectively (Figure 4C).

To explore the prognostic value of dynamic MRD during treatment, four groups were defined according to the MRD levels of EOI and post-C1: EOI negative/post-C1 negative (n = 173, 50%), EOI positive/post-C1 negative (n = 48, 13.9%), EOI negative/post-C1 positive (n = 5, 1.4%), EOI positive/post-C1 positive (n = 120, 34.7%). The OS of patients with an early and durable MRD response (EOI negative/post-C1 negative) was significantly superior to that of the others, with 5-year OS rate of 72.4% (95% CI: 64.6%–80.24%). There was no significant statistical difference in OS between patients with MRD conversion from positivity at EOI to negativity post-C1 and those with persistent MRD from EOI to post-C1 (5-year OS rate: 46.7% vs 46.0%, P = 0.363). The number of patients transitioning from MRD negativity at EOI to positivity post-C1 was very small (n = 5), and patients with such changes also had extremely poor OS (median OS 12.6 months) (Figure 4D).

Univariate and multivariate analyses of OS

Supplementary Table 6 showed the prognostic factors of OS based on the univariate and multivariate analyses. Compared with AYAs, patients aged ≥ 40 years had a significantly worse survival (Figure 5A). Multivariate analysis showed that age ≥ 40 years and positive post-induction MRD were independent predictors of for OS. Supplementary Figure 1 presented the forest plot for multivariate Cox regression of OS.

Integrating risk stratification and MRD to define new clinically prognostic subgroups

Based on their MRD status at EOI (negativity vs. positivity) and risk stratification at diagnosis (SR vs. HR), patients were divided into four groups: SR patients with MRD negativity (SR-MRD^{neg}; n = 73), SR patients with MRD positivity (SR-MRD^{pos}; n = 69), HR patients with MRD negativity (HR-MRD^{neg}; n = 120), and HR patients with MRD positivity (HR-MRD^{pos}; n = 127). The 5-year OS rates of patients with SR-MRD^{neg}, SR-MRD^{pos}, HR-MRD^{neg}, HR-MRD^{neg} were 82.6% (95% CI: 73.2%–92.0%), 58.7% (95% CI: 45.4%–72.0%), 58.3% (95% CI: 47.3%–69.3%), and 36.1% (95% CI: 25.5%–46.7%), respectively (P < 0.001) (Figure 5B). The 5-year CIRs were 24.2%, 41.1%, 36.3%, and 50.1%, respectively (P < 0.001) (Figure 5C).

The four patient groups were further stratified according to their transplantation status to explore the impact of allo-HSCT on survival. The baseline characteristics of patients with and without allo-HSCT in CR1 in the same group are shown in Supplementary Table 7. In landmark analysis, the 5-year OS of patients with SR-MRD^{neg} who received allo-HSCT in CR1 (n = 28) and those who did not (n = 37) were 44.5% (95% CI: 11.8%–77.2%) and 89.0% (95% CI: 78.8%–99.2%), respectively (P = 0.044) (Figure 5D). The 5-year OS rates of patients with HR-MRD^{neg} who received allo-HSCT in CR1 (n = 67) and those who did not (n = 41) were 64.8% (95% CI: 49.5%–80.1%) and 54.6% (95% CI: 36.8%–72.4%), respectively (P = 0.150) (Figure 5E). Allo-HSCT in CR1 did not improve OS in patients in the same risk group with MRD negativity at EOI. However, in patients with MRD positivity at EOI, allo-HSCT in CR1 improved OS significantly both in the SR and HR groups. In landmark analysis, the 5-year OS rates of patients with SR-MRD^{pos} received allo-HSCT in CR1 (n = 38) and those who did not (n = 22) were 59.3% (95% CI: 61.7%–90.3%) and 36.4% (95% CI: 15.2%–57.6%), respectively (P = 0.016) (Figure 5F). The 5-year OS rates of patients with HR-MRD^{pos} who received allo-HSCT in CR1 (n = 67) and those who did not (n = 41) were 59.3% (95% CI: 45.0%–73.6%) and 8.4% (95% CI: 0%–18.6%), respectively (P < 0.001) (Figure 5G).

Without considering the risk stratification, the 5-year OS rates of patients with MRD positivity at EOI received allo-HSCT in CR1 (n = 105) and those who did not (n = 63) were 65.3% (95% CI: 54.5%–76.1%) and 18.0% (95% CI: 7.0%–29.0%) in landmark analysis, respectively (P < 0.001) (Figure 5H). There was no statistical difference in cumulative incidence of TRM among patients with SR-MRD^{neg}, SR-MRD^{pos}, HR-MRD^{neg} and HR-MRD^{pos} underwent allo-HSCT in CR1, 3-year TRM was 5.55%, 22.22%, 15.56% and 19.24%, respectively (P = 0.335) (Figure 5I).

Toxicities

In total, 12 (2.89%) patients with a median age of 47.5 years died during the induction phase. The primary causes of mortality were infection (75%) and intracranial hemorrhage (16.7%). There were additional four treatment-related deaths caused by infection during consolidation therapy. Therefore, the overall treatment-related mortality of the chemotherapy was 3.86%. Table 3 shows the induction and early consolidation cycles related grade 3–5 toxicities. As shown in the table, hematologic toxicity and infections (including bloodstream infection) were the most common toxicities, followed by liver toxicity. Asparaginase-related thromboembolic events were relatively infrequent.

Discussion

Nowadays, novel immunotherapy has greatly changed the treatment paradigm of ALL, especially in the field of B-ALL,[14] nevertheless, the accessibility of novel immunotherapy is limited for the majority of Chinese patients in the frontline setting. Chemotherapy remains the cornerstone of treatment for newly diagnosed patients with Ph$^-$ ALL, and combined with immunotherapy can further improve the survival of patients.[15] Compared with adult protocols, pediatric protocols have higher accumulated doses of non-myelosuppressive agents such as vincristine, glucocorticoids and asparaginase, emphasize more intensive and prolonged CNS prophylaxis.[16] Due to significant survival benefits, the pediatric-inspired regimens have been recommended as an international standard treatment for AYAs with Ph$^-$

ALL.[12] However, the approach to treating adult patients is still a subject of controversy. Some studies have shown that the efficacy and tolerability of pediatric-inspired regimens are acceptable in adult patients aged up to 60 years. However, adult patients have a higher prevalence of treatment-related toxicities than AYAs.[17,18] Our study showed that the 5-year OS of AYAs (aged < 40 years) and other adult patients (aged ≥ 40 years) were 59.2% and 38.3%, respectively. Patients who died during induction therapy were older (median age: 47.5 vs. 27 years for the cohort). The efficacy and tolerability of pediatric-inspired regimen in adult patients was significantly lower than those in AYAs in our study.

The incidence of asparaginase-induced toxicities in our study was lower than that reported in other studies,[8,20,21] which may be related to factors such as a lower median age of enrolled patients, using preventive treatment for hepatotoxicity, consuming a low-fat diet, and being at a lower risk of thrombotic events for Chinese people.[22] Even then, a small number of patients who were unable to tolerate toxicities of chemotherapy and achieve early MRD negativity received auto-HSCT as an alternative treatment option.[23] More considerations should be given to the patients who were intolerant to pediatric-inspired chemotherapy. Dose modification to chemotherapy regimens and the introduction of novel immunotherapies can be the future directions for decreasing toxicity and improving survival in adult patients, particularly those aged ≥ 40 years.[24,25]

The prognostic factors of ALL mainly include genetic and molecular prognostic factors as well as clinical prognostic factors.[26,27] The identification of genetic prognostic subgroups requires the combination of molecular diagnostic techniques including polymerase chain reaction, G-banding, fluorescence in situ hybridization, RNA sequencing, and targeted next-generation sequencing. However, in Chinese patients, the abovementioned tests are less likely available at diagnosis. Therefore, the application of genetic and molecular prognostic classification is limited by the inaccessibility of the molecular diagnostic techniques. Meanwhile, risk stratification based on traditional prognostic factors such as white blood cell count, age, and

immunophenotype is still widely used in clinical practice due to its simplicity and lower requirements for testing techniques.[12,27,28] In addition to the abovementioned static prognostic indicators, MRD is currently considered the most powerful predictor in patients with ALL.[29] The time points and thresholds for MRD monitoring in pediatric ALL have been well established.[30] However, surveillance strategy in adult ALL has not yet been standardized. Stock W and colleagues[8] showed that AYAs with an MRD of > 0.01% at EOI had a significantly inferior DFS than those with an MRD of < 0.01%. Bassan R et al. have shown that MRD persistence at 10 weeks is an indicator for allo-HSCT in adult patients after induction therapy.[31] Our study confirmed that MRD negativity at EOI is an independent prognostic factor of OS. Patients with MRD-lp and MRD-hp at EOI had a lower OS and a higher relapse incidence than those with MRD negativity. In addition, only 13.9% of patients with MRD positivity at EOI achieved MRD negativity after post-C1, and their OS was not improved compared to those with persistent MRD from EOI to post-C1. Therefore, an early and deep MRD clearance is important, and even extremely low MRD but detectable levels can predict poor prognosis.

Patients were further grouped based on both post-induction MRD levels and traditional risk factors. The 5-year OS rates of patients with SR-MRD^{neg}, SR-MRD^{pos}, HR-MRD^{neg}, and HR-MRD^{pos} were 82.6%, 58.7%, 58.3%, and 36.1%, respectively. The survival rates did not significantly differ between patients with HR-MRD^{neg} who received allo-HSCT in CR1 and those who did not. This finding is consistent with that of a multicenter prospective clinical study conducted by the Spanish Collaborative Group.[12] Allo-HSCT improves survival in post-induction MRD-positive patients, regardless of stratification at diagnosis. However, this conclusion was only based on the use of pediatric-inspired chemotherapy and/or allo-HSCT, not incorporating novel immunologic agents in the frontline setting. Given the promising efficacy of blinatumomab in clearing MRD,[32] more clinical evidence is needed to confirm the timing and necessity of allo-HSCT for CR1 patients with MRD response after blinatumomab treatment in the context of standard chemotherapy combined with

immunotherapy.

There were certain constraints in the present study. First, it was performed at a single center. Second, the protocol design of this study did not dynamically adjust the intensity of chemotherapy or introduce immunotherapy according to MRD levels. Third, the relatively high rate of allo-HSCT is not entirely consistent with the principle of pediatric regimen.

Conclusion

AYAs are more likely to benefit from pediatric-inspired regimens compared with patients aged ≥ 40 years. The pediatric-inspired regimen is safe and well tolerated by patients. Age ≥ 40 years and persistent detectable MRD post-induction were independent prognostic factors. Traditional risk factors combined with MRD post-induction exhibit good predictive significance for survival and recurrence, which is helpful in guiding the selection of allo-HSCT.

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Tables

Table 1. Patient baseline characteristics

Characteristics	N (%)
	Total (N=415)
Sex	
Male	244 (58.8)
Female	171 (41.2)
Age at diagnosis, yr	
Median	27
Range	14-65
< 20	120 (28.9)
20-29	105 (25.3)
30-39	81 (19.5)
40-49	62 (14.9)
50-59	37 (8.9)
≥ 60	10 (2.4)
Immunophenotype	
B-ALL	N=312 (75.2% of all patients)
Pro-B	36 (11.5)
Common B	205 (65.7)
Pre B	71 (22.8)
T-ALL	N=103 (24.8% of all patients)
Pro-T	42 (40.8)
Pre-T	33 (32.0)
Cortical T	12 (11.7)
Medullary T	16 (15.5)
WBC, $\times 10^9$/L	
Median	13.2
Range	0.59-504.00
B-ALL with WBC ≥ 30×10^9/L	87 (27.9)
B-ALL with WBC < 30×10^9/L	225 (72.1)
T-ALL with WBC ≥ 100×10^9/L	21 (20.4)
T-ALL with WBC < 100×10^9/L	82 (79.6)
Cytogenetics	
Normal	216 (52.0)
t(v;11q23) or *KMT2A* rearrangements	23 (5.5)
t(1;19)/*TCF3-PBX1*	13 (3.1)
Hypodiploidy (< 44 chromosomes)	2 (0.5)
Hyperdiploidy (51–65 chromosomes)	12 (2.9)
Complex karyotype (≥ 5 chromosomal abnormalities)	20 (4.8)
Other abnormalities	101 (24.3)

No metaphase	25 (6.0)
Not done	3 (0.7)
Ph-like screening since 2017 (N=194)	
Positive	24 (12.4)
Negative	170 (87.6)
Risk Stratification	
Standard risk	148 (35.7)
High risk	267 (64.3)

1

Table 2. 5-year EFS and OS rates of the overall cohort and subgroups

Types of patients		5y-EFS		5y-OS	
	No.	%	95% CI	%	95% CI
Overall	415	45.0	31.3-50.3	53.8	48.1-59.5
B-ALL	312	47.0	40.9-53.1	57.5	51.0-64.0
Ph-like B-ALL*	24	49.7	27.6-71.8	70.7	50.5-90.9
T-ALL	103	39.4	29.4-49.4	42.1	30.5-53.7
ETP-T-ALL	42	24.0	9.3-38.7	13.8	0-34.6
Non-ETP-T-ALL	61	49.7	36.6-62.8	56.2	42.3-70.1
MRD negative at EOI	193	60.5	52.7-68.3	68.0	60.2-75.8
MRD-lp at EOI	52	44.5	29.2-59.8	44.8	28.9-60.7
MRD-hp at EOI	55	43.2	27.3-59.1	52.4	34.1-70.6
MRD-vhp at EOI	89	19.3	10.9-27.7	36.7	24.2-49.2
Standard risk (SR)	148	57.6	48.6-66.6	70.9	62.7-79.1
High risk (HR)	267	38.0	31.5-44.5	44.0	36.7-51.3
B-ALL with SR	143	57.8	48.8-66.8	71.5	63.3-79.7
B-ALL with HR	169	37.6	29.4-45.8	44.9	35.7-54.1
T-ALL with SR	5	60.0	17.1-100.0	53.3	4.7-100.0
T-ALL with HR	98	38.6	28.4-48.8	42.0	30.2-53.8
Age < 40y	306	49.8	43.5-56.1	59.2	52.7-65.7
Age ≥ 40y	109	31.5	21.9-41.1	38.3	27.5-49.1

Abbreviations: EFS, event-free survival; OS, overall survival; ETP-T-ALL, Early T-cell precursor (ETP) acute lymphoblastic leukemia; MRD, minimal residual disease; EOI, end of induction; MRD-lp, MRD low positive; MRD-hp, MRD high positive; MRD-vhp, MRD very high positive.

* The screening of Ph-like ALL started since 2017.

Table 3. Selected grade 3 to 5 adverse events in induction and induction and early consolidation cycles

Adverse event **N (%)**	Induction (N=415)			Consolidation I (N=382)			Consolidation II (N=336)			Interim maintenance I (N=271)		
	Grade 3	Grade 4	Grade 5	Grade 3	Grade 4	Grade 5	Grade 3	Grade 4	Grade 5	Grade 3	Grade 4	Grade 5
Elevated ALT	31（7.5）	2（0.5）	—	17（4.5）	4（1.0）	—	22（6.5）	1（0.3）	—	—	—	—
Elevated AST	25（6.0）	4（1.0）	—	8（2.1）	—	—	5（1.5）	—	—	—	—	—
Hyperbilirubinemia	21（5.1）	—	—	13（3.4）	—	—	11（3.3）	—	—	—	—	—
Hyperglycemia	11（2.7）	3（0.7）	—	3（0.8）	—	—		—	—	—	—	—
Bloodstream infection (BSI)	66（15.9）	6（1.4）	4（1.0）	54（14.1）	2（0.5）	—	29（8.6）	1（0.3）	—	—	—	—
Infection (except BSI)	366（88.2）	10（2.4）	5（1.2）	302（79.1）	3（0.8）	—	208（61.9）	—	—	8（3.0）	—	—
Intracranial hemorrhage	—	—	2（0.5）	—	—	—	—	—	—	—	—	—
Gastrointestinal hemorrhage	—	1（0.2）	—	3（0.8）	—	—	—	—	—	—	—	—
Tumor lysis syndrome	4（1.0）	2（0.5）	—	—	—	—	—	—	—	—	—	—
Pancreatitis	3（0.7）	1（0.2）	—	2（0.5）	—	—	1（0.3）	—	—	—	—	—
Neutropenia	4（1.0）	408（98.3）	—	12（3.1）	363（95.0）	—	23（6.8）	307（91.4）	—	11 (4.1)	32（11.8）	—
Thrombocytopenia	49（11.8）	319（76.9）	—	24（6.3）	348（91.1）	—	29（8.6）	296（88.1）	—	13 (4.8)	26（9.6）	—
Intestinal obstruction	18（4.3）	—	—	10（2.6）	—	—	1（0.3）	—	—	1（0.4）	—	—
Thrombosis	3（0.7）	—	1（0.2）	—	—	—	—	—	—	—	—	—
heart failure	3（0.7）	—	—	2（0.5）	—	—	—	—	—	—	—	—
Elevated serum creatinine	3（0.7）	—	—	—	—	—	—	—	—	13（4.8）	1（0.4）	—

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase

Figure legends

Figure 1. Flowchart of study patients.

Figure 2. Survival outcomes. (A) Overall survival (OS), event-free survival (EFS) and disease-free survival (DFS) curves for the cohort. (B) OS curves according to allo-HSCT vs. auto-HSCT. (C) OS curves for ETP-ALL patients according to transplantation status, survival curves originate at a landmark of 6 months (median time from enrollment to allo-HSCT) to adjust for bias related to early events. (D) OS curves for Ph-like ALL patients according to transplantation status.

Figure 3. Schematic graphs of minimal residual disease (MRD) levels. (A) MRD status at three time points: day 14 of induction, post induction, post the first consolidation (post-C1). (B) MRD levels at end of induction stratified by disease types. The numbers and proportions of patients for each group are shown in tables below figures.

Figure 4. Survival outcomes. (A) Overall survival (OS) curves according to minimal residual disease (MRD) levels at end of induction. (B) OS curves censored at time of hematopoietic stem cell transplantation according to MRD levels at end of induction. (C) Cumulative incidence of relapse curves according to MRD levels at end of induction. (D) OS curves according to the combination of MRD levels at end of induction (EOI) and post the first consolidation (post-C1).

Figure 5. Survival outcomes. (A) Overall survival (OS) curves according to ages (age <40 vs. ≥ 40). (B) OS curves according to risk stratification combined with minimal residual disease (MRD) levels post-induction. (C) Cumulative incidence of relapse curves according to risk stratification combined with MRD levels post-induction. (D) OS curves for standard risk (SR) patients with MRD negativity (SR-MRD^{neg}) post-induction according to allo-HSCT status. (E) OS curves for high risk (HR) patients with MRD negativity (HR-MRD^{neg}) post-induction according to allo-HSCT status. (F) OS curves for SR patients with MRD positivity

(SR-MRD^{pos}) post-induction according to allo-HSCT status. (G) OS curves for HR patients with MRD positivity (HR-MRD^{pos}) post-induction according to allo-HSCT status. (H) OS curves for patients with MRD positivity post-induction according to allo-HSCT status. For D to H, survival curves originate at a landmark of 6 months (median time from enrollment to allo-HSCT) to adjust for bias related to early events. (I) Cumulative incidence of TRM after allo-HSCT in CR1 according to risk stratification combined with MRD levels post-induction.

Figure 1

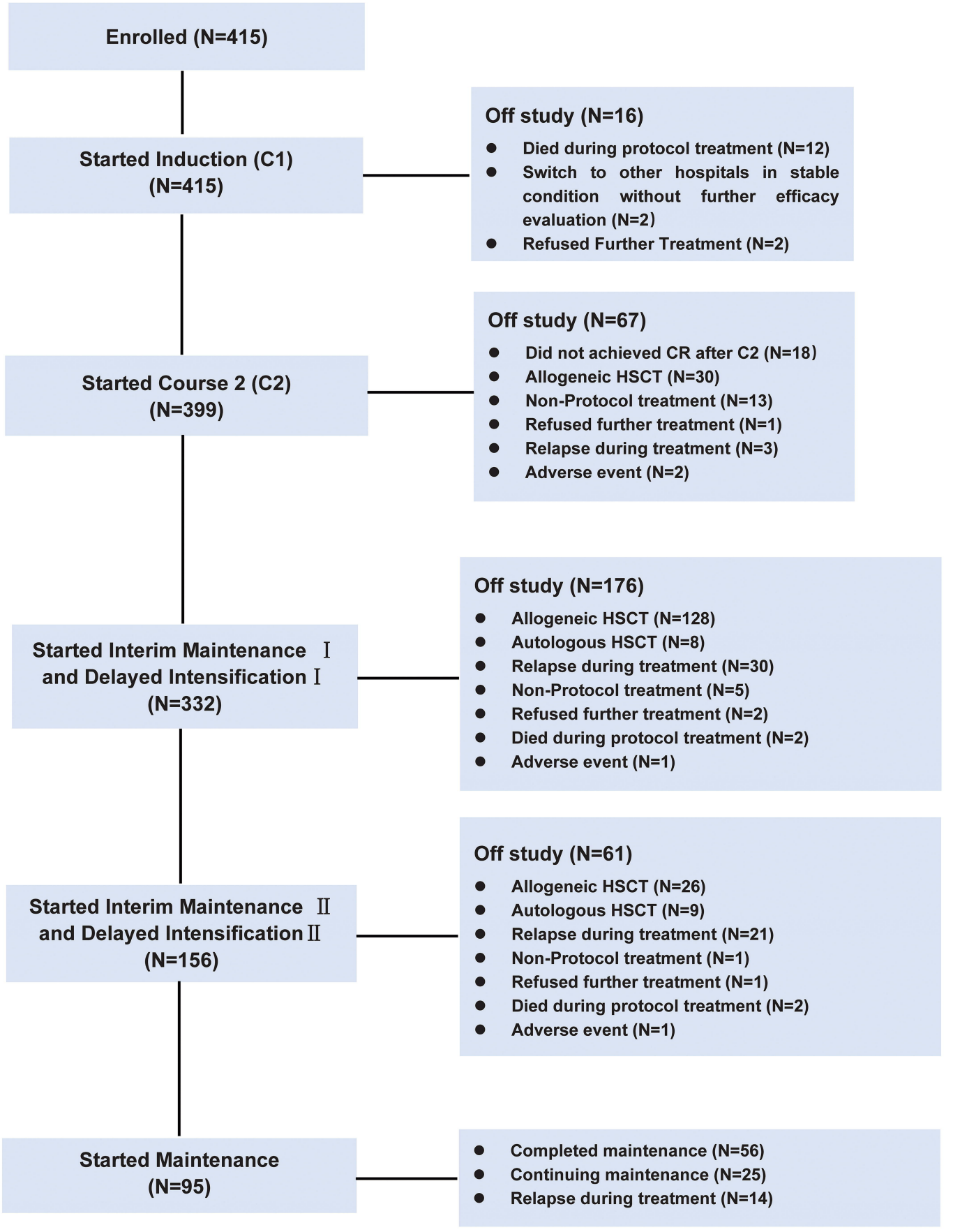

Enrolled (N=415)
Started Induction (C1) (N=415)
Off study (N=16)
Died during protocol treatment (N=12)
Switch to other hospitals in stable condition without further efficacy evaluation (N=2)
Refused Further Treatment (N=2)
Started Course 2 (C2) (N=399)
Off study (N=67)
Did not achieved CR after C2 (N=18)
Allogeneic HSCT (N=30)
Non-Protocol treatment (N=13)
Refused further treatment (N=1)
Relapse during treatment (N=3)
Adverse event (N=2)
Started Interim Maintenance Ⅰ and Delayed Intensification Ⅰ (N=332)
Off study (N=176)
Allogeneic HSCT (N=128)
Autologous HSCT (N=8)
Relapse during treatment (N=30)
Non-Protocol treatment (N=5)
Refused further treatment (N=2)
Died during protocol treatment (N=2)
Adverse event (N=1)
Started Interim Maintenance Ⅱ and Delayed Intensification Ⅱ (N=156)
Off study (N=61)
Allogeneic HSCT (N=26)
Autologous HSCT (N=9)
Relapse during treatment (N=21)
Non-Protocol treatment (N=1)
Refused further treatment (N=1)
Died during protocol treatment (N=2)
Adverse event (N=1)
Started Maintenance (N=95)
Completed maintenance (N=56)
Continuing maintenance (N=25)
Relapse during treatment (N=14)

Figure 2

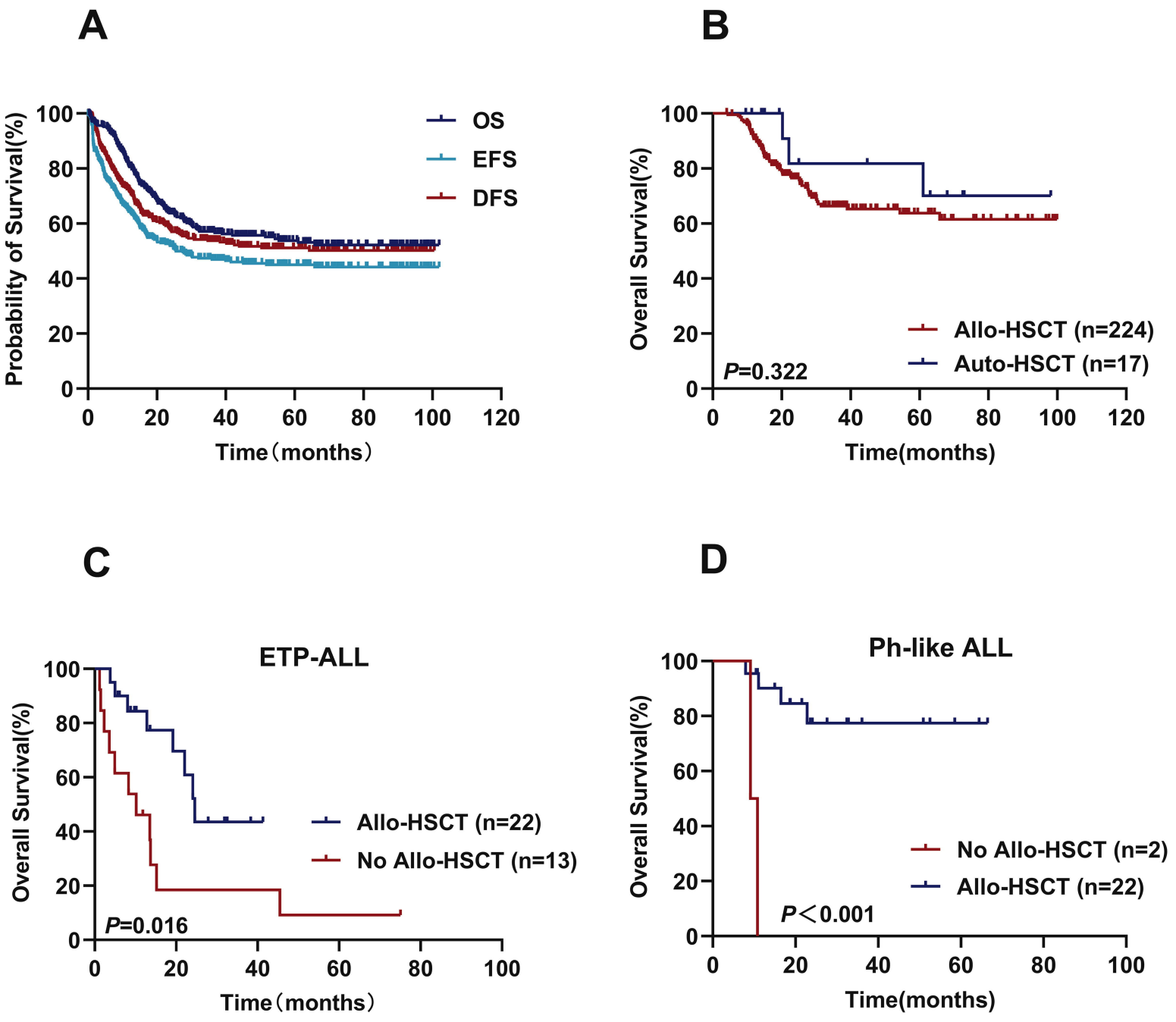
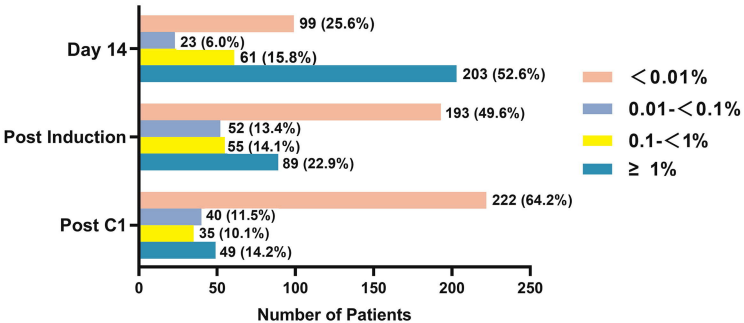

A
Probability of Survival(%)
100
80
60
40
20
0
0
20
40
60
80
100
120
Time（months）
OS
EFS
DFS
B
Overall Survival(%)
100
80
60
40
20
0
0
20
40
60
80
100
120
Time(months)
Allo-HSCT (n=224)
Auto-HSCT (n=17)
P=0.322
C
ETP-ALL
Overall Survival(%)
100
80
60
40
20
0
0
20
40
60
80
100
Time（months）
Allo-HSCT (n=22)
No Allo-HSCT (n=13)
P=0.016
D
Ph-like ALL
Overall Survival(%)
100
80
60
40
20
0
0
20
40
60
80
100
Time(months)
No Allo-HSCT (n=2)
Allo-HSCT (n=22)
P＜0.001

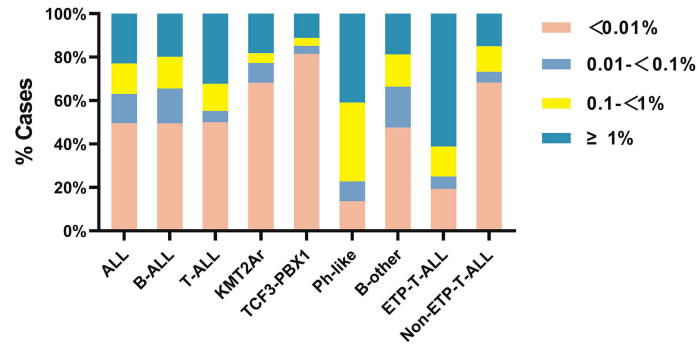
Figure 3

A



Time points	Number of CR patients with different MRD levels (%)			
	＜0.01%	0.01-＜0.1%	0.1-＜1%	≥1%
Day 14 of induction	99 (25.6%)	23 (6%)	61 (15.8%)	203 (52.6%)
Post induction	193 (49.6%)	52 (13.4%)	55 (14.1%)	89 (22.9%)
Post-C1	222 (64.2%)	40 (11.5%)	35 (10.1%)	49 (14.2%)

B



Types of patients	Number of CR patients with different MRD levels at end of induction (%)			
	＜0.01%	0.01-＜0.1%	0.1-＜1%	≥1%
Overall	193 (49.6%)	52 (13.4%)	55 (14.1%)	89 (22.9%)
B-ALL	145 (49.5%)	47 (16.0%)	43 (14.7%)	58 (19.8%)
T-ALL	48 (50.0%)	5 (5.2%)	12 (12.5%)	31 (32.3%)
KMT2A-r	15 (68.2%)	2 (9.1%)	1 (4.5%)	4 (18.2%)
TCF3-PBX1	22 (81.5%)	1 (3.7%)	1 (3.7%)	3 (11.1%)
Ph-like	3 (13.6%)	2 (9.1%)	8 (36.4%)	9 (40.9%)
B-other	106 (47.6%)	42 (18.8%)	33 (14.8%)	42 (18.8%)
ETP-T-ALL	7 (19.4%)	2 (5.6%)	5 (13.9%)	22 (61.1%)
Non-ETP-T-ALL	41 (68.3%)	3 (5.0%)	7 (11.7%)	9 (15.0%)

Figure 4

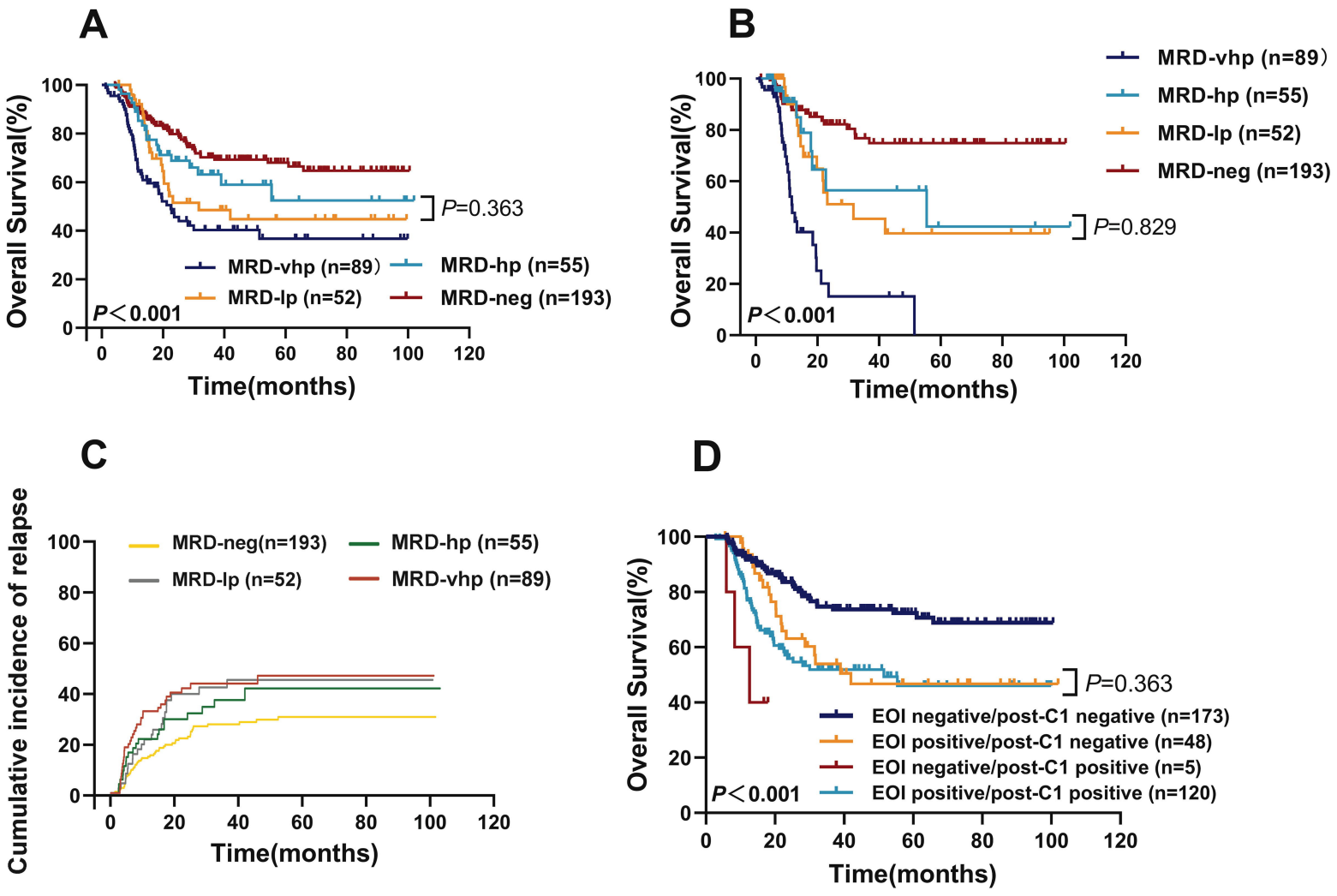
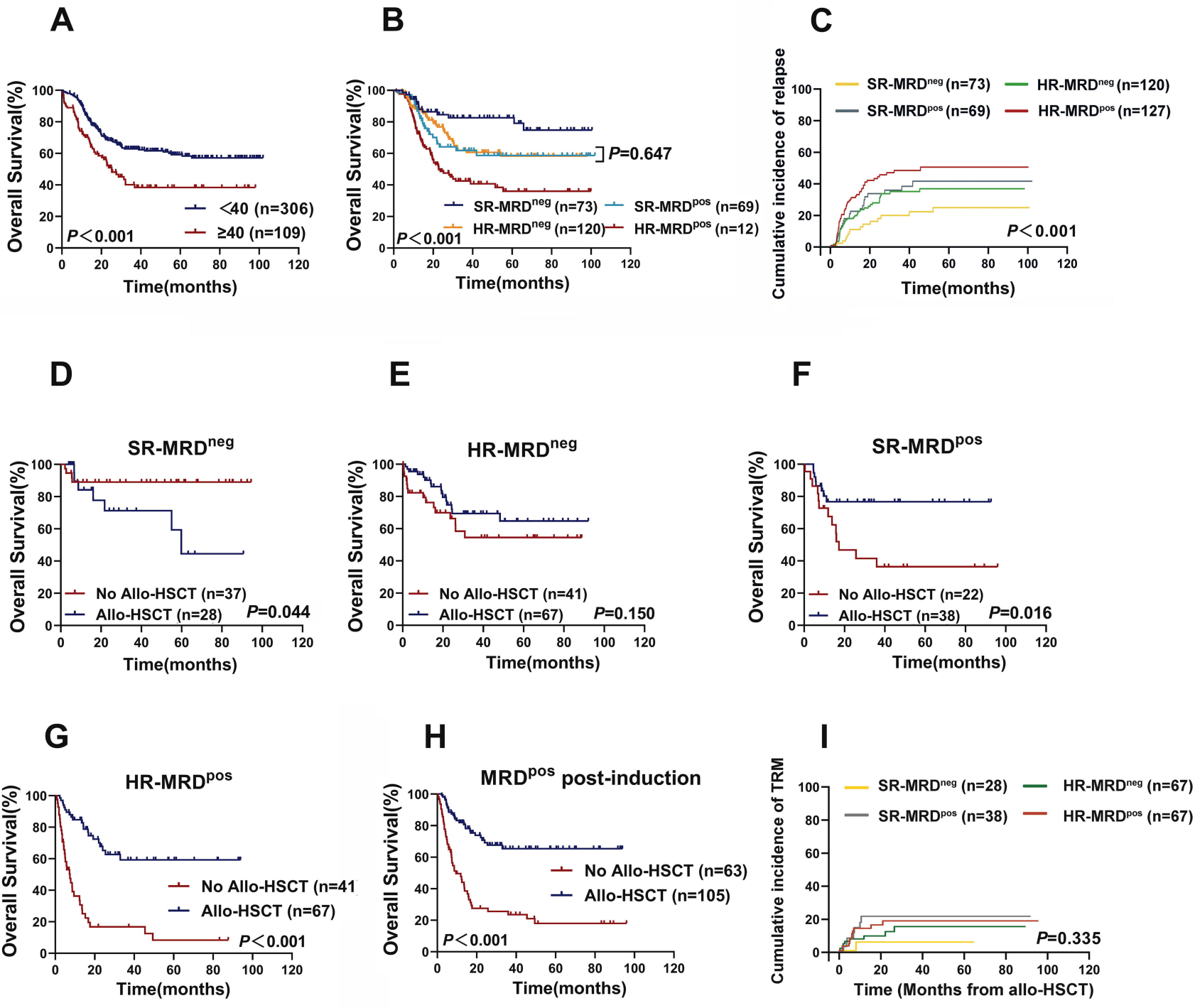

A
Overall Survival(%)
100
80
60
40
20
0
0
20
40
60
80
100
120
Time(months)
P=0.363
P<0.001
MRD-vhp (n=89)
MRD-hp (n=55)
MRD-lp (n=52)
MRD-neg (n=193)
B
Overall Survival(%)
Time(months)
P=0.829
P<0.001
MRD-vhp (n=89)
MRD-hp (n=55)
MRD-lp (n=52)
MRD-neg (n=193)
C
Cumulative incidence of relapse
Time(months)
MRD-neg(n=193)
MRD-hp (n=55)
MRD-lp (n=52)
MRD-vhp (n=89)
D
Overall Survival(%)
Time(months)
P=0.363
P<0.001
EOI negative/post-C1 negative (n=173)
EOI positive/post-C1 negative (n=48)
EOI negative/post-C1 positive (n=5)
EOI positive/post-C1 positive (n=120)

Figure 5


A
Overall Survival(%)
<40 (n=306)
≥40 (n=109)
P<0.001
Time(months)
B
Overall Survival(%)
SR-MRDneg (n=73)
SR-MRDpos (n=69)
HR-MRDneg (n=120)
HR-MRDpos (n=12)
P=0.647
P<0.001
Time(months)
C
Cumulative incidence of relapse
SR-MRDneg (n=73)
HR-MRDneg (n=120)
SR-MRDpos (n=69)
HR-MRDpos (n=127)
P<0.001
Time(months)
D
SR-MRDneg
Overall Survival(%)
No Allo-HSCT (n=37)
Allo-HSCT (n=28)
P=0.044
Time(months)
E
HR-MRDneg
Overall Survival(%)
No Allo-HSCT (n=41)
Allo-HSCT (n=67)
P=0.150
Time(months)
F
SR-MRDpos
Overall Survival(%)
No Allo-HSCT (n=22)
Allo-HSCT (n=38)
P=0.016
Time(months)
G
HR-MRDpos
Overall Survival(%)
No Allo-HSCT (n=41
Allo-HSCT (n=67)
P<0.001
Time(months)
H
MRDpos post-induction
Overall Survival(%)
No Allo-HSCT (n=63)
Allo-HSCT (n=105)
P<0.001
Time(months)
I
Cumulative incidence of TRM
SR-MRDneg (n=28)
HR-MRDneg (n=67)
SR-MRDpos (n=38)
HR-MRDpos (n=67)
P=0.335
Time (Months from allo-HSCT)

Supplementary Material

Methods

Diagnosis

A diagnosis of ALL was established by counting more than 20% lymphoblasts in either peripheral blood (PB) or bone marrow (BM) and the immunophenotypic features of lymphoblasts were confirmed via flow cytometry (FCM). The identification of early T-cell precursor (ETP) ALL relies on the immunophenotypic criteria as outlined in the classification provided by the World Health Organization.[1] The ETP-ALL immunophenotype is defined as follows: (1) expression of cytoplasmic CD3; (2) absent (<5% positive blasts) CD1a and CD8 expression; (3) absent or dim (<75% positive blasts) CD5 expression; and, (4) expression (≥25% positive blasts) of 1 or more myeloid (CD11b, CD13, CD33, CD65, CD117) and/or stem cell (CD34, HLA-DR) markers. To determine cytogenetic analysis, conventional G-banding techniques were utilized. To identify *KMT2A (MLL)* gene rearrangements, fluorescence in situ hybridization (FISH) tests were performed. The Leukemia-Related Fusion Gene Detection Kit (Yuanqi Bio-Pharmaceutical) was utilized to conduct reverse transcription polymerase chain reaction (RT-PCR) for the examination of 43 prevalent fusion genes (Supplementary Table 1), following the guidelines provided by the manufacturer. Since 2017, screening for Ph-like related genes (Supplementary Table 2) via RT-PCR, targeted next-generation sequencing (NGS, Illumina Novaseq), RNA sequencing (RNA seq, Novaseq 6000), and FISH (*CRLF2* rearrangement) were performed on patients with Ph-negative B-ALL who did not present with t(1;19)/*TCF3-PBX1* or t(v;11q23)/*KMT2A* rearrangements. Central nervous system leukemia (CNSL) was defined as a CNS-3 status.[2]

Treatments

Based on the CCG-1961 protocol, we adjusted the protocol according to our medication habits and previous treatment experience. The treatment protocol of this study comprised induction phase, consolidation period, interim maintenance phase, delayed intensification phase, and long-term maintenance therapy. The primary changes to the CCG-1961 protocol are as follows: the use of daunorubicin once a week during induction has been changed to continuous use for 3 days; the use of prednisone in the induction cycle has been changed from 60 mg/m^2 to 1 mg/ kg; the use of methotrexate has been changed from multiple low doses (Capizzi-style) to continuous infusion of high-dose methotrexate; due to the lack of indications for adult patients with ALL in China, all of the pegasparaginase are replaced by Escherichia coli L-

asparaginase; the dosage of vincristine and glucocorticoid in the consolidation and maintenance cycles has been reduced by 1/4 compared to the CCG-1961 protocol. Within the first 3 days of induction, daunorubicin was administered at a dose of 30 mg/m^2/day. On day 14 of induction phase, all patients underwent BM aspiration. Patients with ≥ 10% BM residual leukemic blasts on day 14 of induction received two additional days of DNR at a dose 30 mg/m^2/day on day 15 and 16. The treatment efficacy was evaluated after completing induction phase. Patients who achieved complete remission (CR) received sequential consolidation treatment. Patients who did not achieve CR received the second cycle of chemotherapy in the protocol as salvage treatment. The protocol was withdrawn in patients not achieving CR after two cycles of chemotherapy. Monthly vincristine, mercaptopurine, methotrexate, and prednisone (VMMP) regimen was prescribed as maintenance therapy after completing intravenous chemotherapy (starting after the end of delayed intensification) and was continued for 3 years in male and 2.5 years in female patients. All patients regularly received prophylactic triple intrathecal therapy (methotrexate 10 mg, cytarabine 50 mg, and dexamethasone 10 mg) to prevent CNSL. The total number of prophylactic triple intrathecal injection doses administered ranged from 6–8 doses in patients with hematopoietic stem cell transplantation (HSCT) in CR1 to 16–18 doses in patients with chemotherapy. Patients who were unable or unwilling to receive intrathecal injection were given prophylactic cranial irradiation at a dose of 18 Gy as CNS prophylaxis.

Response evaluation

CR was defined as the disappearance of PB blast cells and the absence of extramedullary disease (ED), and recovery of normal hematopoiesis with neutrophils ≥ 1 × 10^9/L, platelets ≥ 100 × 10^9/L, and lymphoblasts < 5% in the BM. Induction failure (IF) was defined as the inability to attain CR after one course of induction chemotherapy. Relapse referred to disease recurrence at any location (BM or ED) after achieving the first CR. Overall survival (OS) was determined as the period from the time of diagnosis until death from any cause or last follow-up. Disease-free survival (DFS) was determined as the period from CR1 to relapse, mortality from any cause, or last follow-up. Event-free survival (EFS) was determined as the period starting from the time of diagnosis until IF, relapse, death from any cause, or last follow-up. The cumulative incidence of relapse (CIR) was calculated from the time interval between first

CR to first relapse (BM or ED) using a competing risks model. Induction-related mortality (IRM) was defined as mortality during induction chemotherapy or within 2 weeks after the EOI chemotherapy. Treatment-related mortality (TRM) was defined as mortality caused by treatment toxicity instead of disease recurrence or progression. Toxicity was assessed and graded using the Common Terminology Criteria for Adverse Events (CTCAE v 4.0).

MRD assessment

FCM was performed using the EuroFlow ALL panel according to EuroFlow protocols and instrument settings.[3] The antibody combinations for B-ALL were as follows: CD38/CD10/CD34/CD19/CD81/CD20/CD33/CD45. The antibody combinations for T-ALL were as follows: CD7/CD99/CD5/CD16/CD56/CD8/CD4/CD45/TDT/CD2/CD34/CD117/CD33/CD7/CD10/cCD3/CD45. Data were analyzed using the Kaluza software (Beckman-Coulter). The calculation of MRD involved determining the proportion of lymphoblasts within the total count of nucleated cells.

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Supplementary Table 1. 43 Fusion genes screened

BCR::*ABL*	*SIL*::*TAL1*	*E2A*::*HLF*	*TEL*::*AML1*	*MLL*::*AF4*
E2A::*PBX1*	*AML1*::*ETO*	*MLL*::*AF9*	*PML*::*RARa*	*MLL*::*AF6*
MLL::*AF10*	*MLL*::*ELL*	*MLL*::*ENL*	*PLZF*::*RARa*	*STAT5b*::*RARa*
NPM::*MLF1*	*TEL*::*PDGFRB*	*FIP1L1*::*PDGFRA*	*AML1*::*MDS1/EVI1*	*AML1*::*MTG16*
CBFβ::*MYH11*	*DEK*::*CAN*	*TEL*::*ABL*	*ETV6*::*PDGFRA*	*NUP98*::*HoxA13*
NUP98::*HoxC11*	*NUP98*::*HoxD13*	*NUP98*::*HoxA9*	*NUP98*::*HoxA11*	*NUP98*::*PMX1*
TEL::*JAK2*	*MLL*::*AF17*	*MLL*::*AF1q*	*MLL*::*AF1p*	*MLL*::*AFX*
MLL::*SEPT6*	*NPM*::*RARa*	*FIP1L1*::*RARa*	*PRKAR1A*::*RARa*	*NUMA1*::*RARa*
NPM::*ALK,*	*SET*::*CAN*	*TLS*::*ERG*		

Supplementary Table 2. Fusion genes associated with Ph-like ALL

Categories	Fusion genes
ABL1-class fusions	*ETV6*::*ABL1*、*ZMIZ1*::*ABL1*、*NUP214*::*ABL1*、*RCSD1*::*ABL1*、*RANBP2*::*ABL1*、*SNX2*::*ABL1*
ABL2-class fusions	*ZC3HAV1*::*ABL2*、*PAG1*::*ABL2*、*RCSD1*::*ABL2*
JAK2-class fusions	*ETV6*::*JAK2*、*SSBP2*::*JAK2*、*PAX5*::*JAK2*、*TPR*::*JAK2*、*ATF71P*::*JAK2*、*PPFIBPI*::*JAK2*、*STRN3*::*JAK2*、*TERF2*::*JAK2*、*BCR*::*JAK2*、*EBF1*::*JAK2*
PDGFRβ-class fusions	*SSBP2*::*PDGFRβ*、*ZEB2*::*PDGFRβ*、*EBF1*::*PDGFRβ*、*TNIPI*::*PDGFRβ*
Others	*MYH9*::*IL2RB*、*SSBP2*::*CSF1R*、*ETV6*::*NTRK3*、*MYB*::*TYK*

Supplementary Table 3. Protocol for patients ≤55ys

Phase		Agents	Dose	Days of Application
Induction	I. VDCLP	VCR	1.4 mg/m^2 (maximum dose 2 mg) IV	D 1,8,15,22
		DNR	30 mg/m^2 IV	D 1~3, D15~16?
		CTX	1200 mg/m^2 IV	D 1,15
		L-ASP	10,000 u IV	D 5,7,9,11,13,15,17, 19,21,23
		Pred	1 mg/kg/d PO	D 1~14
			0.5 mg/kg/d PO	D 15~28
Consolidation	II. CAMVL	CTX	1000 mg/m^2/d IV	D 1,8
		AraC	100 mg/m^2/d IV	D 1~3, D 8~10
		6-MP	60 mg/m^2/d PO	D 1~14
		VCR	1.4 mg/m^2 (maximum dose 2 mg) IV	D 1,8
		L-ASP	10,000 u	D 5,7,9,11,13,15
	III. CAMVL	Same as cycle II		
Interim maintenance I	IV. HVL	HD-MTX	3 g/m^2 over 24 hours IV	D 1,14
		VCR	1.4 mg/m^2 (maximum dose 2 mg) IV	D 1,14
		L-ASP	10,000u IV	D 3~4, d16~17
Delayed intensification I	V. VDLD	VCR	1.4 mg/m^2 (maximum dose 2 mg) IV	D 1,8,15
		DNR	40 mg/m^2/d IV	D 1,8,15
		L-ASP	10,000 u IV	D 5,7,9,11,13,15
		DEX	8 mg/m^2/d IV or PO	D 1~7, 15~21
	VI. CAMVL	Same as cycle II		
Interim maintenance II	VII. HVL	Same as cycle IV		
Delayed intensification II	VIII. VDLD	Same as cycle V		
	IX. CAMVL	Same as cycle II		
Maintenance	VMMP	VCR	1.4 mg/m^2 (maximum dose 2 mg) IV	D 1
		Pred	1 mg/kg/d PO	D 1~5
		6-MP	60 mg/m^2/d PO	D 1~14
		MTX	20 mg/m^2/week PO	D 8,15

Abbreviations: VCR, vincristine; DNR, daunorubicin; CTX, cyclophosphamide; L-ASP, native Escherichia coli L-asparaginase; Pred, prednisone; AraC, cytarabine; 6-MP, mercaptopurine; HD-MTX, high-dose methotrexate; DEX, dexamethasone; IV, intravenous injection; PO, orally

Supplementary Table 4. Protocol for patients > 55ys

Phase		Agents	Dose	Days of Application
Induction	I. VDCLP	VCR	1.4 mg/m^2 (maximum dose 2 mg) IV	D 1,8,15,22
		DNR	30 mg/m^2 IV	D 1~3, D15~16？
		CTX	1000 mg/m^2 IV	D 1,15
		L-ASP	10,000 u IV	D 5,7,9,11,13,15,17,19
		Pred	1 mg/kg/d PO	D 1~14
			0.5 mg/kg/d PO	D 15~28
Consolidation	II. CAMVD	CTX	750 mg/m^2/d IV	D 1,8
		AraC	100 mg/m^2/d,分 2 次	D 1~3, D 8~10
		6-MP	60 mg/m^2/d PO	D 1~14
		VCR	1.4 mg/m^2 (maximum dose 2 mg) IV	D 1,8
		DEX	10 mg/d IV or PO	D 1~7
	III. CAMVD	Same as cycle II		
Interim maintenance	IV. HVL	HD-MTX	3g/m^2 over 24 hours IV	D 1,8,15
		VCR	1.4 mg/m^2 (maximum dose 2 mg) IV	D 1,8,15
		L-ASP	10,000u IV	D 3~4, D 10~11, D 17~18
Delayed intensification	V. VDLD	VCR	1.4 mg/m^2 (maximum dose 2 mg) IV	D 1,8,15
		DNR	30 mg/m^2/d IV	D 1,8,15
		L-ASP	10,000 u IV	D 5,7,9,11,13,15
		DEX	10 mg/d IV or PO	D 1~7, 15~21
	VI. CAMVL	Same as cycle II		
Maintenance	VMMP	VCR	1.4 mg/m^2 (maximum dose 2 mg) IV	D 1
		Pred	1 mg/kg/d PO	D 1~5
		6-MP	60 mg/m^2/day PO	D 1~14
		MTX	20 mg/m^2/week PO	D 8,15

Abbreviations: VCR, vincristine; DNR, daunorubicin; CTX, cyclophosphamide; L-ASP, native Escherichia coli L-asparaginase; Pred, prednisone; AraC, cytarabine; 6-MP, mercaptopurine; HD-MTX, high-dose methotrexate; DEX, dexamethasone; IV, intravenous injection; PO, orally

Supplementary Table 5. Genomic alterations and frequency in Ph-like ALL

Active 3’ gene	Partner 5’ gene	NO.(%)
CRLF2	IGH	6 (25)
	P2RY8	3 (12.5)
ABL1	NUP214	1 (4.2)
ABL2	RCSD1	3 (12.5)
PDGFRβ	EBF1	2 (8.3)
	TEL	1 (4.2)
JAK2	PAX5	3 (12.5)
	TERF2	2 (8.3)
EPOR	IGH	3 (12.5)

Supplementary Table 6. Univariable and multivariable analysis of prognostic factors for OS

Variable	**NO.**	**Univariate *log rank* Chi-square**	***P* value**	**Multivariate cox regression HR (95% CI)**	***P* value**
Age (≥40y *vs* < 40y)	109/306	15.992	< 0.001	1.653（1.159~2.359）	0.006
Sex (Male *vs* Female)	244/171	0.555	0.456	-	-
WBC count, $\times 10^9$/L (≥30 *vs* < 30)	145/270	2.260	0.133	1.236（0.869~1.758）	0.238
Phenotype (T *vs* B)	103/312	5.711	0.017	1.356（0.932~1.971）	0.111
MRD status at EOI (Positive *vs* Negative)	196/193	18.273	< 0.001	2.089（1.478~2.952）	< 0.001

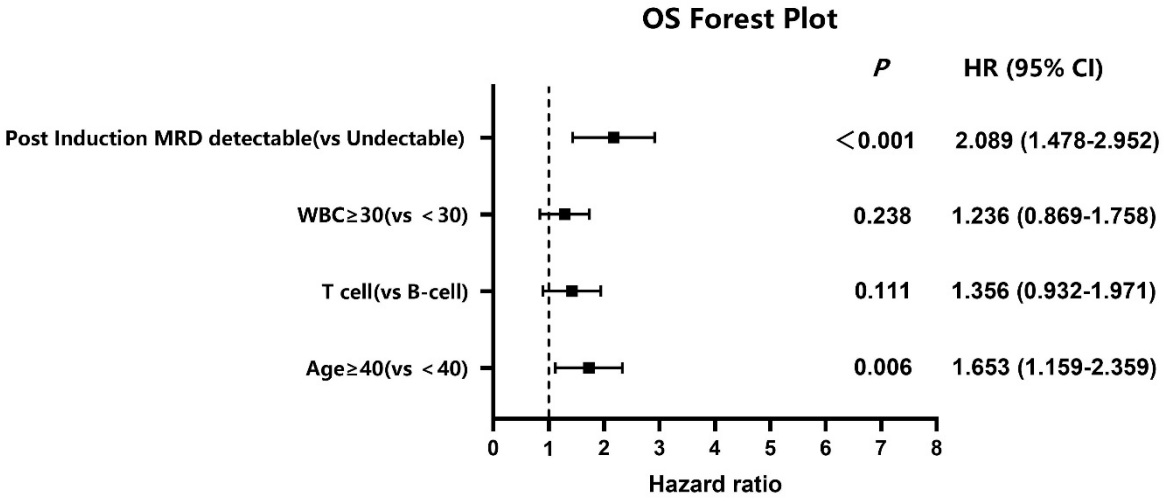
Abbreviations: WBC, white blood cell; MRD, minimal residual disease; EOI, end of induction; -, not included in the model for multivariate analysis; HR, hazard ratio

Supplementary Table 7. Baseline characteristics stratified by allo-HSCT status for patients grouped based on the combination of risk groups and MRD status at EOI

Characteristics	SR-MRD^{neg}			SR-MRD^{pos}			HR-MRD^{neg}			HR-MRD^{pos}		
	Allo (n=28)	No-allo (n=37)	*P*	Allo (n=38)	No-allo (n=22)	*P*	Allo (n=67)	No-allo (n=41)	*P*	Allo (n=67)	No-allo (n=41)	*P*
Proportion of male patients (%)	64.3	56.8	0.54	73.7	45.5	0.029	61.2	51.2	0.309	59.7	51.2	0.388
Median age (range, years)	20 (14-39)	19 (14-37)	0.198	23 (15-39)	23 (14-38)	0.747	28 (14-60)	42 (14-65)	0.016	30 (14-58)	44 (14-64)	0.007
Proportion of B cell immunophenotype (%)	89.3	100	0.075	100	100	-	37.3	63.4	0.008	56.7	73.2	0.086
Median WBC, $\times 10^9$/L	4.95	4.67	0.199	7.89	5.15	0.361	41.05	26.70	0.148	26.85	15.03	0.192
5-year CIR (%)	25.62	15.52	0.174	6.86	68.18	< 0.001	23.54	42.28	0.012	23.14	88.57	< 0.001
3-year TRM (%)	5.55	7.27	0.746	22.22	0	0.047	15.56	2.44	0.166	19.24	4.00	0.112

Abbreviations: allo-HSCT, allogeneic hematopoietic stem cell transplantation; MRD, minimal residual disease; EOI, end of induction; WBC, white blood cell; MRD; CIR, cumulative incidence of relapse ; TRM, treatment-related mortality.

Supplementary Figure 1



Forest plot for multivariate Cox regression of overall survival (OS). Hazard ratio (HR) is depicted on the x-axis, and each prognostic variable is listed on the y-axis. Estimates to the right of 1.0 indicate worse OS.