ARTICLE - Acute Lymphoblastic Leukemia

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

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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.

References

1.Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-405.

2.National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). Acute Lymphoblastic Leukemia (Version 1.2022). https://www.nccn.org/professionals/physician_gls/pdf/all.pdf.

3.Kalina T, Flores-Montero J, van der Velden VH, et al. EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. Leukemia. 2012;26(9):1986-2010.

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

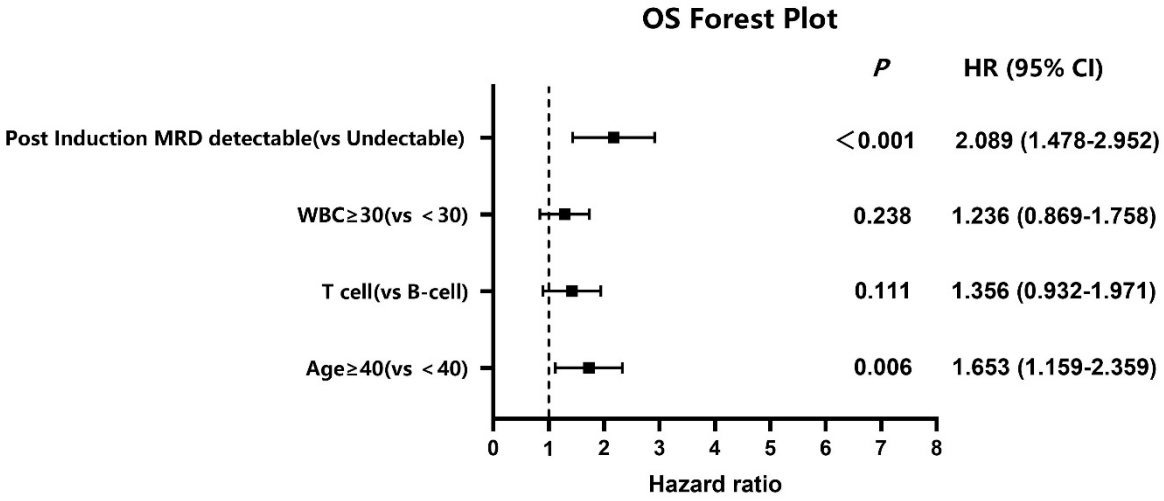
Abbreviations: WBC, white blood cell; MRD, minimal residual disease; EOI, end of induction; -, not included in the model for multivariate analysis; HR, hazard ratio

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.