CCCG-ALL-2015

Initial version, dated: January 1, 2015

Amendment 1: March 1, 2019

Chinese Children Cancer Group Acute Lymphoblastic Leukemia

Study: CCCG-ALL-2015

(Version 2019)

Scientific Committee

Chairman: Prof. TANG Jingyan, Shanghai Children's Research Hospital, Shanghai

Vice Chairmen:
Prof. LI Chi-kong, Prince of Wales Hospital, Hong Kong
Prof. ZHU Xiaofan, Chinese Academy of Medical Sciences &Peking Union Medical College Institute of Hematology & Hospital for Blood Diseases, Tianjin

Members:
Prof. YU Jie, Chongqing Children's Hospital, Chongqing
Prof. HU Shaouyan, Soozhou Children's Hospital, Suzhou
Prof. ZHU Yiping, West China Medical University, Chengdu
Prof. LI Chunfu, South China University Hospital, Guangzhou
Prof. HU Qun, Tongji Hospital of Tongji Medical School, Wuhan
Prof. SHEN Shunhong, Shanghai Children's Medical Center, Shanghai.
Prof. Xiaowen Zhai, Children's Hospital of Fudan University, Shanghai
Prof. Xiuli Ju, Qilu Hospital of Shandong University, Jinan

Contents:

1. Objectives

1.1 Primary Objectives

1.1.1 To conduct a randomized controlled study of the effects of prolonged vincristine and dexamethasone pulses in the maintenance phase of treatment on event-free survival in children with acute lymphoblastic leukemia (ALL)

1.1.2 To conduct a randomized controlled study comparing the effects of imatinib and dasatinib on event-free survival of patients with Philadelphia chromosome positive (Ph+) ALL (**Closed**).

1.2 Secondary Objectives

1.2.1 To establish a platform for clinical research of childhood ALL

1.2.2 To assess the outcome of treatment according to the standardization of risk stratification

1.2.3 To assess the impact of minimal residual disease-directed treatment on clinical outcome in various risk groups

2. Background

Childhood acute lymphoblastic leukemia (ALL) is the commonest pediatric malignancy. However, its incidence is low and multi-center trial is the widely-accepted method to conduct meaningful study to improve outcome. In China, multi-center trial on childhood ALL dated back to year 2005 and established the fundamental elements of large-scale study among mainland centers in treating childhood ALL. In the multi-center SCMC-ALL-2005 study, it showed favorable outcome for low-risk patients but the long-term survival rates for intermediate- and high-risk patients were relatively low. There is a need for further improvement through cooperative study on new treatment regimen, particularly for the intermediate- and high-risk patients. However, there is also higher treatment-related mortality in these high-risk patients, especially chemotherapy-induced bone marrow toxicity. Recently L-asparaginase has been studied extensively on its impact on survival, and some studies suggested that patients received more than 26 weeks of L-asparaginase had increased survival by up to 20%. In this study, we will also adopt a more intensive L-asparaginase treatment approach to achieve a better leukemia control including extramedullary disease.

Despite the high cure rates (70% to 80%) in childhood ALL, resistant forms of this disease still represent a leading cause of cancer-related deaths in children. While efforts are being made to identify new effective anti-leukemia agents or new approaches to therapy, current treatment emphasizes early and vigorous assessment of the risk of relapse in individual patients, so that higher-risk patients are not under-treated or lower-risk patients over-treated.

Risk stratification is therefore the corner stone in deciding treatment regimen and intensity for individual children with ALL. B-cell ALL cases with age between 1 and 10 years and

presenting leukocyte count <50 x 10^9 /L, leukemic cell DNA index ≥1.16, hyperdiploidy or TEL-AML1 fusion have an overall excellent prognosis and are generally considered to have low-risk leukemia. Based on clinical presenting features, leukemia blast genetic abnormalities and response to treatment, patients can be divided into risk group which reflects the probability of leukemia relapse. Advancement in assessing treatment response by means of minimal residual disease (MRD) monitoring which is more sensitive and specific than morphologic examination has significantly improved outcome and its predictive value on survival has gained world-wide acceptance. Flow cytometry and polymerase-chain-reaction for specific leukemia markers are the commonly used methods. Researchers from St Jude Children's Research Hospital have shown that despite favorable presenting features, patients with ≥1% bone marrow blasts at day 19 or 26 of remission induction and those with MRD ≥0.01% on remission date (~ day 46) were at high risk of relapse. A 3-colored flow cytometry MRD assessment on day 19 of induction chemotherapy is capable to reliably assess early treatment response in 98% of patients and this serves as a good indicator for risk group assignment . Another MRD study on day 46 which requires more markers and more technical skill to differentiate leukemic blasts from normal hematogones arising from regenerating bone marrow, will further refine the risk-group and allow escalation of treatment, if necessary. Based on current data, performing MRD study after day 46 improves risk stratification in a small fraction of provisional low-risk patients and identifies another small group of patients with high-risk leukemia with 5% or more blasts at the end of remission induction, but there is also risk of false positive MRD result in patients with active regenerating marrow after chemotherapy if the assay was performed by technologists who lack experience and use fewer leukemia-associated markers. Therefore, regular MRD monitoring after day 46 is not recommended, except for patients who have MRD of 1% or more on day 19 of induction and have persistent positive MRD results.

Because of the generally poor outcome of patients with CNS3 disease, testicular leukemia, or hypodiploidy<44 chromosomes, these patients are treated on the intermediate-risk arm. Patients with T-cell ALL, pre-B-cell ALL with the t(1;19) or *E2A-PBX1* fusion or *MLL* rearrangement fare well provided they receive intensive therapy. Hence, they are also assigned to intermediate-risk group in current study.

In recent decade, there are emergence of new information and data on disease markers and genetic abnormalities which have impact on treatment outcome of childhood ALL. Philadelphia chromosome-like ALL (Ph-like ALL) is one of the recently identified subtype of ALL having a higher proportion of patients with induction failure or relapse. It has similar patterns of gene expression as Ph+ ALL with *IKZF1* gene alteration with activation of tyrosine kinase signaling pathway. Unlike Ph +ve ALL, Ph-like ALL lacks *BCR-ABL1* gene fusion. It is, however, technically demanding to perform testing for Ph-like leukemia and it is not feasible, at least at this moment, to implement screening for this sub-group in daily clinical practice. Nevertheless, majority of these patients with poor outcome could be identified by MRD monitoring with poor early treatment response and excluded from low-risk arm. This protocol will not stratify patients risk category based on the diagnosis of Ph-like ALL but recommend the use of florescent-in-situ-hybridization (FISH) technique to detect presence of gene fusion products with subsequent activation of tyrosine kinase which is not uncommonly found in Ph-like ALL. This includes

detection of ABL1 rearrangement by 3-colors BCR-ABL1- FISH probe and PDEGFRB rearrangement by PDGFRB break-apart FISH probe. Tyrosine kinase inhibitors (TKI) imatinib and dasatinib are reported to be useful in suppressing activity of the tyrosine kinase in a small cohort of Ph-like ALL patient with refractory disease. In this study, Ph-like ALL patients with ABL1 or PDGFRB rearrangement will receive combined TKI and chemotherapy, same as Ph +ve ALL patients.

Early-T-precursor ALL (ETP-ALL) is another recently recognized T-ALL sub-group which carries very poor prognosis with 5-years overall survival less than 20%. These cases can be identified by flow cytometry analysis though not every center includes these markers for ETP-ALL as part of regular immunocytochemistry. It is reported that with intensive treatment, the survival outcome of these patient may be improved and treatment escalation for those who did not respond well to early treatment is necessary. Whether this protocol could offer a satisfactory outcome in this group of patients is a question to be addressed and the pre-requisite to answer this question is to correctly identify this group of patients with proper flow cytometry analysis. Intra-chromosomal amplification of chromosome 21 (iAMP21), which can be detected by FISH analysis, is another genetic abnormality that attracts clinical attention. In general patients with iAMP21 carries poor prognosis. However, it has been shown that the outcome of iAMP21 patients could be improved with intensive chemotherapy and therefore patients with iAMP21 will be stratified to intermediate risk group in this study.

The role of TKI treatment for Ph +ve ALL patients is well established with recent data showing that TKI treatment could yield comparable outcome to hematopoietic stem cell transplant (HSCT). In view of the success of TKI therapy, this study will reserve HSCT only for high-risk Ph +ve ALL patients with suboptimal treatment response (i.e., minimal residual disease ≥1% at the end of remission induction around Day 46). This study will randomize Ph +ve ALL patients to receive either imatinib or dasatinib. Dasatinib has, in theory, higher inhibitory effect in suppressing ABL1 activation than does imatinib and can also inhibit other tyrosine kinase such as Src kinase. In some patients with ABL1 mutation and resistant to imatinib treatment, dasatinib may still have inhibitory effect against kinase activity. In addition, dasatinib has relatively better penetration to CNS with probable better efficacy in treating CNS leukemia than imatinib. Despite the theoretical advantages, randomized clinical trials are needed to examine for any survival benefit or different toxicity profile of dasatinib in comparison with imatinib.

This protocol is based on the St Jude total XV protocol and Total XVI protocol with the following modifications that suit the Chinese patients, and also based on results of some recent research discoveries.

1) 4 days of Dexamethasone (Dex) is used to replace 4 days of methotrexate (MTX) in the window phase of Total-XV
2) Increase number of doses of L-Asparaginase (L-ASP) in induction and reinduction to 10 times in low risk (LR). PEG-Asparaginase (PEG-ASP) is used to replace native L-Asparaginase in intermediate-risk (IR) and high-risk (HR) groups. (In centers that do not have Peg-Asparaginase as frontline treatment, native L-Asparaginase can be used).

3) In the early intensification CAT phase, duration of 6-mercaptopurine (6-MP) is reduced from 14 days to 7 days, whereas Cytarabine (Ara-C) is given in 7 consecutive days. In patients with day 19 MRD ≥ 1% or T-cell ALL, one more course of CAT with Peg-asparaginase and vincristine will be given.
4) High dose methotrexate (HDMTX) will be increased from 2.5 gm/m^2 to 3 gm/m^2 in LR. Dose of 6-mercaptopurine will be reduced from 50 mg/m^2 to 25 mg/m^2 per day.
5) In continuation therapy, Daunorubicin (DNR) is used instead of doxorubicin. PEG-Asparaginase (PEG-ASP) is used to replace native L-Asparaginase in IR and HR groups. Ara-C in reinduction 2 of IR and HR risk arm is advanced to days 1 and 2.
6) For IR and HR groups, CXT＋VCR＋Ara-C+Dex are given in the first week in the maintenance treatment to reduce clinic visit frequency. In the latter phase of maintenance treatment, patients will be randomized to have vincristine and dexamethasone pulses or not.

3. Amendments

Amendment 1

3.1 Amendment regarding Ph+ ALL

Ph+ ALL patients will all be treated with dasatinib

Between January 1, 2015 and September 18, 2018, 225 patients with Ph+ALL were enrolled on the study, of whom 189 were randomized to receive imatinib (n=97) or dasatinib (n=92). Based on an interim analysis, on October 4, 2018, the Data and Safety Monitoring Committee recommended stopping the randomization and treating all Ph+ ALL patients with dasatinib because of superior event-free survival among patients treated with that drug. The scientific advisory committee of the study group reached the same conclusion, leading to implementation of the recommended changes. The study will remain open for Ph+ALL.

3.2 Subsequent adjustments following high MTX concentrations

Even among patients with normal serum creatinine tests, some of them occasionally had serum MTX concentration at hour 44 exceeding 1μM. In the original protocol, we recommended that the MTX dose be reduced in subsequent courses of HDMTX. However, in most patients, the MTX concentration dropped during the subsequent courses. We therefore will not reduce the methotrexate dosage but rather closely monitor these patients for serum creatinine concentration. If the serum creatinine is normal, methotrexate dosage will not be reduced but a 16-hour methotrexate level will be obtained with dosage adjusted as following.

3.2.1 16-hour MTX concentration lower than 100μM

1) For patients with serum creatinine level increased less than 26mM from the baseline, leucovorin rescue will remain the same according to the 44-hour MTX concentration.

2) For patients whose serum creatinine increased by more than 26mM from the baseline but remained in the normal range, the hydration rate should be increased to 150ml/m2/hr and acetazolamide should be given. (Furosemide is not recommended as it may increase renal toxicity and affect urine alkalization). The methotrexate concentration and renal function should be

monitored again at 24 hours.

a. If there is no markedly rise in MTX concentration, measurement of serum creatinine and leucovorin rescue will remain the same, according to the 44-hour MTX concentration.

b. If the 24-hour MTX concentration or creatinine are progressively elevated, leucovorin rescue at 30mg/m^2 will be started at 36 hours, and renal function will be monitored again. For patients with rising creatinine level, leucovorin rescue will be increased to 60mg/m^2.

3) For patients with confirmed impaired renal function, methotrexate infusion should be stopped immediately, and MTX concentration and renal function monitoring should be performed at 24 hours or earlier (at least 4 hours after cessation of MTX administration), the 44-hour MTX concentration could be predicted:

$$MTX_{44h} \approx MTX_{t2} * \left[\frac{MTX_{16h} - MTX_{t2}}{MTX_{16h}}\right]^{\left(\frac{44-t2}{t2-t1}\right)+1}$$

Here, t1 is the time of MTX infusion stopped, t2 is the time of blood drawn for MTX retesting a few hours after stoppage of administration, MTX44h, MTX16h, MTXt1, and MTXt2 are the respective MTX concentrations at 44 and 16 hours from the start of MTX infusion as well as at t1 and t2.

a. For patients whose predicted 44-hour MTX concentration is less than 1μM, leucovorin will still be performed according to the 44-hour MTX concentration.

b. For patients whose predicted 44-hour MTX concentration is 1-10μM, 30mg/m2 leucovorin should be given early from 36 hours.

c. For patients whose predicted 44-hour MTX concentration is greater than 10μM, hemodialysis should be started as soon as possible (The MTX plasma protein binding rate is about 50 %, and earlier dialysis can reduce binding rate and increase the dialysis effect).

3.2.2 16-hour MTX concentration greater than 100μM

1) If MTX is 100-149.9μM, MTX infusion should be stopped at 20 hours, and the hydration rate should be increased to 175ml/h/m^2.

2) If MTX is MTX150>μM, MTX infusion should be stopped at 18 hours, and the hydration rate should be increased to 200ml/h/m^2.

3) The MTX concentration and renal function were monitored again at 24 hours;

a. If the 24-hour MTX is <50μM, leucovorin will be given according to the 44-hour MTX concentration; (anticipating a 6-hour half-life, the 48-hour MTX concentration will drop to 1 μM or less).

b. If the 24-hour MTX is >50μM, but less than half of the 16-hours level, leucovorin should be given earlier than 36 hours and adjusted according to the 44-hour concentration (anticipating a 6-hour half-life, the 48-hour MTX concentration will fall to between 1-10μM).

c. If the 24-hour MTX concentration is greater than half the 16-hour level, hemodialysis should be started as soon as possible.

4. Eligibility criteria and subject enrollment

4.1 Inclusion criteria

1. Diagnosis of acute lymphoblastic leukemia and
2. Age range from 1 month to 18 years (inclusive).

4.2 Exclusion criteria

1. Diagnosis of mature-B ALL, acute mixed phenotype leukemia (not including ALL with aberrant expression of myeloid markers)
2. Secondary malignancy OR blastic transformation from CML
3. Presence of underlying immunodeficiency syndromes
4. Had exposure to glucocorticoid for more than 1 week, in the period between 1 month to 1 week before enrollment, or had chemotherapy or radiotherapy in the 3 months prior to enrollment (with the exception of emergency radiotherapy to alleviate compression symptoms)

4.3 Randomization studies

4.3.1 Low risk group

Starting from week 54, low risk arm A (LR-A) will receive Dex / VCR / 6-MP / MTX and arm B (LR-B) will receive 6-MP / MTX

4.3.2 Intermediate / high risk group

Starting from week 54, arm A (I/HR-A) will receive Dex / VCR / CTX / Ara-C and arm B (I/HR-B) will receive CTX / Ara-C

4.3.3 Ph + ALL: **(closed)**

Upon confirmation of the diagnosis, these patients will be randomized to receive either Imatinib or Dasatinib and continued till completion of chemotherapy (closed). This randomization is terminated on Oct. 2018 and Ph + patients will receive Dasatinib (see section 3.1); Ph + ALL are not eligible for the randomized study on the effect of prolonged vincristine and dexamethasone pulses.

4.4 Informed consent

All patients need to have informed consent signed by parents / legal guardian before enrollment into the study including consent for chemotherapy treatment, consent for randomization, and consent for banking of biological samples and the use of left-over samples for future research. For patients whose parents / legal guardian consented for study but declined randomization, parents / legal guardian has the right to select their preferred treatment arm, but they will be excluded from statistical analysis in the corresponding randomized study.

5. Diagnostic work up and follow up investigations

5.1 All newly diagnosed patients must have the following investigations

5.1.1 Complete blood picture, peripheral blood blast count

5.1.2 Chest X-ray

5.1.3 Liver function test, renal function test, uric acid, lactate dehydrogenase (LDH), PT / APTT, virology study for hepatitis B (HBsAg, anti-HBs, anti-HBc) and hepatitis C (anti-HCV),

blood group

5.1.4 ECG, echocardiogram

5.1.5 CT / MRI brain if suspected CNS disease

5.1.6 Bone marrow aspirate for morphology and cytochemisry

5.1.7 Immunohistochemistry

-B lineage: CD10, CD19, TdT, cyμ, IgM, CD20, cyCD22, CD22, cyCD79a

-T lineage: CD1a, CD2, CD3, CD4, CD5, CD7, CD8, TCRαβ, TCRγδ, cyCD3

-Myeloid: CD11b, CD13, CD14, CD15, CD33, CD41, CD61, CD64, CD65, CD71, GPA, cyMPO

-Others: CD34, HLA-DR, CD117, CD45

5.1.8 MRD flow cytometry markers

B-ALL

	FITC	PE	PerCP	PE-cy7	APC	V500
1	IgG1	IgG1	CD34	CD10	CD19	CD45
2	CD38	CD24	CD34	CD10	CD19	CD45
3	CD38	CD58	CD34	CD10	CD19	CD45
4	CD38	CD73	CD34	CD10	CD19	CD45
5	CD38	CD86	CD34	CD10	CD19	CD45
6	CD38	CD97	CD34	CD10	CD19	CD45
7	CD38	CD99	CD34	CD10	CD19	CD45
8	CD38	CD200	CD34	CD10	CD19	CD45
9	CD9	CD44	CD34	CD10	CD19	CD45
10	CD66c	CD123	CD34	CD10	CD19	CD45
11	CD15	CD133	CD34	CD10	CD19	CD45
12	CD15	NG2	CD34	CD10	CD19	CD45

T-ALL

	FITC	PE	PerCP	APC	V500
1	CD5	CD34	cyCD3	CD19+CD33+HLA-DR	CD45
2	TdT	CD5	cyCD3	CD19+CD33+HLA-DR	CD45
3	CD5	CD99	cyCD3	CD19+CD33+HLA-DR	CD45

Simplified bone marrow MRD assessment on day 19 can be performed with the following flow cytometry combination, which has been shown to be applicable in 98% of patients, irrespective of the selected specific panel of flow cytometry markers determined at diagnosis and should be performed in all cases.

B-ALL: CD19, CD10, CD34

T-ALL: CyCD3, TdT

5.1.9 Cytogenetic studies

Conventional cytogenetic study by G-banding of metaphases together with florescent in-situ

hybridization (FISH) analysis for the following gene fusion / rearrangements:

-BCR-ABL1: 3-color FISH probe will be used to identify rearrangements involving ABL1 gene and is applicable to B-ALL and T-ALL. For follow up purpose in patients with known BCR-ABL1 gene fusion, dual color FISH probe can be used.

-MLL rearrangement: MLL break-apart FISH probe will be used to look for MLL gene rearrangement in B-ALL and T-ALL.

-TEL-AML1: FISH study will be used to confirm the finding of PCR study. It can also help to look for the presence of iAMP21 in B-ALL.

-PDGFRB rearrangement: PDGFRB break-apart FISH probe will be used in B-ALL

-C-myc rearrangement: C-myc break-apart FISH probe will be used in cases with mature B-ALL and lymphoma. The test should also be performed in patients with cyμ and / or sIgM positivity and patients with L3 FAB morphology.

5.1.10 PCR for gene fusion study

-TEL/AML1

-E2A/PBX1

-MLL/AF4

-BCR/ABL1

5.2 Performance status by Eastern Cooperative Oncology Group (ECOG) scoring

ECOG Grade				
0	1	2	3	4
Fully active, able to carry on all pre-disease performance without restriction	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work	Ambulatory and capable of all self-care but unable to carry out any work activities	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair

5.3 Treatment response assessment

5.3.1 Bone marrow morphological evaluation criteria: M1: blast cells <5%; M2: blast cells 5% to <25%; M3: blast cells >25%.

5.3.2 Early treatment response is assessed on day 19 with bone marrow examination and MRD assessment. MRD ≥1% signifies poor early treatment response.

5.3.3 Induction remission status assessment (Day 46 assessment): After CAT chemotherapy, bone marrow examination is performed when the following hematological criteria are fulfilled:

WBC ≥ 1.5x10^9/L, ANC ≥ 0.3x10^9/L, platelet count ≥ 50x10^9/L (usually between day 43 to day 46 and may be postponed for a maximum of 7 to 10 days). MRD assessment is performed only for intermediate-risk/high-risk patients or provisional low-risk patients with MRD >0.1% on Day 19. For MRD ≥ 1%, provisional low-risk patient will be escalated to high risk; for MRD ≥ 0.01%, provisional low-risk patient will be escalated to intermediate risk (For patients with MRD ≥ 1%, the result should be reconfirmed with alternative method of analysis. Consider repeating the test in SCMC laboratory).

5.3.4 High-risk patients will have bone marrow examination with MRD monitoring at 1 to 2 months before HSCT. MRD of < 0.01% before HSCT is preferable. Re-intensification chemotherapy (DAEL) can be given once or twice for those with positive MRD status (i.e. ≥ 0.01%) before transplant.

5.3.5 For patients do not have MRD marker (simplified MRD on day 19 included), bone marrow morphology status M2 is equivalent to MRD >1%.

5.3.6 For D46 MRD-positive (≥0.01%) patients, MRD will be tested after 4 courses of HDMTX treatment. If still positive, MRD will be tested before each reinduction treatment course for L/I/HR patients and before the 3rd continue treatment for I/HR patients until MRD-negative.

6. Risk stratification

6.1 Risk stratification criteria

Low Risk Group	Moderate Risk Group	High Risk Group
1. Required condition (B-ALL fulfilling one of the following conditions) ① Age ≥ 365 days and < 10 years and presenting WBC ≤50 x 10^9/L; or ②Hyperdiploidy with chromosome number ≥ 50 or DNA index ≥1.16; or ③TEL-AML1 gene fusion positive 2. The following situations must be excluded ① CNS 3 and/or testicular leukemia ② t (1;19), t (9;22), MLLr, hypodiploidy (<44 chromosomes), iAMP21 ③19 MRD ≥ 1%	1. Ph＋ALL 2. T-ALL 3. MLLr: Age 6≥ months or WBC<300×10^9/L 4. hypodiploidy (<44 chromosomes) 5. All cases that do not meet the criteria for low risk or high risk	1. Day 46 MRD≥1%; 2. MLLr-ALL: Age <6 months, and WBC≥300×10^9/L

Patients are classified into one of three categories (low-, intermediate-, or high-risk) based on the presenting age, leukocyte count, presence or absence of CNS-3 status or testicular leukemia, immunophenotype, cytogenetic and molecular genetics abnormalities, hyperdiploidy or DNA index, and early response to therapy. Hence, definitive risk assignment (for provisional low-risk or intermediate-risk cases based on presenting features) will be made after completion of remission induction therapy. The criteria for entry into each category are provided below:

6.1.1 Criteria for low-risk ALL

- B-cell ALL fulfilling one of the following criteria:

1. Age ≥ 365 days and < 10 years and presenting WBC ≤50 x 10^9/L; or
2. Hyperdiploidy with chromosome number ≥ 50 or DNA index ≥1.16; or
3. TEL-AML1 gene fusion positive

- MUST not have:

1. CNS 3 status (≥ 5 WBC/μL of cerebrospinal fluid with morphologically identifiable blasts or cranial nerve palsy)
2. Overt testicular leukemia
3. Adverse genetic features: t(9;22) or BCR-ABL fusion; t(1;19) or E2A-PBX1 fusion; rearranged MLL (as measured by FISH and / or PCR); or hypodiploidy (<44 chromosomes); iAMP21
4. Poor early response with day 19 MRD ≥ 1%, OR day 19 MRD ≥0.1% and day 46 MRD ≥0.01%.

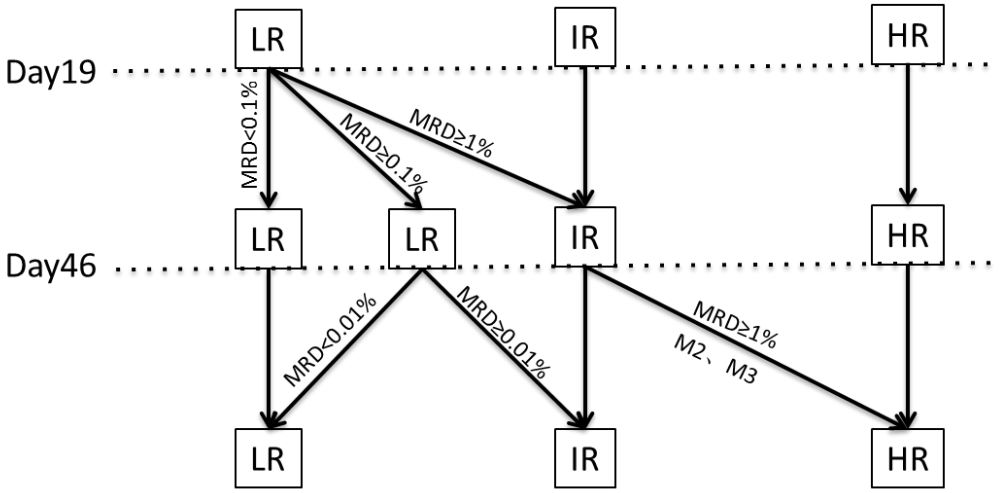
6.1.2 Criteria for intermediate-risk ALL

1. T-cell ALL
2. Ph +ve ALL
3. Presence of rearranged MLL with age ≥ 6 months at presentation or presenting WBC < 300x10^9/L
4. Hypodiploidy with chromosome number < 44
5. All cases that do not meet the criteria for low risk or high risk

6.1.3 Criteria for high-risk ALL

1. Intermediate risk patient with induction failure (presence of ≥ 5% blast by morphological assessment of bone marrow aspirate on day 46 for patients without MRD marker or MRD ≥ 1%)
2. Infants <6 months with MLL rearrangement and presenting WBC ≥ 300x10^9/L.

6.2 Flow diagram of risk stratification adjustment



7. Treatment regimen

7.1 Induction chemotherapy

Drug	R G^	Dose	Route	Frequency	Time (Day)	Remark
Window phase and PVDL induction						
Dex*	L / I / H	6 mg/m^2/day	IV / PO	Bid	1-4	For WBC ≥ 50x10^9/L, give one dose of Dex 3 mg/m^2 on day 0
Pred#	L / I / H	45 mg/m^2/day T-cell:60 mg/m^2/day	PO	Bid or Tid	5-28	taper over 7 days from day 29 to day 35
VCR	L / I / H	1.5 mg/m^2	IV	Once	5, 12, 19, 26	Max 2 mg
DNR§	L / I / H	25 mg/m^2	IV	Once	5, 12	
L-Asp	L	6,000 U/m^2	IM	Alternate days	6 to 24, 10 doses.	If D 19 MRD >1%, immediately switch to PEG-Asp 2,000 U/m^2 for 1 dose (alternatively 6 doses more L-Asp).
Peg-Asp	I/H	2000U/ m^2	IM		6,26	May be interchanged with Erwinase 10,000 U/m^2 x 6, or L-asp 6000 U/m^2 x 6 over two weeks for each dose of Peg-ASP,
IT@	L	Refer to section 6.1.2			5, 19	If CNS-2 or traumatic tap with blast, add IT on day 8, 12 and 15
	I				5, 12, 19	Add IT on day 8, 15 for T-ALL, CNS-2, CNS-3, or traumatic tap with blast.
	H				5, 8, 12, 15, 19	
CAT						
CTX	L / I / H	1000mg/m^2	IV over 1 hour	Once	29	If WBC > 4.0 x 10^9/L, and ANC > 1.0 x 10^9/L, start CAT on D 27. If WBC < 2.0 x 10^9/L or ANC < 0.8 x 10^9/L, postpone CAT till D 33.
Ara-C	L / I / H	50mg / m^2	SC	Every 12 hours	29 to 35, 14 doses	
6-MP	L / I / H	60mg/m^2/day	PO	Daily	29 to 33, 7	

					doses	If counts still below above cutoff, start CAT with halved 6-MP and Ara-C.
IT	L / I / H	Refer to section 6.1.2			29	
CAT$^+$ for T-ALL or day 19 MRD ≥ 1%						
VCR		1.5 mg/m^2	IV	Once	50, 57	To start at least 2 weeks after completion of CAT. If WBC < 2.0 x 10^9/L or ANC < 0.8 x10^9/L or platelet < 80 x10^9/L, can postpone *CAT$^+$* by 1 week. If counts remain below above cutoff, start *CAT$^+$* with halved 6-MP and Ara-C.
Peg-Asp		2000 U/m^2	IM	Once	50	
CTX		1000 mg/m^2	IV over 1 hour	Once	50	
Ara-C		50 mg/m^2	SC	Every 12 hours	50- 56(14doses)	
6-MP		60 mg/m^2/day	PO	Daily	50 -56(7doses)	
IT		Refer to section 6.1.2			50	

^ RG, risk group; L, Low risk; I, Intermediate risk; H, High risk

***** Shortening of duration of Dexamethasone is allowed for prior exposure to glucocorticoid for more than 4 days in the week before induction, but a minimum of 2 consecutive days of full dose corticosteroid is needed before L-Asp. The day after Dexamethasone window period is counted as day 5. Perform PB blast count on day 5.

Pred, prednisolone

§ Day 12 DNR in LR patients with WBC < 1.0x10^9/L or (absolute neutrophil count) ANC < 0.3x10^9/L can be postponed for 4 to 7 days. After 7 days, if WBC or ANC remains below the above mentioned criteria and there is absence of blast in peripheral blood (PB), 2nd dose DNR can be omitted.

7.1.1 Induction

Dexamethasone window (Days 1 to 4)

Dexamethasone 6 mg/m^2 PO / IV in BID dose from days 1 to 4

For presenting WBC ≥ 50 x 10^9/L, give one dose of dexamethasone 3 mg/m^2 and count as day 0. Shortening of duration of dexamethasone is allowed for prior exposure to glucocorticoid for more than 4 days in the week before induction, but a minimum of 2 consecutive days of full dose corticosteroid is needed before L-Asp. The day after dexamethasone window phase is counted as day 5. Perform peripheral blood (PB) blast count on day 5.

PVDL (Days 5 to 28)

- **Prednisolone (Pred)**
  45 mg/m^2/day (T-cell ALL: 60 mg/m^2/day) PO from days 5 to 28 in tid, taper over 1 week from days 29 to 35.
- **Vincristine (VCR)**
  1.5 mg/m^2/dose (Max. 2mg) IV bolus on day 5, 12, 19, 26
- **Daunorubicin (DNR)**
  25 mg/m^2/dose IV infusion over 1 hour on day 5 and day 12
- Day 12 DNR in patients with WBC < 1.0x10^9/L or (absolute neutrophil count) ANC < 0.3x10^9/L can be postponed for 4 to 7 days. After 7 days, if WBC or ANC remains below the above mentioned criteria and without blast in peripheral blood, 2nd dose DNR can be omitted in LR patients.
- **E. Coli L-asparaginase (L-Asp)**

LR patients: 6000 U/m^2/dose IM on alternate days from day 6 to 24, for total of 10 doses (i.e. days 6, 8, 10, 12, 14, 16, 18, 20, 22, 24) [Alternatively, 1 dose pegylated E.coli asparaginase (PEG-Asp) 2,000 U/m^2]. For MRD on day 19 ≥ 1%, immediately switch to PEG-Asp at 2000U/m^2/dose for 1 dose ((alternatively 6 doses more L-Asp, i.e. 3 times per week for 2 weeks).

IR/HR patients: Peg-asparaginase 2000 U/m^2/dose IM infusion over 1 hour on days 6 and 26.

Note:

Peg-asparaginase 2000 U/m^2 one dose, Erwinase 10000 U/m^2 three times per week for 2 weeks, or L-asp 6000 U/m^2 3 times per week may be interchangeable each other if necessary.

CAT (Day 29 to day 35)

- Cyclophosphamide (CTX) 1000 mg/m^2 iv on day 29
- Ara-C 50mg/m^2, q12h, day 29 to day 35 (14 doses)
- 6-MP 60mg/m^2/day, p.o., day 29 to 35 (7 doses)
- IT chemotherapy: day 29 with age-based dosage as in section 6.1.2

Requirement for beginning CAT chemotherapy

- Good general status
- No severe infection
- WBC ≥ 2.0x10^9/L, ANC ≥ 0.8x10^9/L, platelet ≥ 80x10^9/L, can postpone start of CAT to day 33 if blood counts not meeting the minimal requirement
- Can start on day 27 if clinical condition is suitable. If WBC > 4.0 x 10^9/L, and ANC > 1.0 x 10^9/L.

7.1.2 Intrathecal (IT) chemotherapy

As a traumatic lumbar puncture with blast in CSF may result in a poorer outcome and the need for extra intrathecal therapy subsequently, the first lumbar punctures will be performed by experienced personnel.

Triple intrathecal chemotherapy (IT) dosage is age-dependent as following:

Age (Months)	Methotrexate (mg)	Cytarabine (mg)	Dexamethasone (mg)	NS
<12	6	15	2.5	6ml
12 to 35	9	25	2.5	6ml
≥ 36	12.5(max)	35	5	10ml

Low-risk patients receive IT on day 5, day 19, day 29.

Low-risk patients with any of the following features will receive additional triple intrathecal treatment on days 8, 12 and 22:

1. CNS-2 disease (< 5WBC/μL of CSF with blasts)
2. Traumatic LP (>10 RBC/μL of CSF)

Intermediate-risk patients receive IT on day 5, day 12, day 19, day 29.

Intermediate-risk patients with any of the following features will receive additional triple intrathecal treatment on days 8 and 15:

1. T-cell ALL
2. CNS-3 status (i.e., ≥5 WBC/μL of CSF with blasts or cranial nerve palsy)
3. CNS-2 status (< 5 WBC/μL of CSF with blasts)
4. Traumatic tap (> 10 RBC/μL of CSF)

High-risk patients receive IT on day 5, day 8, day 12, day 15 and day 19, day 29.

7.1.3 CAT^+ (Day 50 – 56)

For patients with T-cell ALL or day 19 MRD ≥ 1%, give CAT^+ at an interval of at least 2 weeks after completion of CAT

Requirement for CAT^+

- WBC ≥ 2.0 x 10^9/L, ANC ≥ 0.8 x10^9/L and platelet ≥ 80 x10^9/L.
- Postpone 1 week if blood count requirement not fulfilled. If blood counts remain below the requirement after 1 week, commence CAT^+ with 50% dose reduction for 6-MP and Ara-C.
- Vincristine 1.5 mg/m^2 (Maximum 2 mg), IV on day 50, day 57
- Peg-Asp 2000 U/m^2, IM on day 50.
  Alternative is to give L-Asp 6000 U/m^2 IM three times per week for 6 doses
- Cyclophosphamide (CTX) 1000 mg/m^2 on day 50
- Ara-C 50mg/m^2, q12h, day 50 to day 56 for 14 doses
- 6-MP 60mg/m^2/day, p.o., day 50 to 56 for 7 doses
- IT chemotherapy: day 50

7.2 Consolidation

The dosage adjustment of HDMTX depends on creatinine clearance and or renogram results to assessment renal function. The dosage for subsequent HDMTX shall be adjusted according to the results of serum MTX concentrations at hour 44 of the previous treatment course. The original dosage will not change if MTX concentrations between 0.5mmol/L ~1mmol/L. The dosage will increase 20% if MTX concentrations less than 0.5mmol/L (maximum for LR is 3g/m2, maximum for IR is 5g/m2). Please see section 3.2 if MTX concentrations at hour 44 exceeded 1μM.

Requirements for starting consolidation

- No severe infection
- ANC ≥ 0.3x10^9/L, WBC ≥ 1.5x$10^{\wedge 9}$/L, and platelet count ≥ 50 x 10^9/L

7.2.1 High dose Methotrexate (HDMTX) and 6-MP dosage

Consolidati	Schedule	1 (Week 8)	2 (Week10)	3 (Week12)	4 (Week14)	Usage Method
	MTX	LR:3g/ m^2 IR/HR:5 g/ m^2	As previous	As previous	As previous	1) Pre-hydration: 100ml/m^2*h 12h or 200ml/m^2*h 2-4h;
	6-MP	25 mg/m^2 qn×14	As previous	As previous	As previous	2) Hydration: 3000ml/m^2/d, d1-d3;

		days				3) 1/10 of the total dose should be administered IV over 30 minutes. 9/10 of the total dose should be administered IV over subsequent 23.5 hours; 4) Alkaline Urine: Starting on d1 use 5% $NaHCO_3$ 5ml/kg for 3 days. Urine pH > 7 and <8 should be maintained. 5) Monitor MTX concentration at 44 hours.
	Leucovorin	15 mg/m^2, q6h×3 times at 42 hours after MTX start	As previous	As previous	As previous	
	IT	D1	As previous	As previous	As previous	
	Adjustments: 1) ANC <0.3×109/L or WBC <1×109/L or platelet <50 x 109/L or ALT> 5 times normal value or TBIL >34μmol/L，DBIL >24μmol/L require delay of HDMTX treatment; 2) The starting dose of MTX for patients with renal dysfunction is adjusted according to sCr ; 3) Leucovorin rescue: Patients with history of severe mucositis or typhlitis after previous HDMTX, 5 doses of leucovorin rescue should be given in subsequent HDMTX. For patients with clinical signs and symptoms of MTX toxicity within 36 hours after start of HDMTX infusion, leucovorin rescue can be advanced to 36 hours after start of HDMTX 4) Leucovorin rescue should be given more times to patients with abnormal sCr until the MTX concentration is less than 0.1μm (or below the lab detection minimum). Daily sCr test will be done until sCr reaches normal. 5) 6-MP may be held in the presence of ANC < 0.3 x109/L, WBC < 1.5x109/L, platelet count < 50 x109/L 6) See section 3.2 for patients with a 44h [MTX] >1μmol/L.				

6-Mercaptopurine (6-MP)

6-MP 25 mg/m^2/day will be given from day 1 to day 56. In patients for whom high dose methotrexate treatment is delayed, 6-MP may be continued until 14 days after the last course of high dose methotrexate. 6-MP may be held in the presence of ANC < 0.3 x10^9/L, WBC < 1.5x10^9/L, platelet count < 50 x10^9/L or grade 3 or 4 mucositis. Dosage of 6-MP in subsequent courses may be reduced in patients who have prolonged neutropenia after HDMTX and 6-MP treatment.

High-dose methotrexate (HDMTX)

HDMTX is administered on day 1, 15, 29 and 43. The dosage of HDMTX is dependent on the risk classification of individual patients. Patients with IR or HR ALL will receive 5 gm/m^2 and those with LR ALL 3 gm/m^2, administered over 24 h IV. The subsequent HDMTX, 6-MP and IT will be delayed if ANC < 0.3 x10^9/L, WBC < 1.5 x10^9/L, platelet count < 50 x10^9/L, ALT > 5 times the normal upper limit or total bilirubin > 34μmol/L, direct bilirubin > 24umol/L or presence of mucositis.

Assessment sCr is needed before administration of each dose of HDMTX. Give HDMTX only if creatinine within normal range for age.

Low risk patient

- **Methotrexate 3 gm/m^2/dose**

-- 1/10 of the total dose (i.e. 300 mg/m^2) should be administered IV over 30 minutes as a loading dose.

- 9/10 of the total dose (i.e. 2700 mg/m^2) should be administered IV over subsequent 23.5 hours.

Intermediate / high risk patient

- **Methotrexate 5 gm/m^2/dose**

-- 1/10 of the total dose (i.e. 500 mg/m^2) should be administered IV over 30 minutes as a loading dose.

- 9/10 of the total dose (i.e. 4500 mg/m^2) should be administered IV over subsequent 23.5 hours.

Hydration before and during MTX infusion

Good urine output should be established at least in the period starting from 4 hours before to 72 hours after HDMTX infusion by administration of adequate IV fluid, at 3000 ml/m^2/day. During the methotrexate infusion, patients should receive hydration fluid with $NaHCO_3$. Urine pH > 7 and <8 should be maintained at least from 4 hours before to 72 hours after HDMTX infusion by alkalinization of IV fluid.

7.2.2 Leucovorin rescue and MTX level monitor

Leucovorin (LCV / Folinic acid) rescue: 15 mg/m^2, IV for 3 doses, at 42 hours, 48 hours, 54 hours after the start of HDMTX infusion.

Serum methotrexate level monitoring is mandatory for all risk groups. Check MTX level at 44 hours after the start of infusion. Give 3 doses of LCV and stop if 44 hours MTX level < 1 μmol/L. Otherwise continue LCV rescue and forced alkaline diuresis until serum MTX level falls to safe level. Adjust dose of LCV according to MTX level, which has to be monitored daily:

[MTX] μM（44~48h）	[MTX] μM（68~72h）	LCV	Hydration rate
≤1.0	≥*DL and ≤0.4	15mg/m^2	
1.0< [MTX] ≤2.0	0.4< [MTX] ≤0.5	30mg/m^2	150ml/m^2.h
2.0< [MTX] ≤3.0	0.5< [MTX] ≤0.6	45mg/m^2	150ml/m^2.h
3.0< [MTX] ≤4.0	0.6< [MTX] ≤0.8	60mg/m^2	175ml/m^2.h
4.0< [MTX] ≤5.0	0.8< [MTX] ≤1.0	75mg/m^2	175ml/m^2.h
5.0< [MTX] ≤6.0	1.0< [MTX] ≤1.5	90mg/m^2	200ml/m^2.h
6.0< [MTX] ≤7.0	1.5< [MTX] ≤2.0	100mg/m^2	200ml/m^2.h
7.0< [MTX] ≤8.0	2.0< [MTX] ≤3.0	120mg/m^2	200ml/m^2.h
8.0< [MTX] ≤9.0	3.0< [MTX] ≤4.0	140mg/m^2	200ml/m^2.h
9.0< [MTX] ≤10	4.0< [MTX] ≤5.0	160 mg/m^2	200ml/m^2.h
>10	>5	200mg/m^2+CRRT+PE	

Patients with history of severe mucositis or typhlitis after previous HDMTX, 5 doses of leucovorin rescue should be given in subsequent HDMTX. For patients with clinical signs and symptoms of MTX toxicity within 36 hours after start of HDMTX infusion, leucovorin rescue can be advanced to 36 hours after start of HDMTX.

7.2.3 Intrathecal chemotherapy

All patients will receive triple intrathecal therapy every other week for four doses on Days 1, 15, 29, and 43 (dosages are age-based, see section 7.1.2) during consolidation. The intrathecal injection should be on the same day of HDMTX infusion.

7.3 Bone marrow assessment post consolidation in HR patients

Bone marrow examination and MRD assessment should be performed for HR patients after consolidation, 1 to 2 months before planned HSCT.

HR patients should be prepared for HSCT after consolidation, which will be performed before start of maintenance chemotherapy. MRD negativity (< 0.01%) before HSCT is desirable. For patient with MRD ≥ 0.01%, consider 1 to 2 courses of re-intensification chemotherapy (DAEL), with number of cycles determined by MRD status.

Re-intensification chemotherapy (DAEL)

Drug	Dosage	Schedule
Dexamethasone	20 mg/m^2/day; BD	Days 1 – 6
Ara-C	2 gm/m^2/dose; q12h (3 hours IV infusion)	Days 1 – 2
VP16	100 mg/m^2/dose; q12h (1-hour IV infusion)	Days 3 – 5
PEG-asp	2,000 U/m^2 (1-hour IV infusion)	Day 6
IT	See section 7.1.2	Day 5

7.4 Continuation therapy

Post-remission continuation therapy begins 2 weeks after the 4th course of HDMTX of consolidation treatment, if ANC ≥ 0.5x10^9/L, WBC ≥ 2x10^9/L and platelet count ≥ 50x10^9/L and no evidence of mucositis. Continuation therapy is composed of 2 treatment phases, the first half being interim maintenance and re-induction therapy; the second half being maintenance therapy.

7.4.1 Table of Continuation Therapy (interim maintenance and reinduction)

Week	LR	IR / HR
1	6-MP + Dex +VCR (IT)	Dexa + DNR + VCR + 6-MP + Peg-Asp (IT)
2	6-MP + MTX	6-MP
3	6-MP + MTX	6-MP
4	6-MP + Dex + VCR (IT)	Dexa + DNR + VCR + 6-MP + Peg-Asp (IT)
5	6-MP + MTX	6-MP
6	6-MP + MTX	6-MP
7	Reinduction 1 (IT)	Dexa + DNR + VCR + 6-MP +Peg-Asp (IT)
8	Reinduction 1	6-MP
9	Reinduction 1	6-MP
10	6-MP + MTX	Dexa + DNR + VCR + 6-MP +Peg-Asp (IT)
11	6-MP + MTX	6-MP
12	6-MP + MTX	6-MP
13	6-MP + Dex +VCR (IT)	Dexa + DNR + VCR + 6-MP +Peg-Asp (IT)
14	6-MP + MTX	6-MP
15	6-MP + MTX	6-MP
16	6-MP + MTX	6-MP
17	Reinduction 2 (IT)	Reinduction (IT)
18	Reinduction 2	Reinduction
19	Reinduction 2	Reinduction

7.4.2 Continuation Therapy drug dosages

6-MP：$ LR 50 mg/m^2 /d, d1~7, qn＃ HR 25 mg/m^2 /d, d1~7, qn＃ MTX：25 mg/m^2 , po, d 1＃$ PEG-Asp：2000U/ m^2 , im/iv，d3＊	VCR：1.5 mg/m^2 (Max.2.0 mg),d 1 DNR：25 mg/m^2, d 1＃ Dex：LR 8mg/m^2 /d, d1~7, BID HR 12 mg/m^2 /d, d1~5, BID

＃ 6-mercaptopurine, methotrexate and Daunorubicin will be held if ANC < 0.5 x 10^9/L, WBC < 2 x 10^9/L or platelet < 50 x 10^9/L.

＊Peg-asparaginase 2000 U/m^2 one dose, Erwinase 20000 U/m^2 two times per week for 3 weeks, or L-asp 20000 U/m^2 3 times per week may be interchangeable each other if necessary.

$ If within 48 hours after termination of dexamethasone, WBC and ANC do not double in number, reduce dose of 6-MP and MTX by 30~50%

If 1 week after start of dexamethasone, WBC and ANC drop below the value before start of dexamethasone with WBC < 4x10^9/L and ANC < 1 x10^9/L:

- Stop 6-MP and MTX for at least one week and resume 6-MP and MTX when WBC ≥ 2x10^9/L and ANC ≥ 0.5x10^9/L.

- **Dex (Dexamethasone)**

  LR 8 mg/m^2/day, day 1 to 7, PO, divided in BID

  IR / HR 12 mg/m^2/day, day 1 to 5, PO, divided in BID

- **DNR (Daunorubicin)**

  IR/HR: 25 mg/m^2/dose IV, on day 1

- **VCR (Vincristine)**

  1.5 mg/m^2 IV bolus (Max. 2mg), on day 1

- **6-MP (6-mercaptopurine)**

  LR 50 mg/m^2/day, PO, day 1 to 7, at bedtime

  IR / HR 25 mg/m^2/day, PO, day 1 to 7, at bedtime

  Withheld 6-MP if ANC < 0.5x10^9/L or WBC < 2x10^9/L or platelet < 50x10^9/L

- **MTX (Methotrexate)**

  25 mg/m^2, PO, on day 1

- **Asparaginase**

  IR / HR: Peg-Asparaginase 2000 U/m^2 IM， on day 3, in week 1, 4, 7, 10 and 13.

  or L-asparaginase 20,000 U/m^2 IV on day 3 of each week, from week 1 to 16

- **IT chemotherapy** (See section 7.1.2 for age-based dose)

  LR Day 1 of week 1, 4, 7 and 13

  IR /HR Day 1 of week 1, 4, 7, 10 and 13

7.4.3 Reinduction

7.4.3.1 LR reinduction 1 and 2

LR patients will receive 2 re-inductions during continuation therapy. Each reinduction lasts for 3 weeks with reinduction 1 from week 7 to 9 and reinduction 2 from week 17 to 19.

Drug	Dose and route	Schedule
Dexamethasone	8 mg/m^2/day, PO, BD	Days 1 - 7, Days 15 - 21
Vincristine	1.5 mg/m^2 IV (Max. 2mg)	Days 1, 8, 15
Daunorubicin	25 mg/m^2 IV	Day 1 (Reinduction 1 only)
L-asparaginase*	6000 U/m^2 IV	Days 3, 5, 7, 9, 11, 13, 15, 17, 19, 21
IT	See section 7.1.2	Day 1

* Alternatively, one dose of PEG-asparaginase 2,000 U/m^2 on day 3 or Erwinase 10000 U/m^2, qod, 10 times.

7.4.3.2 IR / HR reinduction

IR / HR patients will receive 3 weeks of reinduction at week 17 to 19 of continuation therapy. High dose (HD) cytarabine will be held if ANC < 0.5 x 10^9/L or WBC < 2 x 10^9/L. It is preferable to start HD-cytarabine when WBC $\geq$ 2.0x10^9/L and ANC $\geq$ 0.5x10^9/L.

Drug, dose and schedule of reinduction therapy for IR / HR

Drug	Dose and route	Schedule
Dexamethasone	8 mg/m^2/day, PO, BD	Days 1 - 7, Days 15 - 21
Vincristine	1.5 mg/m^2 IV (Max. 2mg)	Days 1, 8, 15
Ara-C	2 gm/m^2, IV, q12h	Days 1, 2
PEG-asparaginase*	2,000 U/m^2 IM or IV	Day 3
IT	See section 7.1.2	Day 1

* L-asparaginase 20,000 U/m^2 IV on day 3, 10, 17 or Erwinase 20000 U/m^2 two times per week for 3 weeks can be used as substitute of Peg-asp.

Daunorubicin, cyclophosphamide, cytarabine, methotrexate and mercaptopurine may be reduced by 30 to 50% if WBC is between 1.0 and 1.5 x10^9/L with ANC $\geq$ 0.3 x10^9/L and platelet count $\geq$ 50 x 10^9/L. Disproportionate dose reduction of one agent compared to another should be avoided unless clinically indicated.

7.4.4 Maintenance chemotherapy

Week	LR		IR / HR	
1	MTX + 6-MP		MTX + 6-MP	
2	MTX + 6-MP		MTX + 6-MP	
3	MTX + 6-MP		CPM + VCR + Ara-C + Dexa + IT	
4	6-MP + Dexa + VCR + IT (IT only in cycles 1 to 4)		Rest week	
4 weekly cycles for 5 cycles				
	LR-A	LR-B	I/HR-A*	I/HR-B*
1	MTX + 6-MP	MTX + 6-MP	MTX + 6-MP	MTX + 6-MP
2	MTX + 6-MP	MTX + 6-MP	MTX + 6-MP	MTX + 6-MP
3	MTX + 6-MP	MTX + 6-MP	MTX + 6-MP	MTX + 6-MP

4	MTX + 6-MP	MTX + 6-MP	MTX + 6-MP	MTX + 6-MP
5	MTX + 6-MP	MTX + 6-MP	MTX + 6-MP	MTX + 6-MP
6	MTX + 6-MP	MTX + 6-MP	MTX + 6-MP	MTX + 6-MP
7	MTX + 6-MP	MTX + 6-MP	CTX + Ara-C + Dex + VCR	CTX + Ara-C
8	MTX+ 6-MP + Dex + VCR	MTX + 6-MP	Rest week	Rest week
	8 weekly cycles for 7 cycles			
	Continue 6-MP and MTX, with total treatment duration being 2.5 years, counting from start of induction chemotherapy			

* ALL Ph+ALL cases are in arm A.

Dose of Chemotherapy during Maintenance phase:

6MP (mercaptopurine) will be given as 50 mg/m^2/day, PO.

MTX (Methotrexate) 25 mg/m^2, PO, on day 1

Dex (Dexamethasone) Cycle 1-5, 8 mg/m^2/day, day 1 to 7, PO, divided in Bid

From randomization onward, 6 mg/m^2/day, day 1 to 7, PO, divided in Bid

VCR (Vincristine) 1.5 mg/m^2 IV bolus (Max. 2mg), on day 1

CTX (Cyclophosphamide) 300 mg/m^2 IV, Day 1

Ara-C (Cytarabine) 300 mg/m^2 IV, Day 1

Mercaptopurine and methotrexate will be reduced if WBC and ANC do not increase by at least 2 folds a week after the start date of dexamethasone pulse. Exception can be made in patients whose WBC and ANC do not double but are over 3 and 1.2 x10^9/L respectively with no history of prior chemotherapy interruption or myelosuppression. In Intermediate Risk patients consider reducing cyclophosphamide and cytarabine dose if suspected to contribute to myelosuppression. On the weeks of vincristine and dexamethasone pulses, mercaptopurine may be reduced by 30% to 50% in patients with WBC $\leq$ 1.5 x10^9/L the week after prior dexamethasone pulse. In Intermediate Risk patients consider reducing cyclophosphamide and cytarabine dose.

Dosage of continuation treatment should be titrated to keep WBC between 1.8 and 3.0 x10^9/L, ANC between 0.5 and 1.2 x10^9/L, and platelet count $\geq$ 50 x 10^9/L.

8. Toxicity，complication and dose adjustment

Table-Therapy related adverse event scoring

	0	1	2	3	4	5
Neutrophils (10^9/L)	Normal	≥1.5 <2.0	≥1.0 <1.5	≥0.5 <1.0	<0.5	Death
PLT (10^9/L)	Normal	≥75 <100	≥50 <75	≥25 <50	<25	Death
Mucositis	Normal	No painful ulcer/mucosal erythema; no mild pain from open wounds	Painful ulcers/mucosal erythema which affect drinking and eating	Painful ulcers/mucosal erythema which require intravenous fluid	Painful ulcers/mucosal erythema which require intravenous nutrition;	Death

				infusions; contact ulcerative bleeding	some tissue necrosis.	
Infection	Normal		Local infections, only require local treatment.	Venous using antimicrobial agents required.	Event posing a risk to life: Shock, hypotension, acidosis, necrosis	Death

8.1 Cardiotoxicity:

If any symptoms related to cardiac insufficiency or arrhythmia arise during the treatment, ECG and cardiac ultrasound or arrhythmia examinations should be performed. When a cardiac function test indicates that the heart ejection fraction <55% or the fractional shortening <28%, if the left ventricular function abnormality can be proved to be connected with bacterial infections, then anthracyclines antibiotic agents can continue to be used. Otherwise, such agents should be suspended, until the ejection fraction ≥ 55% or the fractional shortening ≥ 28%.

8.2 Hepatotoxicity:

During the chemotherapy, except as required for monitoring tumor lysis syndromes in the initial treatment course, liver functions should be re-examined before the next treatment course.

8.2.1. Aminotransferase increase: No adjustment is needed if a pre-chemotherapeutic pure ALT or AST increase did not exceed 5 times the normal upper limit, whereas in the case of 5 times or more, only HDMTX should be postponed, and no change of treatment plans is needed for other chemotherapeutic drugs. All chemotherapies should be postponed if ALT reaches 10 or more times its normal upper limit. Simple elevated ALT in a given treatment course will not affect any chemotherapy. The effect of liver-protective drugs is unclear, and they might affect the curative efficacy of chemotherapeutic drugs; thus they are not suggested for routine use.

8.2.2. Bilirubin increase: For a DBIL increase during chemotherapy, chemotherapeutic dosages are adjusted according to the following table after leukemic infiltration can be excluded as the cause; otherwise the chemotherapy is to be performed as normal. For DBIL≥24μmol/L before each treatment course, postpone the chemotherapy for 1 week; then if DBIL does not decrease to an optimal level, the chemotherapy can be started by adjusting chemotherapeutic dosages according to the following table.

DBIL	Dosage
24~<51μmol/L	Reducing 50%
51~<85 μmol/L	Reducing 75%
≧85 μmol/L	Stop drugs

When TBIL≥34μmol/L, DNR and VCR should be adjusted accordingly, and returned to full dose after DBIL has recovered to<24μmol/L. When TBIL ≥ 34μmol/L, especially when there is mucositis, L-ASP should be stopped, and low and high dose MTX should be stopped or reduced in dosage if TBIL was ≥ 34μmol/L.

8.3 Neurotoxicity:

8.3.1. Cytarabine: Ataxia, nystagmus, dysarthria or dysmetria are commonly seen symptoms related to the nervous system toxicity caused by cytarabine. However, a large dosage of cytarabine might also result in convulsions or other cerebropathy symptoms. Cytarabine should be omitted if the symptoms are significant and affect normal life of child patients. The patients should be drop off the protocol and switch to other regimen to avoid such cytarabine related neurotoxicity.

8.3.2. Vincristine dosage should not exceed 2mg. Commonly-seen mild toxicity includes mandibular pain, constipation, and weakening of deep reflexes. Sometimes there may be dysphonia, but it should be identified to avoid being misunderstood as candida laryngitis. If there is continuous presence of abdominal cramps, unsteady gait, severe pain, or syndrome of inappropriate antidiuretic hormone secretion (SIADH), vincristine dosages should be reduced or vindestine instead, which is less neurotoxic. Vincristine should be stopped if there is any cecitis, and vindestine instead after the recovery of the patient.

8.4 Pulmonary toxicity:

If patients have dyspnea, hypoxemia (SpO_2<92%), and bilateral pulmonary infiltrations as indicated by chest X-ray, with infections excluded, and left ventricular insufficiency with anthracyclines excluded as a cause, then ARDS caused by cytarabine should be considered. Such patients should receive chest CT scans to identify whether they are suffering pulmonary infections. Meanwhile, an appropriate number of cardiac ultrasound examinations on left ventricular function should be performed and cardiotoxicity caused by anthracyclines should be excluded. As conditions allow, pediatric pulmonologists may be invited to perform consultations. Methylprednisolone is recommended to be used for ARDS that has been identified as caused by cytarabine: 1 mg/kg IV qd for patients with mild symptoms; 500 mg/m^2 IV qd for patients with severe symptoms (reducing dosages after 4 days). The exact treatment course is to be determined according to varying disease situations. Other glucocorticoids also could be used.

8.5 Renal dysfunction

The corrected creatinine clearance (CCR) value, Y = reported CCR value×1.73/the actual body surface area.

8.5.1. Cytarabine: patients with renal dysfunction might have delayed elimination of cytarabine, leading to hematological and non-hematological toxic adverse effect. Therefore, hydration fluid should be given orally or intravenously to patients who have serum creatinine >176μmol/L or > 2 times of normal value. Creatinine clearance rate (CCR) or isotope renogram should be done to check the glomerular filtration rate (GFR). A cytarabine dosage of more than 1000mg/m^2 may be given if CCR ≥ 60 ml/min/1.73m^2. The dosing interval is extended to one time per day if CCR <60 ml/min/1.73m^2.

8.5.2. HDMTX：Nephrotoxic drugs (e.g., acyclovir) may cause subclinical renal dysfunction with decreased glomeration filtration rate. That kind of drugs should be postponed to 20 hours after

completion of HDMTX or after MTX has been adequately eliminated. MTX should be postponed or its dosage adjusted based on CCR.

Corrected CCR (Y) (ml/min)	Dosage (%)
70-85	80%
55-70	70%
40-55	50%
20-40	40%

8.6 L-Asparaginase allergy:

L-ASP allergy may be manifested as positive skin test, rashes, bronchospasm, shock, laryngeal edema, or red, hot, swollen and tender injection site. If there is any allergic reaction, alternative L-ASP agent may be used. Allergic responses may be accompanied by the L-ASP neutralizing antibody that inactivates the enzyme. Therefore, cross-reactions between different L-ASP agents should be taken into consideration when an alternative drug is being chosen. Currently there are three types of L-ASP agents commonly used in our country: native Escherichia coli L-ASP, PEG-ASP (Pegaspargase) and Erwinia L-ASP. The former two are extracted from Escherichia coli with similar antigenicity, and are cross-reactive; therefore, it is preferable to monitor plasma drug concentration when native Escherichia coli L-ASP is replaced by PEG-ASP. Erwinia L-ASP has a smaller probability of cross-reaction with the former two, but its half-life is only one half of that of the native Escherichia coli L-ASP. The Table below shows the dosage and schedule for optimal use of the three types of L-ASP agents.

Agents \ Dosage	1,000 units	2,000 units	6,000 units	10,000 units	20,000 units
PEG-ASP	2 weeks	3 weeks	--	--	--
Native Escherichia coli L-ASP	--	--	3 days	--	1 week
Erwinia L-ASP	--	--	--	2 days	4 days

8.7 L-Asparaginase and pancreatitis:

For abdominal pain that is suspected to relate to pancreatitis, an abdominal ultrasound or CT examination must be performed. The treatment for pancreatitis induced by L-ASP is the same as that for pancreatitis caused by other reasons. Sandostatin may be used, 2.5~4μg/kg/1~3 times/day, by subcutaneous or intravenous.

Mild to moderate pancreatitis is defined by abdominal pain lasting less than 72 hours, and

serum amylase and lipase levels less than 3 times the normal upper limits. For patients with mild or moderate pancreatitis, L-ASP must be suspended and may be resumed only when the clinical symptoms and physical signs have disappeared, and amylase and lipase levels have returned to normal. Because the recurrent risk is approximately 15% with the resumption of L-ASP, we suggested to resume with Erwinia L-ASP at 10000U/m^2 qod as the first choice because of its shorter half-life. The second choice is native L-ASP given at 6000U/m^2 qod. PEG-ASP is not recommended. During the re-challenge period, Sandostatin may be administered concurrently to prevent recurrence of pancreatitis.

Severe pancreatitis is defined by abdominal pain lasting 72 hours or longer and the serum amylase and lipase levels 3 times or more of their normal upper limits, and evidenced by ultrasound or CT imagine. All L-ASPs should be immediately stopped when a severe pancreatitis occurred. If the pancreatitis is caused by glucocorticoids, purinethol or other reasons not related to L-ASP, L-ASP may be considered again after pancreatitis is resolved. L-ASP should be permanently omitted if severe pancreatitis is caused by ASP. After L-ASP has been omitted, MTX and/or 6-MP dosages may be adjusted higher to the limit of tolerance in the continuation treatment stage; no extra chemotherapeutic drugs are added.

For asymptomatic cased with increased amylase or lipase level, L-ASP should also be suspended. It may be used again under strict monitoring after pancreatitis has been excluded as a cause by a gastroenterologist. Magnetic resonance cholangiopancreatography should be performed to exclude pancreatemphraxis before re-challenge with L-ASP, similar to patients with mild to moderate pancreatitis .

8.8 Individualized maintenance treatment:

During the maintenance treatment, routine blood test is needed twice a week initially and then once every 1-2 week after the dosage is titrated. Liver and kidney functions (including ALT, AST, total bilirubin level, direct bilirubin level, BUN, Creatinine) should be checked monthly.

During the maintenance treatment, dosages should be adjusted to keep WBC between 1.8×10^9/L and ~3.0×10^9/L, absolute neutrophil count (ANC) between 0.5×10^9/L and ~1.2×10^9/L (except for the week after dexamethasone treatment), and platelet count ≥50×10^9/L. When WBC<2.0×10^9/L, ANC <0.5×10^9/L or platelet <50×10^9/L, chemotherapy should be stopped for at least a week until the count is recovered. The dosages should be reduced by 30% when the chemotherapy is re-started. When WBC >4.0×10^9/L（or ANC >1.5×10^9/L) and 6-MP dosage had been held <25%, then the dosage should be increased by 30%. When WBC≥2.0×10^9/L (or ANC≥0.5×10^9/L), and platelet ≥50×10^9/L, then full dosages may be resumed.

Dexamethasone and vincristine can be given regardless of the blood counts as long as the general condition of patient is good.

If WBC and ANC do not double in count a week after dexamethasone administration, 6-MP and MTX dosages should be reduced by 30~50%; if WBC and ANC are less than or equal to their initial value or WBC<$4.0x10^9$/L or ANC<$1.0x10^9$/L after dexamethasone treatment, 6-MP and MTX

should be stopped for at least one week, until WBC≥2.0x10^9/L and ANC≥0.5x10^9/L, and then resume 6-MP and MTX with dosages decreased by 30%.

Human parvovirus B9 infections, hemolysis or other factors not related to chemotherapeutic drugs should be considered (e.g., septra or bactrim) when any anemia (HGB<70 g/l) or neutropenia (ANC<0.3×10^9/L) lasts for more than 3 weeks and fails to be explained by other reasons during complete remission period.

Maintenance therapy adjustment because of abnormal liver or kidney functions is the same as HDMTX. Liver and kidney functions should be checked once a week during the adjustment period.

8.9 Neutropenia and fever or mucositis symptoms:

All chemotherapeutic drugs, except VCR/glucocorticoids/L-ASP, should be suspended until temperature/CRP/mucositis return to normal with negative blood culture after an episode of neutropenia with fever or overt mucositis. If the patient has received 20 days or 80% of the chemotherapy dosages of the course, this course of treatment should be considered completed and will not be repeated.

8.10 Requirements for blood counts, liver and kidney function before each treatment course:

The therapy can be commenced if WBC ≥2.0×10^9/L, ANC ≥0.5×10^9/L, platelet ≥80×10^9/L, total bilirubin level <34μmol/L, direct bilirubin level <24μmol/L, and ALT<5 times of its normal upper limit. If WBC or ANC do not meet required conditions, but platelet count has recovered to normal level for more than one week, then chemotherapy may be commenced. Otherwise, one week of observation is required during which the chemotherapy may be commenced if required conditions are met. A bone marrow examined may be performed for evaluation if patients still fail to meet the required conditions. For patients with bone marrow suppression, additional week of observation is required, and parvovirus infection should be suspected for which IVIG 500mg/Kg x 4 times if diagnosis is confirmed. The chemotherapy could be commenced as soon as the patients meet the required conditions. In case they still fail to meet the required conditions, then chemotherapy with 20% dosage reduction, except VCR, glucocorticoids and L-ASP, is commenced.

8.11 Prophylaxis Antibiotics

8.11.1 During the entire chemotherapy and 3 months after completion of chemotherapy, SMZco 25mg/kg per day, in two divided doses, is applied orally, 3 consecutive days per week, to prevent Pneumocystis Carinii infection. SMZco should be stopped 24 hours before starting HDMTX for 4 days.

8.11.2 During the remission induction and reinduction phase treatment for intermediate-risk, high-risk ALL, levofloxacin could be used for infection prophylaxis.

8.11.3 For patients with recurring infections during the maintenance treatment, gamma immunoglobulin could be applied, 200-300mg/kg, once per 1-2 months.

9. Extramedullary disease and management

9.1 CNS disease and management

Definition of CNS disease

(1) CNS 1: CSF negative, no blast in CSF identified. No clinical evidence of CNS disease

(2) CNS 2: Blast identified in CSF but WBC < 5/μL of CSF

A traumatic tap is considered when ≥10 RBC /μL of CSF with blasts

(3) CNS 3: CNS leukemia

WBC > 5/μL of CSF and presence of blast in CSF cytospin preparation

Cranial nerve palsy even when there were no identifiable blasts in CSF

Mass lesion as shown on cranial CT / MRI

For CNS3 disease, administer triple intrathecal chemotherapy according to treatment protocol schedule. If blast is present in 3 consecutive CSF samples or neuro-imaging showed evidence of persistent CNS disease, this is regarded as resistant CNSL, which is rare. For patients older than 3 years, 18 Gy of cranial radiotherapy (CRT) should be performed after completion of re-induction. Mercaptopurine and methotrexate must be stopped for at least 1 week before CRT and substitute with dexamethasone (8 mg/m^2, day 1 to 7, day 15 to 21), VCR (2 mg/m^2, maximum 2 mg, on day 1, 8 and 15) during CRT.

For relapsed CNS leukemia, it should be treated according to relapse protocol of individual institute.

9.2 Testicular leukemia

Ultrasonographic assessment should be performed to look for testicular involvement in patients with testicular enlargement. Repeated assessment by USG scan should be performed after CAT and HDMTX. For persistent USG findings suggestive of testicular leukemic infiltration, testicular biopsy should be performed and protocol principal investigator should be contacted in cases with biopsy proven leukemic infiltration.

For relapsed testicular leukemia, it should be treated according to relapse protocol of individual institute.

10. Hematopoietic Stem Cell Transplant (HSCT)

All HR patients are candidates for HSCT, and transplantation should be performed after HDMTX and before maintenance treatment. It is preferable to achieve MRD negativity before HSCT. MRD-positive patients may be treated with 1 to 2 courses of DAEL chemotherapy (outlined in the table below), depending on the timing of MRD negativity. If there is no suitable donor or family refuses HSCT, patients should be treated according to HR arm.

Drug	Dosage	Schedule
Dexamethasone	20 mg/m^2/day; BD	Days 1 – 6
Ara-C	2 gm/m^2/dose; q12h (3 hours IV infusion)	Days 1 – 2
VP16	100 mg/m^2/dose; q12h	Days 3 – 5

	(1-hour IV infusion)	
PEG-asp	2,000 U/m^2 (1-hour IV infusion)	Day 6
IT	See section 7.1.2	Day 5

11. Ph + ALL

Once Ph+ ALL is confirmed, the patient should be treated according to IR arm, and dasatinib 80 mg/m^2 per day should be added. HSCT will only be offered to those with poor treatment response and fulfill the high-risk criteria (i.e., MRD≥1% at the end of remission induction). Dasatinib should be stopped during HSCT and may be resumed for 6 months after HSCT. Dasatinib should be given throughout the whole chemotherapy course for patients not transplanted.

Adjustment of dasatinib dosages:

(1) Non-hematopoietic system toxicity:

If there is serious toxicity, dasatinib should be suspended until toxicities resolved, and the subsequent starting dose of dasatinib should be reduced by 25%. The dose should be gradually escalated to recommended dose as tolerated; otherwise imatinib should be considered.

(2) Hematopoietic system toxicity:

During the induction treatment, tyrosine kinase inhibitor (TKI) should not be stopped if there are no infections or other severe complications.

When there is significant marrow toxicity (ANC <0.5x10^9/L or platelet <50x10^9/L), the concurrent myelosuppressive agents such as DNR, Ara-C, CTX, MTX, 6MP should be stopped. If the marrow toxicity persists or progresses, withholding TKI should be considered.

If significant marrow toxicity happens with fever and infection cannot be ruled out, all chemotherapy including TKI should be stopped. When serious marrow toxicity occurs (ANC <0.3x10^9/L or platelet < 20x10^9/L), all chemotherapy including TKI should also be stopped. In the induction treatment, glucocorticoids, VCR and L-Asp may continue to be used if there is no evidence of infection.

When the blood counts are persistently low (ANC <1.0x10^9/L or platelet <100x10^9/L) despite 50% reduction of myelosuppressive chemotherapy dosage, TKI dose may be reduced by 20%. In the maintenance treatment, dosage of TKI may be considered to be reduced if 6-MP dosage is reduced to 10% of recommended dosage and marrow toxicity is still unresolved.

12. Ph-Like ALL

Philadelphia chromosome-like ALL (Ph-like ALL) is one of the recently identified subtypes of ALL having poor prognosis. Recently it was reported that this subtype of ALL may have satisfactory outcome with appropriate treatment. It has similar gene expression patterns as that of Ph +ve ALL, and some cases have activation of tyrosine kinase signaling pathway, e.g. JAK2, ABL1, PDGFRB. Tyrosine kinase inhibitor (TKI) such as imatinib or dasatinib has been reported to be effective in a small cohort of refractory Ph-like ALL patients with ABL-class fusion. In this study, Ph-like ALL

patients with ABL1, ABL2 or PDGFRB rearrangement may receive combined dasatinib and chemotherapy same as that for Ph +ve ALL patients.

13. Off-protocol and off-study criteria

13.1 Off-protocol criteria

- Failure to achieve complete remission
- Relapse at any site
- Withdraw from protocol-defined treatment

13.2 Off-study criteria

- Death due to any cause
- Withdraw of consent
- Lost to follow up

14. Statistical Considerations

The Statistical Design and considerations of this multi-institution clinical trial is driven by

Primary Objective 1: ***To conduct a randomized controlled study on the effect of prolonged vincristine and dexamethasone pulses in maintenance phase of treatment on event-free survival.***

The goal of this objective is to establish the non-inferiority on the event-free survival (EFS) of replacing 6MP+DEX+VCR pulses (control) with 6MP+MTX (experiment) in the low-risk (LR) patients and removing DEX+VCR (experiment) in the CTX+VCR+AraC+DEX (control) pulses in intermediate/high risk (I/HR) patients during the last half of the maintenance therapy (week 55 to week 126 of the whole therapy). Non-inferiority (or lack of) will be assessed separately in LR and I/HR patients. It should be noted that Ph+ patients (automatically assigned I/HR) will NOT be included in this study, but rather they will be consented to participate in Primary Objective 2 study (see below).

The primary endpoint is EFS. Secondary endpoints include relapse and OS.

Design and non-inferiority margin: We will conduct an open-label, randomized controlled study. We will declare that the experimental treatment is non-inferior to the control (conventional) therapy if the five-year EFS rate of the patients receiving the experimental treatment is NO more than 0.05 (5%) lower than that of the control.

Definition of failures: For EFS, death due to any reason, relapse of ALL, second malignancies, and induction failures (by morphology) are considered as failures. For EFS estimation, the time to failure for patients who fail remission induction is set to zero.

Randomization schema: Stratified block randomization [1] will be applied. We set the block size to 6 and randomization ratio 1:1. Stratification factors for both risk cohorts include institution/hospital, gender, and age at diagnosis (infant, 1 year to 9 year, 10 years or older). In the I/HR cohort the randomization is additionally stratified by lineage (pre-B, T-cell). In the LR cohort, randomization is additionally stratified by TEL-AML1 status.

Randomization time: **Randomization of a patient should be completed within the period of therapy weeks 51-53 (Cycle 5 of Maintenance phase), by which time all information**

required for stratification should be available.

Sample size: For sample size determination we consider 80% statistical power at the 0.05 significance level to declare non-inferiority based on the 0.05 margin if in fact the experimental and control treatments have the same 5-year EFS rate. The following table shows the required number of patients to be randomized in each risk cohort under several postulated 5-year survival probabilities. The computation is done by East6.3 (Cytel, Boston, MA, USA).

5-year EFS of Control	5-year EFS of experiment	Hazard rate to 0.05 lower EFS	Sample size
0.6	0.6	0.511	1476
0.7	0.7	0.357	1249
0.8	0.8	0.223	971
0.9	0.9	0.105	704

Therefore, in each risk cohort we will randomize 1500 patients.

Analyses: The analyses will be performed separately in each risk cohort (LR, I/HR). In each risk cohort all randomized patients will be included in the assessment of treatment outcome, and the analyses will be done according to intent-to-treat. EFS functions will be estimated using the Kaplan-Meier estimator of survival functions along with 95% confidence interval at 5 year of follow up. The 95% upper confidence bound of the difference in 5-year EFS probability (control minus experiment) will be computed; we declare the experimental treatment is non-inferior if this upper confidence bound is less than 0.05 (5%).

Analysis time: To assure an adequate amount of follow up, **the analyses in each risk group will begin at two years *after* the enrollment date of the 1500th (the last) patient in that risk group.**

Interim analysis: An interim analysis after 3.5 years of accrual will be conducted. The analysis will be performed separately in each risk cohort (LR, I/HR). In each risk cohort all randomized patients will be included in the assessment of treatment outcome, and the analyses will be done according to intent-to-treat. EFS functions will be estimated with the Kaplan-Meier estimator, and compared by log-rank test. If in a risk cohort significant difference (at the 0.005 significance level) is observed, we will suspend accrual in that risk cohort and investigate.

Primary Objective 2: ***To conduct a randomized controlled study comparing the effects of imatinib and dasatinib on the event-free survival of patients with Philadelphia chromosome positive (Ph+) ALL.*** **(closed)**

Patients with Ph+ ALL will be randomized at a 1:1 ratio to receive either imatinib or dasatinib. The randomization is stratified by institution/hospital, and age at diagnosis (infant, 1 year to 9 year, 10 years or older). EFS is the primary endpoint and will be estimated by the Kaplan-Meier method and compared by a two-sided log-rank test. The study of this objective is limited by the number of available patients. Historical data showed that 4% of childhood ALL patients are Ph+. Thus, we expect to randomize 68 patients per year and totally 204 patients in 3 years of accrual. With this sample size (102 per arm), at the 0.05 significance level we will have 80% power to detect a difference between the two groups if the 5-year EFS results are, for example, 0.8 vs. 0.885, 0.75 vs. 0.845, or 0.7 vs. 0.81 respectively. The secondary endpoints are relapse and toxic death, as well as overall survival. To assure adequate follow up time, the final analysis will begin 2 years after the 204th patient is randomized.

Interim analysis (in case accrual is slower than anticipated): An interim analysis after 3.5 years of accrual will be conducted. All randomized patients will be included in the assessment of treatment outcome, and the analyses will be done according to intent-to-treat. EFS functions will be estimated using the Kaplan-Meier estimator, and compared by the log-rank test. If significant difference (at the 0.01 significance level) is observed, we will suspend accrual and investigate.

Secondary Objective 1: ***To establish a platform on clinical research of childhood acute lymphoblastic leukemia (ALL)***. This objective is administrative and does not require a statistical design.

Secondary Objectives 2 and 3: To assess the outcome of the treatment according to the standardization of risk stratification, and to assess the impact of minimal residual disease-directed treatment on clinical outcome in various risk groups

Levels of minimal residual disease (MRD) will be described using summary statistics: mean, standard deviation, quartiles and range for continuous measurements and proportions for categorized measurements (e.g., <0.01%, 0.01% to <1% and ≥1%).

All enrolled eligible patients will be included in the assessment of treatment outcome, which will be described by overall survival (OS) and event-free survival (EFS) using the Kaplan-Meier estimator of survival functions along with 95% confidence intervals at given years of follow up (e.g., 5 years). Cumulative incidence (CIN) of ALL relapse will be estimated using Kalbfleisch-Prentice method [2].

For OS, only death due to any reason is considered as failure (event). For EFS death due to any reason, relapse of ALL, second malignancies, and induction failures (by morphology) are considered as failures. For EFS and relapse CIN estimation, the time to failure for patients who fail remission induction is set to zero.

Associations of MRD with EFS and relapse CIN will be analyzed by log-rank and Gray's test [3], and by Cox and Fine-Gray regression models [4] with MRD and other known prognostic factors (age, leukocyte count, immunophenotype and molecular subtype at diagnosis, and gender) as explanatory variables.

The analyses of treatment outcome will begin at two years *after* the last enrollment date. The last enrollment date is the enrollment date of last randomized patient in Primary Objective 1 (see above).

15. References

1. Zelen M. The randomization and stratification of patients to clinical trials. J Chron Dis. 27:365-375, 1974.
2. Kalbfleisch JD and Prentice RL. The Statistical Analysis of Failure time Data, Second Edition. John Wiley and Sons, Inc. 2002. Pp.254-255.
3. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. Annals of Statistics, 16:1141-1154, 1988.
4. Fine JP and Gray RJ. A proportional hazards model for the subdistribution of a competing risk. Journal of American Statistical Association, 94:496-509, 1999.

5. Pui CH, Campana D, Pei D, et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. N Engl J Med 360:2730-41, 2009.
6. Pui CH, Campana D, Sandlund JT, et al. Treatment of Childhood Acute Lymphoblastic Leukemia without Cranial Irradiation. Ann Hematol 90 (Suppl 1): S61-S63, 2011.
7. Liu Y, Chen J, Tang J, Ni S, Xue H, Pan C. Cost of childhood acute lymphoblastic leukemia care in Shanghai, China. Pediatr Blood Cancer 53:557–562, 2009.
8. Conter V, Aricò M, Basso G, et al. Long-term results of the Italian Association of Pediatric Hematology and Oncology (AIEOP) Studies 82, 87, 88, 91 and 95 for childhood acute lymphoblastic leukemia. Leukemia. 2010 24:255-264, 2010
9. Möricke A, Zimmermann M, Reiter A, et al: Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000. Leukemia 24:265-284, 2010
10. Escherich G, Horstmann MA, Zimmermann M, et al. Cooperative study group for childhood acute lymphoblastic leukemia (COALL): long-term results of trials 82,85,89,92 and 97. Leukemia. 24:298-308, 2010
11. Hunger SP, Lu X, Devidas M, et al: Improved survival for children and adolescents with acute lymphoblastic leukemia between 1990 and 2005: a report from the children's oncology group. J Clin Oncol 30:1663-1669, 2012
12. Veerman AJ, Kamps WA, van den Berg H, et al: Dexamethasone-based therapy for childhood acute lymphoblastic leukemia: results of the prospective Dutch Childhood Oncology Group (DCOG) protocol ALL-9 (1997-2004). Lancet Oncol 10:957-966, 2009
13. Vrooman LM, Stevenson KE, Supko JG, et al: Postinduction dexamethasone and individualized dosing of Escherichia Coli L-asparaginase each improve outcome of children and adolescents with newly diagnosed acute lymphoblastic leukemia: results from a randomized study--Dana-Farber Cancer Institute ALL Consortium Protocol 00-01. J Clin Oncol 31:1202-1210, 2013
14. Domenech C, Suciu S, De Moerloose B, et al for EORTC – CLG. Dexamethasone (6 mg/m2/day) and prednisolone (60 mg/m2/day) were equally effective as induction therapy for childhood acute lymphoblastic leukemia in the EORTC CLG 58951 randomized trial. Haematologica 99:1220-1227, 2014
15. Stary J, Zimmermann M, Campbell M, et al: Intensive chemotherapy for childhood acute lymphoblastic leukemia: results of the randomized intercontinental trial ALL IC-BFM 2002. J Clin Oncol 32:174-184, 2014
16. Yamaji K, Okamoto T, Yokota S, et al. Minimal residual disease-based augmented therapy in childhood acute lymphoblastic leukemia: a report from the Japanese Childhood Cancer and Leukemia Study Group. Pediatr Blood Cancer 55:1287-95, 2010
17. Yeoh AE, Ariffin H, Chai EL, et al: Minimal residual disease-guided treatment deintensification for children with acute lymphoblastic leukemia: results from the Malaysia-Singapore acute lymphoblastic leukemia 2003 study. J Clin Oncol 30:2384-2392, 2012
18. Vora A, Goulden N, Mitchell C, et al: Augmented post-remission therapy for a minimal residual disease-defined high-risk subgroup of children and young people with clinical standard-risk and intermediate-risk acute lymphoblastic leukemia (UKALL 2003): a randomised controlled trial. Lancet Oncol 15:809-818, 2014

19. Schmiegelow K, Forestier E, Hellebostad M, et al: Long-term results of NOPHO ALL-92 and ALL-2000 studies of childhood acute lymphoblastic leukemia. Leukemia 24:345-354, 2010
20. Conter V, Bartram CR, Valsecchi MG, et al. Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: results in 3184 patients of the AIEOP-BFM ALL 2000 study. Blood. 115:3206-3214, 2010
21. Schrappe M, Valsecchi MG, Bartram CR, et al. Late MRD response determines relapse risk overall and in subsets of childhood T-cell ALL: results of the AIEOP-BFM-ALL 2000 study. Blood. 118:2077-2084, 2011
22. Eden T, Pieters R, Richards S, et al: Systematic review of the addition of vincristine plus steroid pulses in maintenance treatment for childhood acute lymphoblastic leukemia - an individual patient data meta-analysis involving 5,659 children. Br J Haematol 149:722-733, 2010
23. Pieters R, Hunger SP, Boos J, et al: L-asparaginase treatment in acute lymphoblastic leukemia: a focus on Erwinia asparaginase. Cancer 117:238-249, 2011
24. Vrooman LM, Stevenson KE, Supko JG, et al: Postinduction dexamethasone and individualized dosing of Escherichia Coli L-asparaginase each improve outcome of children and adolescents with newly diagnosed acute lymphoblastic leukemia: results from a randomized study--Dana-Farber Cancer Institute ALL Consortium Protocol 00-01. J Clin Oncol 31:1202-1210, 2013
25. Aricò M, Valsecchi MG, Camitta B, et al: Outcome of treatment in children with Philadelphia chromosome-positive acute lymphoblastic leukemia. N Engl J Med 342:998-1006, 2000
26. Aricò M, Schrappe M, Hunger SP, et al: Clinical outcome of children with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia treated between 1995 and 2005. J Clin Oncol 28:4755-4761, 2010
27. Schultz KR, Carroll A, Heerema NA, et al: Long-term follow-up of imatinib in pediatric Philadelphia chromosome-positive acute lymphoblastic leukemia: Children's Oncology Group Study AALL0031 Leukemia 28:1467-1471, 2014
28. Biondi A, Schrappe M, De Lorenzo P, et al: Imatinib after induction for treatment of children and adolescents with Philadelphia-chromosome-positive acute lymphoblastic leukemia (EsPhALL): a randomised, open-label, intergroup study. Lancet Oncol 13:936-945, 2012
29. Zwaan CM, Rizzari C, Mechinaud F, et al: Dasatinib in children and adolescents with relapsed or refractory leukemia: results of the CA180-018 phase I dose-escalation study of the Innovative Therapies for Children with Cancer Consortium. J Clin Oncol 31:2460-2468,2013
30. Den Boer ML, van Slegtenhorst M, De Menezes RX, et al: A subtype of childhood acute lymphoblastic leukemia with poor treatment outcome: a genome-wide classification study. Lancet Oncol 10:125-134, 2009
31. Roberts KG, Pei D, Campana D, et al: Outcome of children with BCR-ABL1-like acute lymphoblastic leukemia treated with risk-directed therapy based on the levels of minimal residual disease. J Clin Oncol 2014 Jul 21 [Epub ahead of print]

32. Roberts KG, Li Y, Payne-Turner D, et al: Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Eng J Med 371:1005-1015, 2014
33. Coustan-Smith E, Mullighan CG, Onciu M, et al: Early T-cell precursor leukemia: a subtype of very high-risk acute lymphoblastic leukemia. Lancet Oncol 10:147-156, 2009
34. Patrick K, Wade R, Goulden N, et al: Outcome for children and young people with Early T-cell precursor acute lymphoblastic leukemia treated on a contemporary protocol, UKALL 2003. Br J Haematol 166:421-424, 2014
35. Buitenkamp TD, Izraeli S, Zimmermann M, et al: Acute lymphoblastic leukemia in children with Down syndrome: a retrospective analysis from the Ponte di Legno study group. Blood 123:70-77, 2014
36. Buitenkamp TD, Pieters R, Gallimore NE, et al. Outcome in children with Down's syndrome and acute lymphoblastic leukemia: role of IKZF1 deletions and CRLF2 aberrations. Leukemia. 2012;26:2204-2211, 2012
37. Schrappe M, Hunger SP, Pui CH, et al: Outcomes after induction failure in childhood acute lymphoblastic leukemia. N Engl J Med 366:1371-1381, 2012
38. Mullighan CG: Genomic characterization of childhood acute lymphoblastic leukemia. Semin Hematol 50:314-24, 2013
39. Weston BW, Hayden MA, Roberts KG, et al: Tyrosine Kinase Inhibitor Therapy Induces Remission in a Patient With Refractory EBF1-PDGFRB-Positive Acute Lymphoblastic Leukemia. J Clin Oncol 31:e413-6, 2013
40. Lengline E, Beldjord K, Dombret H, et al: Successful tyrosine kinase inhibitor therapy in a refractory B-cell precursor acute lymphoblastic leukemia with EBF1-PDGFRB fusion. Haematologica 98:e146-e148, 2013
41. Zhang J, Ding L, Holmfeldt L, et al: The genetic basis of early T-cell precursor acute lymphoblastic leukemia. Nature 481:157-63, 2012
42. Yeoh AE, Tan D, Li CK, et al. Management of adult and paediatric acute lymphoblastic leukemia in Asia: resource-stratified guidelines from the Asian Oncology Summit 2013. Lancet Oncol. 14:e508-523, 2013

16. Appendix. Treatment tables

LR			I/HR			TKI	Remark
Week	**Date**	**Chemotherapy**	**Week**	**Date**	**Chemotherapy**	I/D (dosage/ days)	
1		Dex (4days)	1		Dex (4days)		
2		PVDL (IT：d5/d19; CNS2/adding d8/d12/d15 for injuries)	2		PVDL (IT：d5/d12/d19; CNS2/HR/adding d8/d15 for injuries)		
3			3				
4			4				d19:BM+MRD
5		CAT (IT：d29)	5		CAT(IT：d29)		
6			6		T-ALL d19MRD≥1%		
7			7		CAT+VCR+Peg-Asp(IT: d50)		d46: BM+MRD
8		HDMTX (IT：d1)	8		HDMTX (IT：d1)		
9			9				
10		HDMTX (IT：d1)	10		HDMTX (IT：d1)		
11			11				
12		HDMTX (IT：d1)	12		HDMTX (IT：d1)		
13			13				
14		HDMTX (IT：d1)	14		HDMTX (IT：d1)		
15			15				
					HR patients could enter into HSCT procedures, MRD>0.01, DAEL could be provided		
16		6-MP+Dex+VCR (IT：d1)	16		Dex＋DNR＋VCR＋6MP+PEG-Asp (IT：d1)		d46MRD+BM/MRD(d0)
17		6MP+MTX	17		6MP		
18		6MP+MTX	18		6MP		
19		6-MP+Dex+VCR (IT：d1)	19		Dex＋DNR＋VCR＋6MP+PEG-Asp (IT：d1)		
20		6-MP+MTX	20		6MP		
21		6MP+MTX	21		6MP		
22		Re-induction 1 （DxVDL） (IT：d1)	22		Dex＋DNR＋VCR＋6MP+PEG-Asp (IT：d1)		Previous MRD+BM/MRD(d0)
23			23		6MP		
24			24		6MP		
25		6MP+MTX	25		Dex＋DNR＋VCR＋6MP+PEG-Asp (IT：d1)		
26		6MP+MTX	26		6MP		
27		6MP+MTX	27		6MP		
28		6-MP+Dex+VCR(IT：d1)	28		Dex＋DNR＋VCR＋6MP+PEG-Asp(IT：d1)		
29		6MP+MTX	29		6MP		
30		6MP+MTX	30		6MP		
31		6MP+MTX	31		6MP		
32		Re-induction 2 （DxVL）(IT：d1)	32		Re-induction (IT：d1)		
33			33				
34			34				
					HR patients should receive transplantation before this time, or continue their subsequent chemotherapy		

LR				IR/HR				**TKI**	Remark
Week	Date	Chemotherapy		Week	Date	Chemotherapy		I/D (dosage/days)	
35		（6-MP+MTX）×3				（6-MP+MTX）×2			
36									
37						CTX+VCR+ara-C+DEX (IT：d1)			
38		6-MP+Dex+VCR(IT：d1)				No Chemotherapy			
39		（6-MP+MTX）×3				（6-MP+MTX）×2			
40									
41						CTX+VCR+ara-C+DEX (IT：d1)			
42		6-MP+Dex+VCR(IT：d1)				No Chemotherapy			
43		（6-MP+MTX）×3				（6-MP+MTX）×2			
44									
45						CTX+VCR+ara-C+DEX (IT：d1)			
46		6-MP+Dex+VCR(IT：d1)				No Chemotherapy			
47		（6-MP+MTX）×3				（6-MP+MTX）×2			
48									
49						CTX+VCR+ara-C+DEX (IT：d1)			
50		6-MP+Dex+VCR(IT：d1)				No Chemotherapy			
51		（6-MP+MTX）×3				（6-MP+MTX）×2			
52									
53						CTX+VCR+ara-C+DEX (IT：d1)			
		Random				Random (Ph-ALL equally subject to I/HR-A)			
		LR-A	LR-B			I/HR-A	I/HR-B		
54~ 61		6-MP+Dex+VCR	6-MP+MTX			（6-MP+MTX）×6	(6-MP+MTX)×6		
		(6-MP+MTX）×7	6-MP+MTX			C+A+Dx+V	CTX + ara-C		
62~ 69		6-MP+Dex+VCR	6-MP+MTX			（6-MP+MTX）×6	(6-MP+MTX)×6		
		(6-MP+MTX）×7	6-MP+MTX			C+A+Dx+V	CTX + ara-C		
70~ 77		6-MP+Dex+VCR	6-MP+MTX			（6-MP+MTX）×6	(6-MP+MTX)×6		
		(6-MP+MTX）×7	6-MP+MTX			C+A+Dx+V	CTX + ara-C		
78~ 85		6-MP+Dex+VCR	6-MP+MTX			（6-MP+MTX）×6	(6-MP+MTX)×6		
		(6-MP+MTX）×7	6-MP+MTX			C+A+Dx+V	CTX + ara-C		
86~ 93		6-MP+Dex+VCR	6-MP+MTX			（6-MP+MTX）×6	(6-MP+MTX)×6		
		(6-MP+MTX）×7	6-MP+MTX			C+A+Dx+V R	CTX + ara-C		

94~ 101		6-MP+Dex+VCR	6-MP+MTX			（6-MP+MTX）×6	(6-MP+MTX)×6		
		(6-MP+MTX）×7	6-MP+MTX			C+A+Dx+V	CTX + ara-C		
102~109		6-MP+Dex+VCR	6-MP+MTX			（6-MP+MTX）×6	(6-MP+MTX)×6		
		(6-MP+MTX)×7	6-MP+MTX			C+A+Dx+V	CTX + ara-C		
110~117		(6-MP+MTX)×8	6-MP+MTX			(6-MP+MTX)×8	(6-MP+MTX)x8		
118~125		(6-MP+MTX)×8	6-MP+MTX			(6-MP+MTX)×8	(6-MP+MTX) x8		
To perform the full examinations two months after stopping drugs: BM biopsy, MRD, original positive fusion genes, immunology (T\B\NK\Ig), endocrinology, abdominal ultrasound B, cardiac ultrasound									