STUDY PROTOCOL

Real-World Study of the Diagnosis, Treatment, and Survival Outcomes of Children With Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia in China

Nationwide Multicenter Retrospective Cohort Study

UNIQUE PROTOCOL ID **2025-IRB-0385-P-01**

DOCUMENT DATE **29 August 2025**

Background

Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL), defined by the presence of the BCR::ABL1 fusion gene, accounts for approximately 3%-5% of childhood ALL and has historically been associated with poor outcomes. Over the past decade, the introduction of tyrosine kinase inhibitors (TKIs) has markedly improved prognosis, with overall survival (OS) rates reaching 70%-80%. Consequently, most affected children no longer undergo hematopoietic stem cell transplantation.

During minimal residual disease (MRD) monitoring in pediatric Ph+ ALL, marked discordance is often observed later in treatment between multiparameter flow cytometry (MFC) and quantitative fusion-gene testing. Some children are MFC-MRD negative while remaining persistently positive by quantitative polymerase chain reaction (qPCR). Discordance may also occur between BCR::ABL1 transcript testing and immunoglobulin/T-cell receptor (Ig/TCR) rearrangement testing, with differences exceeding 1 log.

International studies using fluorescence in situ hybridization (FISH) on residual BCR::ABL1-positive cells have shown that the fusion gene may be present not only in leukemic cells, but also in nonleukemic monocytes, granulocytes, and T lymphocytes. This biological subtype has been termed CML-like ALL. Limited international evidence suggests that CML-like ALL and classic Ph+ ALL have similar outcomes; however, BCR::ABL1 transcript levels may have limited prognostic value in the CML-like subgroup. Overtreatment is therefore a concern, and treatment-related mortality, rather than disease relapse, may be a major cause of treatment failure in these patients.

In clinical practice, some children rapidly revert to BCR::ABL1 positivity after completing chemotherapy and discontinuing TKI therapy, which further complicates treatment decisions. These observations highlight the need for more refined risk stratification and individualized treatment strategies for CML-like ALL. The present multicenter retrospective study is designed to provide clinical data to support these goals.

Study Objectives

The primary objective is to characterize the diagnosis and treatment of children with Ph+ ALL across China. The study will also estimate the frequency of CML-like ALL; describe current diagnostic and treatment patterns; evaluate treatment response and survival outcomes; and generate clinical evidence to support standardized, individualized management of this patient population.

Study Design and Methods

This is a nationwide, multicenter, retrospective cohort study designed to generate real-world evidence. Children newly diagnosed with Ph+ ALL between 1 January 2015 and 31 December 2024 will be identified through participating centers of the Hematology Group of the Chinese Pediatric Society, Chinese Medical Association. Clinical characteristics, treatment, MRD monitoring, and outcomes will be collected in a standardized Excel-based case report form (CRF) and subjected to statistical analysis.

> **Study scope.** Nationwide multicenter retrospective cohort; diagnosis period 2015-2024; target sample size: 500 patients.

Study Population

The study plans to enroll approximately 500 children diagnosed with Ph+ ALL between 1 January 2015 and 31 December 2024. Eligibility will be determined according to the following criteria.

Inclusion Criteria

1. Age at diagnosis from 1 month through 18 years.
2. Positive for the BCR::ABL1 fusion gene.
3. Serial quantitative MRD testing performed during treatment using both MFC and PCR (qRT-PCR or ddPCR), with samples for both assays collected at the same time points.
4. Receipt of standardized TKI therapy, including imatinib, dasatinib, or another TKI.

Exclusion Criteria

5. Mixed-phenotype acute leukemia.
6. Irregular treatment or treatment not administered in accordance with the applicable chemotherapy protocol.
7. A previous or concurrent malignancy.
8. Failure to receive standardized TKI therapy because of TKI intolerance, a contraindication to TKI treatment, or another relevant factor.

Statistical Analysis Plan

Descriptive and Survival Analyses

Continuous variables will be summarized as medians with interquartile ranges (IQRs), and categorical variables as counts and percentages, n (%). Time-to-event outcomes will be estimated using the Kaplan-Meier method and compared using the log-rank test. Multivariable Cox proportional hazards models will be used for survival analyses. The proportional hazards assumption will be assessed, and time-dependent covariates will be used when necessary.

Subgroup Analyses

Pre-specified subgroup analyses will consider age, white blood cell (WBC) count, central nervous system (CNS) involvement, BCR::ABL1 transcript type, CML-like ALL status, IKZF1 deletion status, center level, and geographic region.

Bias and Confounding Control

Selection bias will be addressed through broad multicenter enrollment and a minimum case-number threshold for participating centers. Information bias will be reduced through use of a standardized CRF, laboratory quality control, and blinded central re-evaluation of key endpoints. Confounding will be addressed using propensity-score methods in parallel with multivariable modeling; center will be modeled as a random effect or stratification variable, and calendar year will be used to account for treatment era.

Sensitivity Analyses and Reporting

Sensitivity analyses will include case-deletion analyses for centers or variables at high risk of missing data. E-values will be calculated to assess the potential impact of unmeasured confounding. A two-sided P value of <0.05 will be considered statistically significant. Where applicable, model-based results will include point estimates with standard errors, the relevant test statistics, and exact P values.