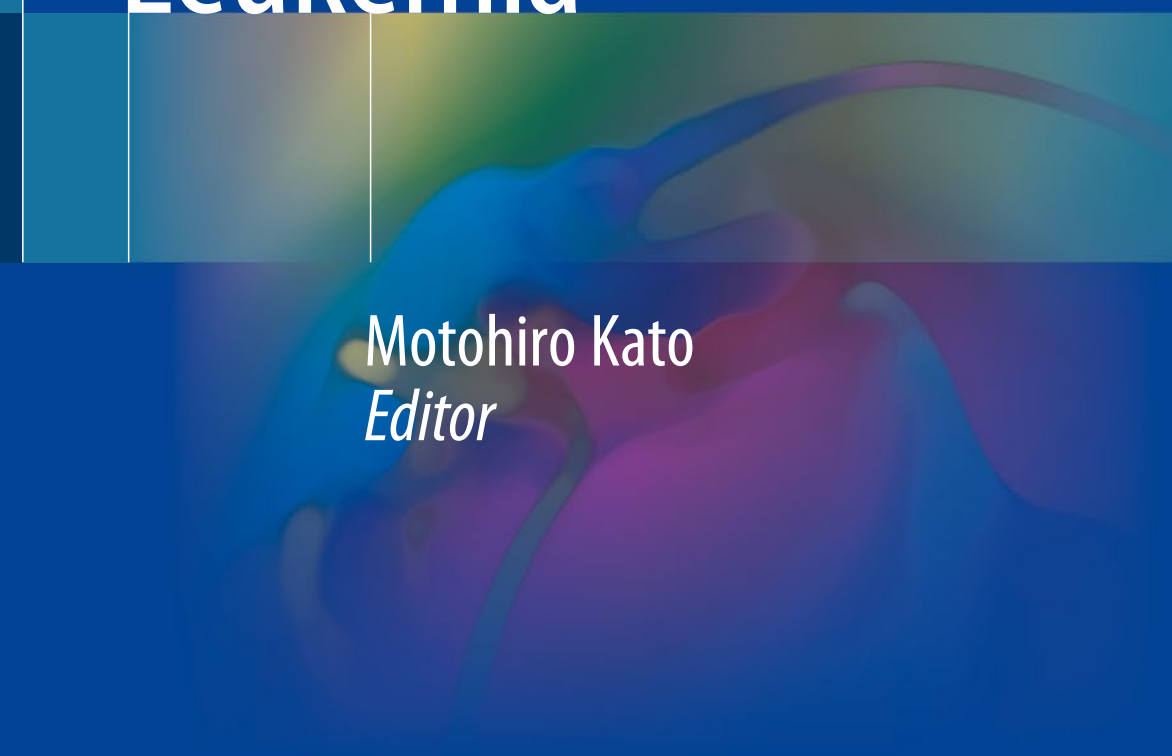
Pediatric Acute Lymphoblastic Leukemia



Motohiro Kato
Editor

Springer

Pediatric Acute Lymphoblastic Leukemia

Motohiro Kato
Editor

Pediatric Acute Lymphoblastic Leukemia

Springer

Editor
Motohiro Kato
Department of Transplantation and Cell Therapy
Children's Cancer Center
National Center for Child Health and Development
Setagaya-ku
Tokyo
Japan

ISBN 978-981-15-0547-8 ISBN 978-981-15-0548-5 (eBook)
https://doi.org/10.1007/978-981-15-0548-5

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

On behalf of all the authors, I am pleased to present the first edition of ***Pediatric Acute Lymphoblastic Leukemia***. Acute lymphoblastic leukemia is the most common malignancy during childhood. Previously, the survival probability had been 10–20%; however, the cure rate has dramatically improved up to 80–90%. Improved supportive care, treatment stratification based on relapse risk, biological features of leukemic cells, and optimization of treatment regimens by nationwide and international collaboration have contributed to this dramatic improvement.

This book consists of 17 chapters written by experts in this field, describing the updated information on biology, diagnostic procedure, treatment, and supportive therapy of pediatric acute lymphoblastic leukemia.

I could not have completed this book without the help of Ms. Saki Kasai and Ms. Kripa Guruprasad of Springer. I dedicate this book to our patients, parents, colleagues, and mentors. I hope that ***Pediatric Acute Lymphoblastic Leukemia*** is useful for not only pediatric hematologists but also medical students, interns, residents, and fellowship doctors.

Setagaya-ku, Tokyo, Japan — Motohiro Kato, M.D., Ph.D

Contents

Part I
Epidemiology and Diagnosis of Pediatric ALL

Chapter 1
Overview

Motohiro Kato

Abstract Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer. Survival probability of pediatric ALL had been dismal at 50 years ago, but the most recent clinical trials with multiagent chemotherapy have achieved overall survival probability of better than 80%, thanks to better supportive care, treatment stratification based on relapse risk, and the biological features of leukemic cells. Diagnosis of ALL was based principally on morphological identification of leukemic blasts in bone marrow, and immunophenotype assessment by flow cytometry is necessary, and most pediatric ALL cases are clinically classified as B-cell precursor, T-cell ALL, or mature B-cell types, comprising 80%, 15%, and 5% of cases, respectively.

Keywords Diagnosis · Bone marrow aspiration

1.1 Introduction

Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer, consisting approximately 25% of malignant diseases in children. A slight male predominance has been observed, with a peak incidence between 1 and 4 years of age [1].

Survival probability of pediatric ALL had been dismal at 50 years ago, and ALL was considered to be an intractable disease. However, beginning from the pivotal paper by Farber et al. showing that temporal remissions of pediatric ALL were achieved by folic acid antagonist (4-aminopteroyl-glutamic acid), new era of chemotherapy aiming to conquer ALL started. The most recent clinical trials have achieved overall survival probability of better than 80% [2, 3]. The main contributors to this dramatic success are better supportive care, treatment stratification based on relapse risk and the biological features of leukemic cells, and the accumulation of evidence obtained by clinical trials through nationwide and international collaboration.

M. Kato (✉)
Department of Transplantation and Cell Therapy, Children's Cancer Center, National Center for Child Health and Development, Setagaya-ku, Tokyo, Japan
e-mail: kato-mt@ncchd.go.jp

M. Kato (ed.), *Pediatric Acute Lymphoblastic Leukemia*,
https://doi.org/10.1007/978-981-15-0548-5_1

1.2 Symptoms and Diagnosis

Symptoms of ALL are generally non-specific and various and include prolonged fever, bone pain, swollen lymph nodes, petechia, and dyspnea due to mediastinum enlargement. Some patients were suspected as having leukemia by image findings, such as X-ray and/or magnetic resonance imaging (MRI) (Fig. 1.1).

Diagnosis of ALL was based principally on morphological identification of leukemic bone marrow blasts exceeding 25% (Fig. 1.2a). In some cases, repeated bone marrow examination is required to confirm the diagnosis [4]. On rare occasions, bone marrow metastasis of solid tumor including neuroblastoma and rhabdomyosarcoma is misdiagnosed as leukemia (Fig. 1.2b). Immunophenotype assessment by

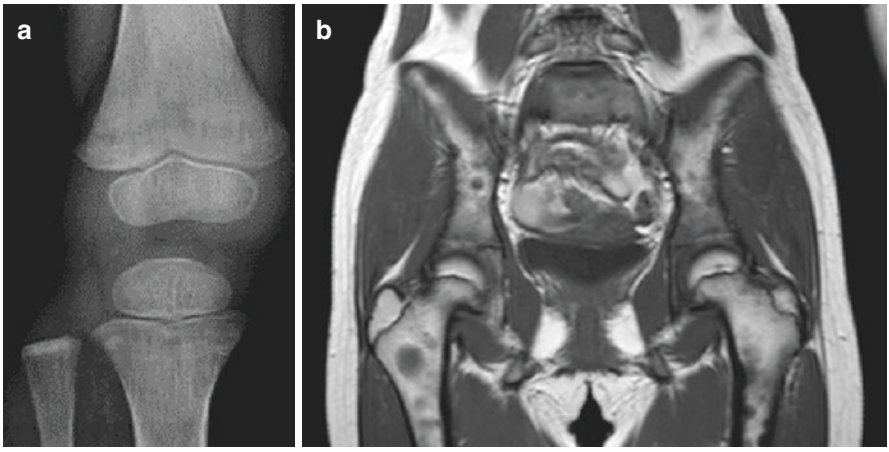


Fig. 1.1 Imaging findings of ALL cases (**a**) X-ray findings of the knee of leukemia case. Metaphyseal lucent band was observed. (**b**) Abnormal signal (low signal in T1-weighted image) by magnetic resonance imaging (MRI)

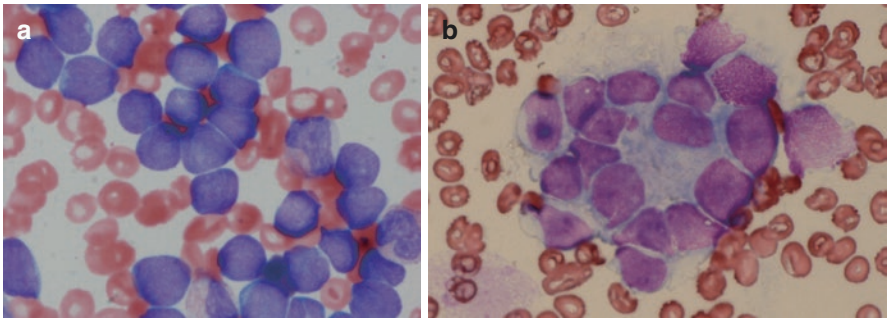


Fig. 1.2 Typical morphology of ALL. May-Giemsa staining of bone marrow specimens of (**a**) a ALL case and (**b**) a neuroblastoma case

flow cytometry (FCM) is necessary, and most pediatric ALL cases are clinically classified as B-cell precursor (BCP), T-cell ALL, or mature B-cell types, comprising 80%, 15%, and 5% of cases, respectively.

Clinical features of T-cell ALL are slightly different from those of BCP-ALL and include older age, male predominance, high frequency of mediastinal mass, and higher leukocyte count at diagnosis. The prognosis of patients with T-ALL was poor compared to that of patients with BCP-ALL, especially due to the higher risk of relapse involving the central nervous system (CNS). Thus, in the past, these cases were normally treated as a part of the higher risk group in clinical trials using the same treatment regimen as for BCP-ALL. However, given the characteristics of T-ALL, recent clinical trials have adopted modifications specific for T-ALL, such as intensification of CNS-directed therapy and more intensive treatment using L-asparaginase and methotrexate based on stratification using minimal residual disease (MRD) kinetics.

Mature B-cell ALL has immunophenotypic and clinical features that are almost identical to those of mature B-cell lymphoma, and they should be treated with short and intensive chemotherapy [5].

1.3 Treatment

Typical treatment duration is 2–3 years, consisting of induction, consolidation, and maintenance therapy. Treatment schedule and intensity are selected based on prognostic factor, such as age, leukocyte count at diagnosis, biological/molecular features of leukemic cells, and early response to treatment. For a small fraction of cases with high risk for relapse, allogeneic stem cell transplantation is indicated. Most of the drugs used for ALL treatment have several adverse effects [6], as shown in Table 1.1. Severe adverse effects potentially fatal, and risk-directed stratification contribute to suppress relapse risk and avoid excess complication.

1.4 Future Directions

Current status is more than 80% of survival, some subsets of ALL still suffer relapse. Further intensification of conventional cytotoxic agents is practically impossible, and new strategies are required. One clue is targeted therapy with small molecules such as tyrosine kinase inhibitor, which has been successfully adopted in BCR-ABL1 positive ALL. The other clue is immunotherapy approach, such as bi-specific antibody and chimeric antigen receptor (CAR) T-cell therapy. Clinical trials showed that these new agents were effective for relapsed/refractory ALL, and we should investigate how to incorporate these hope into standard therapy.

Table 1.1 Chemotherapeutic agents used for pediatric ALL

Agents	Adverse events
Steroids	
Prednisolone/ predonine	Hypertension, hyperglycemia, immunosuppression, avascular necrosis
Dexamethasone	Hypertension, hyperglycemia, irritability/menta depression, immunosuppression, avascular necrosis
Antimetabolite	
Mercaptopurine	Hepatotoxicity, mucositis
Methotrexate	Hepatotoxicity, mucositis, renal dysfunction, leukoencephalopathy
Cytarabine	Fever, conjunctivitis, mucositis
Anthracyclines	
Doxorubicin	Cardiotoxicity
Daunorubicin	
Idarubicin	
Mitoxantrone	
THP-adriamycin	
Vinca alkaloid	
Vincristine	Neuropathy, constipation
Vinblastine	
Asparaginase	
L-asparaginase	Allergy, coagulation disorder, pancreatitis
Erwinia asparaginase	
PEG-asparaginase	
Alkylating agents	
Cyclophosphamide	Cystitis, cardiotoxicity
Ifosphamide	Cystitis, renal dysfunction
Busulfan	Seizure

Considering improved outcomes of pediatric ALL, survival probability is not always the best endpoint to assess superiority of new treatment strategy. Quality of line (QOL) assessment might be an alternative endpoint for clinical trial, as well as other diseases in similar situation, such as acute promyelocytic leukemia [7].

References

1. Horibe K, Saito AM, Takimoto T, et al. Incidence and survival rates of hematological malignancies in Japanese children and adolescents (2006-2010): based on registry data from the Japanese Society of Pediatric Hematology. Int J Hematol. 2013;98:74–88.
2. Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. Lancet. 2013;381:1943–55.
3. Pui CH, Yang JJ, Hunger SP, et al. Childhood acute lymphoblastic leukemia: progress through collaboration. J Clin Oncol. 2015;33:2938–48.

4. Kato M, Koh K, Kikuchi A, et al. Case series of pediatric acute leukemia without a peripheral blood abnormality, detected by magnetic resonance imaging. Int J Hematol. 2011;93:787–90.
5. Kobayashi R, Sunami S, Mitsui T, et al. Treatment of pediatric lymphoma in Japan: current status and plans for the future. Pediatr Int. 2015;57:523–34.
6. Schmiegelow K, Attarbaschi A, Barzilai S, et al. Consensus definitions of 14 severe acute toxic effects for childhood lymphoblastic leukaemia treatment: a Delphi consensus. Lancet Oncol. 2016;17:e231–9.
7. Burnett AK, Russell NH, Hills RK, et al. Arsenic trioxide and all-trans retinoic acid treatment for acute promyelocytic leukaemia in all risk groups (AML17): results of a randomised, controlled, phase 3 trial. Lancet Oncol. 2015;16:1295–305.

Chapter 2
Genetic Alterations of Pediatric Acute Lymphoblastic Leukemia

Toshihiko Imamura

Abstract Recent genetic studies of pediatric acute lymphoblastic leukemia (ALL), both in B cell precursor and T cell ALL (B/T-ALL), clarified the landscape of genetic alterations due to great progress of comprehensive genome sequencing technologies including next generation sequencing. These studies revealed genetic alterations such as somatic structural DNA rearrangement and sequence mutations that affect multiple pathways including lymphocyte development, cytokine signaling, JAK-STAT pathway, MAP kinase and RAS signaling pathway, transcriptional, and epigenetic regulation to provide us new insight of leukemogenesis of pediatric B/T-ALL. In addition, recent comprehensive genetic studies of paired diagnostic and relapse samples clarified the mechanism of clonal evolution of leukemic cells to provide novel insights of mechanism of therapeutic resistance of pediatric ALL. Owing to huge success of genetic studies, several new subtypes of pediatric ALL have been identified, and some of them are clinically important to be candidate of targeted therapy. Here, we provide a review of recent genetic studies of pediatric ALL including B/T-ALL, acute leukemia ambiguous lineage, and relapsed ALL and discuss the importance of genetic basis of pediatric ALL.

Keywords Pediatric acute lymphoblastic leukemia · Genetic basis · Genetic analysis · Chromosomal translocation · Genetic alteration

T. Imamura (✉)
Department of Pediatrics, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, Kyoto, Japan
e-mail: imamura@koto.kpu-m.ac.jp

M. Kato (ed.), *Pediatric Acute Lymphoblastic Leukemia*,
https://doi.org/10.1007/978-981-15-0548-5_2

2.1 B Precursor ALL with Recurrent Fusion or Chromosomal Abnormality

Pediatric B precursor ALL (B-ALL) is classified into several subtypes according to specific chromosomal abnormalities such as chromosomal rearrangement and aneuploidy. It is well known that these abnormalities are deeply associated with therapeutic responsiveness and prognosis. It is also interesting that distribution of chromosomal abnormalities is age-dependent [1], suggesting that some of leukemogenic mechanisms are age-dependent.

2.1.1 KMT2A *Rearrangement*

KMT2A (*MLL*) located on 11q23 encodes histone methyl transferase and plays an important role in hematopoiesis [2]. KMT2A regulates expression of homeobox gene and *Meis 1* [3, 4]. However, KMT2A fusion protein, most of which recruit aberrant histone methyltransferase, Dot1L, alters the histone code of these genes to perturb expression of them, resulting in developing leukemia [5–8]. *KMT2A* rearrangement including t(4;11)(q21;q23)/*KMT2A-AFF1*, t(9;11)(p22;q23)/*KMT2A-MLLT3*, and t(11;19)(q23;p13.1)/*KMT2A-MLLT1*, is present in more than 80% in infant B-ALL which still shows dismal prognosis [9]. New therapeutic agents should be explored to achieve better outcome of infant B-ALL with *KMT2A* rearrangement [10, 11].

2.1.2 ETV6-RUNX1 *and High Hyperdiploid*

Pediatric B-ALL with *ETV6-RUNX1* or high hyperdiploid (HHD, 51–65 chromosomes) is the most popular subtype showing excellent outcome [12]. These two types of B-ALL are frequently observed in pediatric patients aged 10 years younger. *ETV6-RUNX1* positive pediatric B-ALL accounts for 20 to 25% of pediatric B-ALL. The outcome of *ETV6-RUNX1* positive pediatric B-ALL is generally excellent [13, 14], but some studies determined genetic alterations such as the mutation of *NR3C1* related to poor prognosis [15]. B-ALL with HHD comprise approximately 20–30% of pediatric B-ALL and another subtype with excellent outcome [12]. Gained chromosomes are usually non-random, and several reports show the frequent gains of chromosomes 4, 6, 10, 14, 17, 18, 21, and X [16]. Children's Oncology Group (COG) showed combined gain of chromosome, 4, 10, and 17 was associated with better prognosis [17]. Our group also showed that presence of +11 or +17 was associated with better prognosis in Japanese pediatric cohort [18]. Although leukemogenic mechanism of HHD positive B-ALL is not fully understood, comprehensive genetic analysis revealed that mutations in receptor tyrosine kinase—RAS signaling pathway including in the *FLT3*, *NRAS*, *KRAS*, and *PTPN11* genes were prevalent in this subtype [19].

2.1.3 TCF3 *Rearrangement*

B-ALL with *TCF3* rearrangement consists of two types of chromosomal translocation such as t(1;19)(q23;p13)/*TCF3-PBX1* and t(17;19)(q23;p13)/*TCF3-HLF*. Although B-ALL with *TCF3-PBX1* was initially associated with poor prognosis, contemporary protocol has improved the outcome of this subtype, resulting in 5-year event free survival rate of 85–90% [20, 21]. However, the prognosis of relapsed patients is poor, and genetic alterations related to poor prognosis should be determined. On the other hand, *TCF3-HLF*-positive B-ALL is uncurable [22]. Comprehensive genetic analysis revealed that intragenic deletion of *PAX5* or *VPREB1* was identified in *TCF3-HLF*- positive B-ALL, suggesting that these genetic alterations might inhibit pro to pre B cell transition [23]. Genetic analysis also identified activating mutations of genes associated with the RAS pathway [23]. Interestingly, gene set enrichment analysis revealed enrichment of stem cell and myeloid signatures in *TCF3-HLF*-positive B-ALL. These findings indicate this cellular reprograming might be associated with drug resistant state. Development of new therapy is warranted to improve the outcome of *TCF3-HLF*-positive B-ALL.

2.1.4 *Hypodiploid*

Hypodiploid ALL is defined as ALL with 44 chromosomes or fewer and predict extremely poor outcome [24]. Hypodiploid ALL is classified into several distinct subtypes based on modal chromosome number. Recent comprehensive genomic analysis of hypodiploid ALL revealed characteristic genomic alterations of these subtypes [25]. Near-haploid cases with 24–31 chromosomes harbor alterations targeting receptor tyrosine kinase signaling and RAS signaling pathway and *IKZF3* mutation. Low-hypodiploid cases with 32–39 chromosomes harbor alterations of *TP53* that are germline mutation in most cases, *IKZF2* and *RB1*. Qian M, et al. demonstrated that ALL patients with germline *TP53* mutation were associated with poor outcome and high incidence of second cancer [26].

2.1.5 *BCR-ABL1*

BCR-ABL1 positive ALL was historically associated with poor outcome. BCR-ABL1 fusion protein accelerates cell proliferation through constitutional phosphorylation of ABL1. Thus, inhibition of ABL1 phosphorylation should inhibit proliferation of BCR-ABL1 positive ALL cells. In line with this hypothesis, tyrosine kinase inhibitor (TKI) greatly improves the outcome of *BCR-ABL1* positive ALL [27].

2.2 New Subtype of B-ALL

B-ALL without classical recurrent chromosomal translocation which are described above have been categorized in B-other ALL, and detailed genetic alterations of this subtype were not investigated. However, recent comprehensive genomic analysis revealed several genetic subtypes in B-other ALL.

2.2.1 IKZF1 *Deletion,* CRLF2 *Deregulation, and Ph-Like ALL*

Mullighan CH, et al. analyzed pediatric high risk B-ALL cohort to identify that deletion of *IKZF1*, a gene that encodes the lymphoid transcriptional factor IKAROS, was strongly associated with poor outcome [28, 29]. This finding was validated in many other pediatric B-ALL cohort [30, 31], resulting in establishing *IKZF1* deletion as poor prognostic factor of pediatric B-ALL. Then, deregulated expression of *CRLF2* mRNA was reported, and relationship between genomic lesion affecting *CRLF2* mRNA expression such as *P2RY8-CRLF2* and *IgH-CRLF2*, clinical characteristics, and treatment outcome was extensively studied [32, 33]. Finally, Den Bore M and Mullighan CH reported subtype of B-other ALL with specific gene expression profile resembling that in Ph+/*BCR-ABL1* positive ALL called as Ph+ like/*BCR-ABL1* like ALL [29, 34]. *IKZF1* deletion is also enriched in this subtype. Interestingly, whole transcriptome analysis revealed that fusion genes related to tyrosine kinase or cytokine receptors such as *ABL1*, *PDGFRB*, *JAK2*, *CRLF2*, and *EPOR* related rearrangement was present in Ph+ like ALL, suggesting that possible treatment of this subtype with TKI [35–38]. Currently, clinical trial is ongoing to evaluate the efficacy of TKI in the treatment of Ph+ like ALL with ABL class or JAK2 related fusions.

2.2.2 iAMP21

Harrison CJ, et al. described B-ALL patients with intrachromosomal amplification of chromosome 21 including the *RUNX1* gene (iAMP21) [39]. The iAMP21 positive B-ALL comprise 1–2% of pediatric B-ALL and associated with poor outcome in UK MRC ALL97 protocol [40]. COG also reported that iAMP21 positive B-ALL showed poor prognosis when treated with standard protocol [41], suggesting that intensive chemotherapy is required to obtain good outcome in this subtype.

2.2.3 MEF2D *and* ZNF384 *Rearranged ALL*

Myocyte enhancer factor 2D (*MEF2D*) and zinc finger 384 (*ZNF384*) rearranged ALL is the distinct subtypes of B-ALL. *MEF2D* rearranged ALL is reported to be 1–4% of pediatric B-ALL and has poor outcome [42–45]. *MEF2D* is the 5′ partner in all described fusions, and a total of 6 3′ fusion partner genes have been described [43]. Apart from *MEF2D-CSF1R*, which shows a Ph+ like gene expression profile, *MEF2D* rearranged cases share distinct gene expression profile and deletion of *CDKN2A/2B* [43–46]. This subtype is related to older age at onset and high WBC count, resulting in most classified in NCI-HR group.

ZNF384 rearrangement positive B-ALL comprise 1–6% of pediatric B-ALL. So far, total 9 5′ fusion partner genes have been identified [1, 45]. Interestingly, this subtype has a characteristic immunophenotype with low CD10 expression and expression of myeloid markers such as CD13 and CD33 [46]. Prognostic relevance of *ZNF384* related fusions should be determined.

2.2.4 DUX4 *Rearranged ALL*

Double homeobox 4 gene (*DUX4*) rearranged B-ALL accounts for approximately 5% of pediatric B-ALL [1, 45]. DUX4 encodes a double homeobox transcription factor located within *D4Z4* repeat in the subtelomeric region on 4q. *DUX4* is not expressed in normal B lymphocyte and translocation to *IGH* results in expression of truncated *DUX4* isoform in leukemic cells [47]. Genomic studies also identified that 50–70% of *DUX4* rearranged cases have intragenic *ERG* deletion which was known to be restricted in this subtype [48]. It is also noteworthy that *DUX4* rearranged ALLs commonly express aberrant *ERG* isoform and truncated C-terminal ERG protein irrespective of *ERG* deletions. This aberrant ERG protein, which retains the DNA binding and transactivating domain of ERG, inhibits transcriptional activity of wild type ERG and is transforming [1, 48]. This subtype is associated with high expression of CD2 and good outcome even if the patients harbor *IKZF1* deletion [49].

2.2.5 *Others*

PAX5 is rearranged to a diverse range of fusion partners in approximately 2% of B-ALLs [1]. Gu Z, et al. identified that two subtypes of B-ALL harbor PAX5 alteration using integrated multimodal genomic analysis such as PAX5alt and PAX5 p.

Pro80Arg. *PAX5* alt ALL has diverse *PAX5* alterations such as rearrangements, intragenic amplifications, or mutations. The second subtype is defined by PAX5 p.Pro80Arg and biallelic PAX5 alteration [50]. They showed that p.Pro80Arg impairs B lymphoid development and promotes the development of B-ALL with biallelic *Pax5* alteration in vivo. These studies highlight the importance of PAX5 for regulating B cell differentiation and of *PAX5* alterations as central events of leukemogenesis of B lineage leukemia. In children treated in COG AALL0232 study of NCI-HR B-ALL, the outcome was intermediate for both PAX5alt (5-year event free survival (EFS) 71.5 ± 7.0%) and PAX5 p.Pro80Arg (5-year EFS 75.0 ± 14.2%) [50].

Rare fusions involving *NUTM1* have been reported in several studies [45, 51]. However, clinical characteristics, treatment outcome, and leukemogenic mechanism of this fusion should be elucidated.

2.3 Genetic Alterations of T-ALL

T-ALL accounts for approximately 15% of pediatric ALLs [1]. Although the prognosis of pediatric T-ALL was poor, recent progress of MRD-guided chemotherapeutic protocol improve the outcome of pediatric T-ALL [52]. Approximately 50% of T-ALL have chromosomal translocations involving T cell receptor α and δ (*TRA* and *TRD* located at 14q11) and T cell receptor β (*TRB* located at 7q34). In these chromosomal translocations, T-cell receptor gene fuses to transcriptional factor such as *TAL1*, *TAL2*, *LMO1*, *LMO2*, *LYL1*, *TLX1*, *TLX3*, *MYC*, and *MYB* [1, 53]. In addition, ABL1 related fusion genes such as *NUP214-ABL1*, *EML1-ABL1*, and *ETV6-ABL1* have been identified in T-ALL, which might be the candidate for targeted therapy by TKI. Moreover, gene expression profile can classify T-ALLs into subgroups based on the specific gene expression patterns and aberrant activation of T-ALL related transcriptional factors such as *TAL1*, *TAL2*, *LMO2*, *TLX1*, and *TLX3* [1, 53].

In terms of point mutations, activating *NOTCH1* mutations and loss-of function mutations of *FBXW7*, leading to inhibition of ubiquitin-mediated degradation of the activated form of NOTCH1, occur in more than 60% and 15% in T-ALLs, respectively.

These findings suggest activation of NOTCH1 pathway is deeply associated with leukemogenesis of T-ALL [54].

Early T-cell precursor (ETP) ALL is a distinct subtype of T-ALL characterized by reduced expression of CD1a, CD5, and CD8 [55]. The gene expression profile of ETP-ALL is similar to that of hematopoietic stem cells, suggesting that this leukemia may arise from very immature cells [55]. Genetic analyses revealed that ETP-ALL has mutations of multiple pathways including hematopoietic and lymphoid development (e.g., *RUNX1*, *IKZF1*, *ETV6*, and *GATA3*), RAS and cytokine receptor signaling (e.g., *NRAS*, *IL7R*, *KRAS*, *JAK1*, *JAK3*, *PTPN11*, and *SH2B3*), and epigenetic regulators (e.g., *EZH2*, *SUZ12*, *EED*, and *SETD2*) [56]. Initially,

the prognosis of ETP-ALL was thought to be poor, but contemporary treatments improve the outcome of ETP-ALL [57].

Recent studies identified pathogenic noncoding mutations in T-ALL. Noncoding mutations upstream of TAL1 generate a binding site of MYB, resulting in recruiting a protein complex including TAL1 and CREBBP, namely oncogenic super-enhancer region with high levels of H3K27 acethylation [58].

Comprehensive genetic analyses reveal a landscape of genomic alterations of T-ALL, but genetic alterations related to poor therapeutic responses are hardly identified. Seki M, et al. identified *SPI1* (encoding PU.1) related fusion gene (*STMN1-SPI1* and *TCF7-SPI1*) positive cases in approximately 4% of pediatric T-ALL [59]. The prognosis of *SPI1* fusion positive T-ALL is extremely poor, but this finding should be validated in independent cohort.

2.4 Genetic Alterations of Acute Leukemia Ambiguous Lineage

Acute leukemia ambiguous lineage (ALAL) consists of mixed phenotype acute leukemia (MPAL) and acute unclassified leukemia (AUL). MPAL demonstrates features of ALL and acute myeloid leukemia (AML), while AUL lacks lineage-defining features. MPAL accounts for 2–3% of pediatric ALL, whereas AUL is quite rare [60]. Alexander et al. demonstrated genetic landscape of MPAL using comprehensive genetic analyses [61]. They determined that rearrangement of *ZNF384* was common in B/myeloid (B/M MPAL), whereas biallelic *WT1* mutations were common in T/myeloid (T/M MPAL). Interestingly, T/M MPAL shares the genetic alterations identified in ETP-ALL cases. They also describe that the ambiguous phenotype of MPAL is the result of acquisition of genetic alterations in immature hematopoietic progenitors.

2.5 Genetic Alterations of Relapsed B-ALL

The prognosis of relapsed ALL is usually poor. Thus, there is great interest in recent comprehensive genomic studies for relapsed ALL. These genomic studies revealed that leukemia evolution leading to relapse follows complex branched pathway instead of linear fashion [62]. Although primary chromosomal translocations are retained, new secondary genetic alterations are emerged mainly from minor clones at diagnosis [62, 63]. Common relapse-acquired lesions include *CREBBP* which impair sensitivity of glucocorticoid therapy [63] and 5′-nucleotidase catalytic enzyme II (*NT5C2*) which confer resistance to purine analogs [64]. Other recurrent somatic mutations of relapsed ALL include mutations in mismatch repair gene (e.g., *MSH2* and *MSH6*) and epigenetic regulators (e.g., *KDM6*, *MLL2*, and *SETD2*).

Interestingly, these alterations in mismatch repair genes and epigenetic regulators are enriched in early-relapsed cases, suggesting mechanistic difference between early and late relapse of B-ALL [65, 66].

RAS pathway mutations (e.g., *NRAS*, *KRAS*, *PTPN11*, and *FLT3*) are selected or acquired in relapsed B-ALL, especially in cases with HHD [67]. Currently, treatment with MEK inhibitor is exploring to treat relapsed B-ALL with RAS pathway mutations in pre-clinical model [67, 68].

2.6 Clinical Implications of Genetic Studies

Comprehensive genetic analyses provide us useful information for accurate diagnosis, precise risk stratification, monitoring of treatment response, and implementation of targeted therapy. It is also important to obtain new insight of leukemogenic mechanism of pediatric ALL.

References

1. Iacobucci I, Mullighan CG. Genetic basis of acute lymphoblastic leukemia. J Clin Oncol. 2017;35:975–83.
2. Ziemin-van der Poel S, McCabe NR, Gill HJ, et al. Identification of a gene, *MLL*, that spans the breakpoint in 11q23 translocations associated with human leukemias. Proc Natl Acad Sci U S A. 1991;88(23):10735–9.
3. Yu BD, Hess JL, Horning SE, et al. Altered Hox expression and segmental identity in Mll-mutant mice. Nature. 1995;378(6556):505–8.
4. Milne TA, Briggs SD, Brock HW, et al. MLL targets SET domain methyltransferase activity to Hox gene promoters. Mol Cell. 2002;10(5):1107–17.
5. Zhang Y, Chen A, Yan XM, et al. Disordered epigenetic regulation in MLL-related leukemia. Int J Hematol. 2012;96:428–37.
6. Okada Y, Feng Q, Lin Y, et al. hDot1L links histone methylation to leukemogenesis. Cell. 2005;121:167–78.
7. Zhang W, Xia X, Reisenauer MR, et al. Dot1a-AF9 complex mediates histone H3 Lys-79 hypermethylation and repression of ENaCalpha in an aldosterone-sensitive manner. J Biol Chem. 2006;281:18059–68.
8. Krivtsov AV, Feng Z, Lemieux ME, et al. H3K79 methylation profiles define murine and human MLL-AF4 leukemias. Cancer Cell. 2008;14:355–68.
9. Pieters R, Schrappe M, De Lorenzo P, Hann I, De Rossi G, Felice M, et al. A treatment protocol for infants younger than 1 year with acute lymphoblastic leukaemia (Interfant-99): an observational study and a multicentre randomised trial. Lancet. 2007;370(9583):240–50.
10. Dawson MA, Prinjha RK, Dittmann A, et al. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. Nature. 2011;478:529–33.
11. Daigle SR, Olhava EJ, Therkelsen CA, et al. Selective killing of mixed-lineage leukemia cells by a potent small-molecule Dot1L inhibitor. Cancer Cell. 2011;20:53–65.
12. Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukemia. Lancet. 2013;381:1943–55.
13. Piette C, Suciu S, Clappier E, Bertrand Y, Drunat S, Girard S, et al. Differential impact of drugs on the outcome of ETV6-RUNX1 positive childhood B-cell precursor acute lymphoblastic leukemia: results of the EORTC CLG 58881 and 58951 trials. Leukemia. 2018;32:244–8.

14. Usami I, Imamura T, Takahashi Y, Suenobu SI, Hasegawa D, Hashii Y, et al. Discontinuation of L-asparaginase and poor response to prednisolone are associated with poor outcome of ETV6-RUNX1-positive pediatric B-cell precursor acute lymphoblastic leukemia. Int J Hematol. 2019;109(4):477–82.
15. Kuster L, Grausenburger R, Fuka G, Kaindl U, Krapf G, Inthal A, et al. ETV6/RUNX1-positive relapses evolve from an ancestral clone and frequently acquire deletions of genes implicated in glucocorticoid signaling. Blood. 2011;117:2658–67.
16. Kawamata N, Ogawa S, Zimmermann M, Kato M, Sanada M, Hemminki K, et al. Molecular allelokaryotyping of pediatric acute lymphoblastic leukemias by high-resolution single nucleotide polymorphism oligonucleotide genomic microarray. Blood. 2008;111:776–84.
17. Sutcliffe MJ, Shuster JJ, Sather HN, Camitta BM, Pullen J, Schultz KR, et al. High concordance from independent studies by the children's cancer group (CCG) and pediatric oncology group (POG) associating favorable prognosis with combined trisomies 4, 10, and 17 in children with NCI standard-risk B-precursor acute lymphoblastic leukemia: a children's oncology group (COG) initiative. Leukemia. 2005;19:734–40.
18. Kato M, Imamura T, Manabe A, Hashii Y, Koh K, Sato A, et al. Prognostic impact of gained chromosomes in high-hyperdiploid childhood acute lymphoblastic leukaemia: a collaborative retrospective study of the Tokyo children's cancer study group and Japan Association of Childhood Leukaemia Study. Br J Haematol. 2014;166(2):295–8.
19. Paulsson K, Lilljebjörn H, Biloglav A, et al. The genomic landscape of high hyperdiploid childhood acute lymphoblastic leukemia. Nat Genet. 2015;47:672–6.
20. Moorman AV, Ensor HM, Richards SM, Chilton L, Schwab C, Kinsey SE, et al. Prognostic effect of chromosomal abnormalities in childhood B cell precursor acute lymphoblastic leukaemia: results from the UK medical research council ALL97/99 randomized trial. Lancet Oncol. 2010;11:429–38.
21. Asai D, Imamura T, Yamashita Y, Suenobu S, Moriya-Saito A, Hasegawa D, et al. Outcome of TCF3-PBX1 positive pediatric acute lymphoblastic leukemia patients in Japan: a collaborative study of Japan Association of Childhood Leukemia Study (JACLS) and Children's Cancer and leukemia study group (CCLSG). Cancer Med. 2014;3(3):623–31.
22. Hunger SP. Chromosomal translocations involving the E2A gene in acute lymphoblastic leukemia: clinical features and molecular pathogenesis. Blood. 1996;87:1211–24.
23. Fischer U, Forster M, Rinaldi A, Risch T, Sungalee S, Warnatz HJ, et al. Genomics and drug profiling of fatal TCF3-HLF-positive acute lymphoblastic leukemia identifies recurrent mutation patterns and therapeutic options. Nat Genet. 2015;47(9):1020–9.
24. Nachman JB, Heerema NA, Sather H, et al. Outcome of treatment in children with hypodiploid acute lymphoblastic leukemia. Blood. 2007;110:1112–5.
25. Holmfeldt L, Wei L, Diaz-Flores E, Walsh M, Zhang J, Ding L, et al. The genomic landscape of hypodiploid acute lymphoblastic leukemia. Nat Genet. 2013;45(3):242–52.
26. Qian M, Cao X, Devidas M, Yang W, Cheng C, Dai Y, et al. TP53 Germline variations influence the predisposition and prognosis of B-cell acute lymphoblastic leukemia in children. J Clin Oncol. 2018;36(6):591–9.
27. Schultz KR, Carroll A, Heerema NA, Bowman WP, Aledo A, Slayton WB, et al. Long-term follow-up of imatinib in pediatric Philadelphia chromosome-positive acute lymphoblastic leukemia: Children's Oncology Group Study AALL0031. Leukemia. 2014;28(7):1467–71.
28. Mullighan CG, Goorha S, Radtke I, et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature. 2007;446(7137):758–64.
29. Mullighan CG, Su X, Zhang J, et al. Deletion of *IKZF1* and prognosis in acute lymphoblastic leukemia. N Engl J Med. 2009;360(5):470–80.
30. Dörge P, Meissner B, Zimmermann M, Möricke A, Schrauder A, Bouquin JP, et al. IKZF1 deletion is an independent predictor of outcome in pediatric acute lymphoblastic leukemia treated according to the ALL-BFM 2000 protocol. Haematologica. 2013;98(3):428–32.
31. Asai D, Imamura T, Suenobu S, Saito A, Hasegawa D, Deguchi T, et al. IKZF1 deletion is associated with a poor outcome in pediatric B-cell precursor acute lymphoblastic leukemia in Japan. Cancer Med. 2013;2(3):412–9.

32. Russell LJ, Capasso M, Vater I, Akasaka T, Bernard OA, Calasanz MJ, et al. Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. Blood. 2009;114(13):2688–98.
33. Mullighan CG, Collins-Underwood JR, Phillips LA, Loudin MG, Liu W, Zhang J, et al. Rearrangement of CRLF2 in B-progenitor- and down syndrome-associated acute lymphoblastic leukemia. Nat Genet. 2009;41(11):1243–6.
34. Den Boer ML, van Slegtenhorst M, De Menezes RX, et al. A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. Lancet Oncol. 2009;10:125–34.
35. Roberts KG, Morin RD, Zhang J, et al. Genetic alterations activating kinase and cytokine receptor signaling in high-risk acute lymphoblastic leukemia. Cancer Cell. 2012;22:153–66.
36. Roberts KG, Li Y, Payne-Turner D, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med. 2014;371(11):1005–15.
37. Weston BW, Hayden MG, Roberts KG, et al. Tyrosine kinase inhibitor therapy induces renission in a patients with refractory EBF1-PDGFRB-positive acute lymphoblastic leukemia. J Clin Oncol. 2013;31(25):e413–6.
38. Imamura T, Kiyokawa N, Kato M, Imai C, Okamoto Y, Yano M, et al. Characterization of pediatric Philadelphia-negative B-cell precursor acute lymphoblastic leukemia with kinase fusions in Japan. Blood Cancer J. 2016;6:e419.
39. Harewood L, Robinson H, Harris R, Al-Obaidi MJ, Jalali GR, Martineau M, et al. Leukemia. 2003;17:547–53.
40. Moorman AV, Richards SM, Robinson HM, Strefford JC, Gibson BE, Kinsey SE, et al. Prognosis of children with acute lymphoblastic leukemia (ALL) and intrachromosomal amplification of chromosome 21 (iAMP21). Blood. 2007;109(6):2327–30.
41. Heerema NA, Carroll AJ, Devidas M, Loh ML, Borowitz MJ, Gastier-Foster JM, et al. Intrachromosomal amplification of chromosome 21 is associated with inferior outcomes in children with acute lymphoblastic leukemia treated in contemporary standard-risk children's oncology group studies: a report from the children's oncology group. J Clin Oncol. 2013;31(27):3397–402.
42. Suzuki K, Okuno Y, Kawashima N, Muramatsu H, Okuno T, Wang X, et al. MEF2D-BCL9 fusion gene is associated with high-risk acute B-cell precursor lymphoblastic leukemia in adolescents. J Clin Oncol. 2016;34(28):3451–9.
43. Gu Z, Churchman M, Roberts K, Li Y, Liu Y, Harvey RC, et al. Genomic analyses identify recurrent MEF2D fusions in acute lymphoblastic leukaemia. Nat Commun. 2016;7:13331.
44. Ohki K, Kiyokawa N, Saito Y, Hirabayashi S, Nakabayashi K, Ichikawa H, et al. Clinical and molecular characteristics of *MEF2D* fusion-positive B-cell precursor acute lymphoblastic leukemia in childhood, including a novel translocation resulting in *MEF2D-HNRNPH1* gene fusion. Haematologica. 2019;104(1):128–37.
45. Lilljebjörn H, Fioretos T. New oncogenic subtypes in pediatric B-cell precursor acute lymphoblastic leukemia. Blood. 2017;130(12):1395–401.
46. Hirabayashi S, Ohki K, Nakabayashi K, Ichikawa H, Momozawa Y, Okamura K, et al. ZNF384-related fusion genes define a subgroup of childhood B-cell precursor acute lymphoblastic leukemia with a characteristic immunotype. Haematologica. 2017;102(1):118–29.
47. Yasuda T, Tsuzuki S, Kawazu M, et al. Recurrent DUX4 fusions in B cell acute lymphoblastic leukemia of adolescents and young adults. Nat Genet. 2016;48:569–74.
48. Zhang J, McCastlain K, Yoshihara H, et al. Deregulation of DUX4 and ERG in acute lymphoblastic leukemia. Nat Genet. 2016;48:1481–9.
49. Clappier E, Auclerc MF, Rapion J, et al. An intragenic ERG deletion is a marker of an oncogenic subtype of B-cell precursor acute lymphoblastic leukemia with a favorable outcome despite frequent IKZF1 deletions. Leukemia. 2014;28:70–7.
50. Gu Z, Churchman ML, Roberts KG, Moore I, Zhou X, Nakitandwe J, et al. PAX5-driven subtypes of B-progenitor acute lymphoblastic leukemia. Nat Genet. 2019;51(2):296–307.

51. Andersson AK, Ma J, Wang J, et al. St. Jude Children's research hospital–Washington university pediatric cancer genome project. The landscape of somatic mutations in infant MLL rearranged acute lymphoblastic leukemias. Nat Genet. 2015;47(4):330–7.
52. Petit A, Trinquand A, Chevret S, Ballerini P, Cayuela JM, Grardel N, et al. Oncogenetic mutations combined with MRD improve outcome prediction in pediatric T-cell acute lymphoblastic leukemia. Blood. 2018;131(3):289–300.
53. Ferrando AA, Neuberg DS, Staunton J, et al. Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. Cancer Cell. 2002;1:75–87.
54. Weng AP, Ferrando AA, Lee W, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science. 2004;306:269–71.
55. Coustan-Smith E, Mullighan CG, Onciu M, et al. Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. Lancet Oncol. 2009;10:147–56.
56. Zhang J, Ding L, Holmfeldt L, et al. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature. 2012;481:157–63.
57. Patrick K, Wade R, Goulden N, et al. Outcome for children and young people with early T-cell precursor acute lymphoblastic leukaemia treated on a contemporary protocol, UKALL 2003. Br J Haematol. 2014;166:421–4.
58. Mansour MR, Abraham BJ, Anders L, et al. Oncogene regulation: an oncogenic superenhancer formed through somatic mutation of a noncoding intergenic element. Science. 2014;346:1373–7.
59. Seki M, Kimura S, Isobe T, Yoshida K, Ueno H, Nakajima-Takagi Y, et al. Recurrent SPI1 (PU.1) fusions in high-risk pediatric T cell acute lymphoblastic leukemia. Nat Genet. 2017;49(8):1274–81.
60. Gerr H, et al. Acute leukaemias of ambiguous lineage in children: characterization, prognosis and therapy recommendations. Br J Haematol. 2010;149:84–92.
61. Alexander TB, Gu Z, Iacobucci I, Dickerson K, Choi JK, Xu B, et al. The genetic basis and cell of origin of mixed phenotype acute leukaemia. Nature. 2018;562(7727):373–9.
62. Mullighan CG, Phillips LA, Su X, et al. Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. Science. 2008;322:1377–80.
63. Mullighan CG, Zhang J, Kasper LH, et al. CREBBP mutations in relapsed acute lymphoblastic leukaemia. Nature. 2011;471:235–9.
64. Meyer JA, Wang J, Hogan LE, et al. Relapse-specific mutations in NT5C2 in childhood acute lymphoblastic leukemia. Nat Genet. 2013;45:290–4.
65. Ma X, Edmonson M, Yergeau D, et al. Rise and fall of subclones from diagnosis to relapse in pediatric B-acute lymphoblastic leukaemia. Nat Commun. 2015;6:6604.
66. Spinella JF, Richer C, Cassart P, Ouimet M, Healy J, Sinnett D. Mutational dynamics of early and late relapsed childhood ALL: rapid clonal expansion and long-term dormancy. Blood Adv. 2018;2(3):177–88.
67. Irving J, Matheson E, Minto L, et al. Ras pathway mutations are prevalent in relapsed childhood acute lymphoblastic leukemia and confer sensitivity to MEK inhibition. Blood. 2014;124:3420–30.
68. Jerchel IS, Hoogkamer AQ, Ariës IM, Steeghs EMP, Boer JM, Besselink NJM, et al. RAS pathway mutations as a predictive biomarker for treatment adaptation in pediatric B-cell precursor acute lymphoblastic leukemia. Leukemia. 2018;32(4):931–40.

Chapter 3
Germline Biology of Pediatric ALL

Motohiro Kato

Abstract Molecular genomic studies for ALL have been focused on somatically acquired genetic alterations in leukemic cells, and germline cells have been used mainly as a control to extract somatic mutation. However, recent studies demonstrated that germline genomics conferred pathogenesis of ALL, and an importance of genetic background in development of pediatric ALL is widely recognized. An association between polymorphism and adverse events has been already reported, and recent genomic analyses for familial ALL cases identified inherited causative genes for ALL. Moreover, some studies showed that a certain fraction of non-syndromic/non-familial ALL cases had pathogenic germline variants in cancer predisposition genes, such as *ETV6*, *IKZF1*, and *TP53*. These variants could contribute to not only poor response but also an increased risk of secondary neoplasms. Comprehensive understanding of biology in both ALL cells and germline cells is required.

Keywords Germline · Single nucleotide polymorphism · Adverse events
NUDT15 · TP53

3.1 Importance of Germline Variants in Leukemia Biology

To understand molecular basis of acute lymphoblastic leukemia (ALL), most of conventional genomic research had focused on somatically acquired genomic alterations which were unique to ALL cells. For this purpose, normal cells are used as a "control" to detect leukemia specific mutations. However, recent advances in population and family studies unraveled an importance of germline biology in etiology of pediatric leukemia. Inherited germline variants confer ALL susceptibility,

M. Kato (✉)
Department of Transplantation and Cell Therapy, Children's Cancer Center, National Center for Child Health and Development, Setagaya-ku, Tokyo, Japan
e-mail: kato-mt@ncchd.go.jp

M. Kato (ed.), *Pediatric Acute Lymphoblastic Leukemia*,
https://doi.org/10.1007/978-981-15-0548-5_3

drug response, and incidence of toxicities during/after ALL therapy with a variety of effect size and frequency.

Knowledge of these germline biology in pediatric ALL can provide several clinical advantages (e.g., dose modification based on germline variants can reduce excess toxicity), but ethical consideration and genetic counselling with sufficient knowledge should be carefully provided when germline genomic analysis is performed.

3.2 Germline Biology for Drug Response

In most of clinical situation, drug doses are calculated based on patients' body weight or body surface area, but sensitivity to the drug markedly differs between each individual. Some cases are occasionally extremely sensitive to the anti-leukemic drugs, leading to life-threatening complication. To assess this inter-individual heterogeneity in the therapeutic effect or adverse events, genomic investigation has been widely performed. This approach is known as "pharmacogenomics," combining human genetics and pharmacology. Especially, with advances of comprehensive genotyping technology, genome wide association studies (GWAS) have been conducted to identify causative single nucleotide polymorphisms (SNPs) for various adverse events (Fig. 3.1).

3.2.1 Pharmacogenomics of Adverse Events

Genomic variants conferring thiopurine metabolism have one of the most validated evidences in the field of pharmacogenomics [1]. Thiopurines, including 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG), are essential purine antimetabolites used in consolidation and maintenance therapy for ALL. Thiopurines are metabolized by various enzymes, eventually to mono-, di-, and triphosphates of 6-thioguanosine, and finally DNA-incorporated thioguanine (DNA-TG), which has cytotoxic effect (Fig. 3.2) [2]. Variability of thiopurine metabolism activity had been well known, and children taking the same dose of thiopurines showed great variability of cytotoxic effect [3]. A missense SNP in the *TMPT* gene was identified in 1990s as a cause of sensitivity to thiopurines [4]. Patients with homozygous SNP had low TPMT enzymatic activity and were extremely sensitive to thiopurines [5]. Currently, *TPMT* genotyping before thiopurine usage has been established to avoid excess toxicity with maintaining therapeutic effect [6, 7].

In 2014, a Korean group identified a missense SNP (c.415C>T, p.R139C) of *NUDT15* gene as a cause of thiopurine-induced myelosuppression [8]. An impact of the *NUDT15* variant on 6-MP sensitivity was confirmed by a GWAS, which was performed by Yang et al. for children with ALL [9]. They confirmed influences of variants in *TMPT* and *NUDT15* on 6-MP sensitivity by unbiased statistical analysis, and homozygous cases showed extremely high sensitivity to 6-MP. The variant

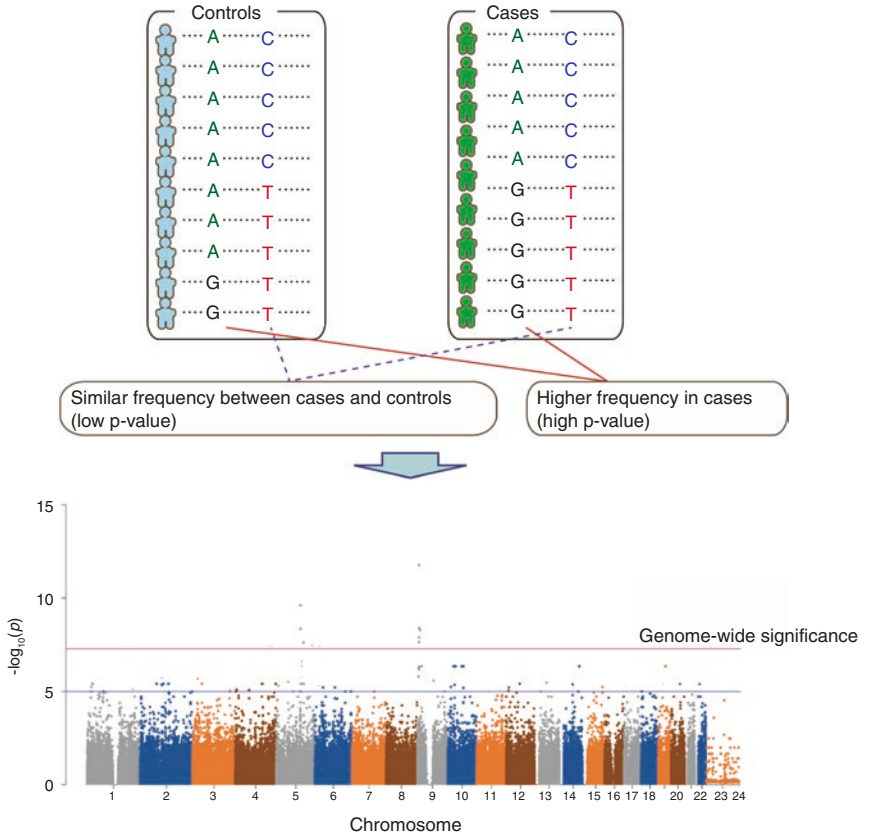


Fig. 3.1 Images of genome-wide association studies. Each point shows a *p*-value to each variant. Accounting for multiple comparison, genome-wide significance threshold should be adjusted by the number of SNPs tested

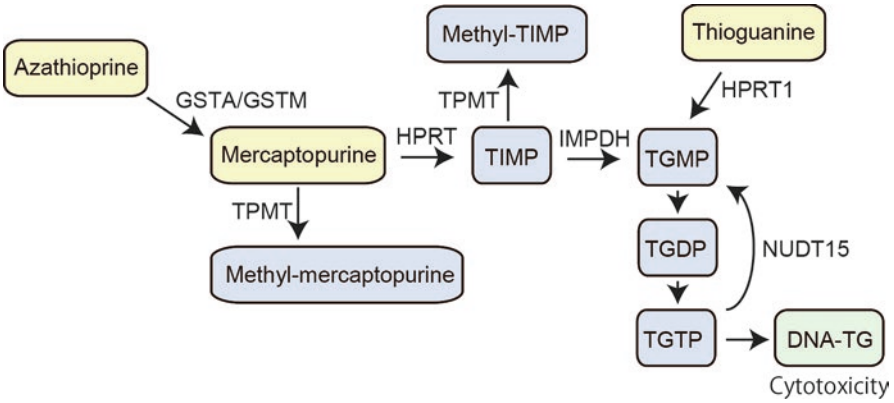


Fig. 3.2 Shema of thiopurine metabolism pathway. Hypomorphic variants of *TMPT* and *NUDT15* cause high sensitivity to thiopurines

allele frequency of *NUDT15* is high in East Asian, which confers relatively high sensitivity to 6-MP in this population [10]. Thus far, a total of seven variants in NUDT15 with low activity were identified, and all of haplotypes with these variants affect tolerability to 6-MP [11]. Bi-allelic variants of *NUDT15* were extremely sensitive to 6-MP and required a reduction in dose to as low as 10% of the intended dose [12].

To date, several GWAS have successfully identified causative SNPs for various adverse events related to ALL therapy (Table 3.1). A variant in the promoter region of *CEP72*, which encoded a centrosomal protein involved in microtubule formation, was identified to be significantly associated with incidence of vincristine-induced neuropathy [13]. The SNP reduces *CEP72* expression in human neurons and leukemia cells, leading to high sensitivity to vincristine.

Germline variants conferring treatment response have also been reported. Intragenic deletion polymorphism in *BIM* gene, which was shown to alter the splicing pattern resulting in loss of proapoptotic isoforms of *BIM*, confers resistance to steroids, although an impact on outcome of ALL is attenuated [16]. A GWAS showed a significant association between minimal residual disease (MRD) status and multiple SNPs, including those located at *IL-15* locus. Presence of the intronic variant induced high expression of IL-15 and lowered sensitivity of leukemic cells to cytotoxic agents such as vincristine or doxorubicine [17].

3.3 Germline Biology for Leukemogenesis

It is generally accepted that most of pediatric leukemia developed without any specific causes, and genomic alterations occur sporadically by chance. However, there are numerous diseases predisposing leukemia, such as primary immunodeficiency, congenital abnormalities, and other systemic syndromes. Furthermore, recent genomic studies demonstrated that prevalence of germline pathogenic variants in cancer-associated genes in children with cancer was much higher than previously estimated, even without family history of pediatric cancer.

Table 3.1 Germline variants associated with adverse events in ALL therapy

Genes	SNPs	Adverse events
NUDT15 [1, 11]	rs116855232 rs746071566 rs147390019 rs186364861	Thiopurine-induced myelosuppression
TPMT [1, 4]	rs1800462 rs1800460 rs1142345	Thiopurine-induced myelosuppression
CEP72 [13]	rs924607	Vincristine-induced neuropathy
ACP1 [14]	rs12714403	Osteonecrosis
ASNS [15]	rs3832526	Asparaginase-associated pancreatitis/allergy

3.3.1 Leukemia Predisposing Syndrome

A number of inherited genetic diseases/syndromes have been known as leukemia predisposition. For example, 21 trisomy (so called Down syndrome) are about 20 times more likely to develop leukemia (see also Chap. 11). Of interest, chromosome 21 is the most frequently gained chromosome in high-hyperdiploid ALL, suggesting that extra copy of this chromosome contributes to leukemogenesis. Germline variants in genes regulating RAS-RAF-MAPK pathway, such as *PTPN11*, *KRAS*, and *NRAS*, cause Noonan syndrome or other related "RASopahty." This RASophathy is also associated with a range of malignancies including ALL [18]. Several primary immunodeficiencies (PID) have a significantly increased risk of developing leukemia occasionally. In some patients, a malignancy can be the first symptom suggesting the underlying PID [19].

These cancer predisposition diseases are found in sporadic ALL cases without other symptoms of underlying diseases. About half of low-hypodiploid (modal number: 32–39) ALL had *TP53* pathogenic mutations in their normal cells, which is characteristic of Li-Fraumeni syndrome [20]. Individuals born with the rare constitutional Robertsonian translocation, rob(15;21)(q10;q10)c, have a significantly higher risk (>2000-fold) of developing iAMP21-positive ALL compared to the general population [20].

Moreover, recent studies identified various germline mutations in genes encoding transcription factors as causes for novel syndrome with familial ALL. Shah S et al. reported two families with multiple pre-B cell ALL with a recurrent germline *PAX5* variant [21]. Recent reports showed germline *ETV6* pathogenic variants caused thrombocytopenia, high erythrocyte mean corpuscular volume (MCV), and familial clustering of leukemia [22]. Another study identified dominant negative *IKZF1* variants conferring immunodeficiency and predisposition to ALL [23]. Perez-Garcia et al. identified *SH2B3* as a recessive tumor suppressor gene and homozygous variant of *SH2B3* gene cause familial leukemia [24].

3.3.2 Leukemia Predisposing in Non-syndromic ALL

Moreover, recently, germline variants are recognized as determinants of not only heterogeneity in drug sensitivity but also ALL susceptibility in non-syndromic cases. A number of inherited pathogenic variants conferring ALL incidence have been identified through GWAS comparing the frequency of SNPs between sporadic ALL cases and controls [25]. Causative genes identified by the GWAS including *IKZF1* [26, 27], *GATA3* [28], *CEBPE* [26], *ARID5B* [26, 27], and *CDKN2A* [29]. Some variants had significant association with specific subtypes. A variant located at *ARID5B* was significantly associated with high-hyperdiploid ALL, and a variant near *GATA3* was correlated with Ph-like ALL through ectopic expression of *GATA3* [30]. Of note, these causative genes are also targeted as somatically acquired

genomic alterations in lymphoid malignancies, suggesting that abnormal expression or dysfunction of these genes can be the first stem for leukemogenesis. Children with multiple risk allele of these variants had ninefold higher incidence of ALL than subjects with fewer risk variants [31]. This impact is statistically significant and important for understanding the pathogenesis of pediatric ALL, but the frequency of ALL is still low even in children with multiple risk alleles, and regular screening is not generally recommended.

Given identification of some novel leukemia-predisposition syndromes, subsequent large-scale studies were performed to search prevalence of pathogenic variants in non-syndromic ALL in children. Moriyama et al. demonstrated that 31 of 4405 (0.7%) non-familial (sporadic) ALL had germline *ETV6* variants [32]. Children with ALL-related *ETV6* variants were significantly older at diagnosis, and higher frequency in high-hyperdiploid ALL. Another study showed that *IKZF1* variants were observed in 43 of 4963 (0.9%) sporadic ALL, and the majority of the variants adversely affected *IKZF1* transcriptional function leading to leukemogenesis [33]. When focusing on *TP53*, 77 of 3801cases had nonsynonymous variants [34]. Children with *TP53* variants had a trend toward inferior prognosis, and pathogenic *TP53* variants were significantly associated with increased risk of secondary malignant neoplasms. Prevalence of these pathogenic variants in each single gene is low, but comprehensive analysis targeting 656 cancer-associated genes showed 4.4% of pediatric ALL had pathogenic variants conferring to leukemia predisposition [35]. These findings led to the recent proposal of surveillance recommendation for children with leukemia-predisposing conditions [36].

References

1. Relling MV, Schwab M, Whirl-Carrillo M, et al. Clinical pharmacogenetics implementation consortium guideline for thiopurine dosing based on TPMT and NUDT15 genotypes: 2018 update. Clin Pharmacol Ther. 2019;105:1095–105.
2. Moriyama T, Nishii R, Lin TN, et al. The effects of inherited NUDT15 polymorphisms on thiopurine active metabolites in Japanese children with acute lymphoblastic leukemia. Pharmacogenet Genomics. 2017;27:236–9.
3. Lennard L, Lilleyman JS. Variable mercaptopurine metabolism and treatment outcome in childhood lymphoblastic leukemia. J Clin Oncol. 1989;7:1816–23.
4. Lennard L, Lilleyman JS, Van Loon J, Weinshilboum RM. Genetic variation in response to 6-mercaptopurine for childhood acute lymphoblastic leukaemia. Lancet. 1990;336:225–9.
5. Lennard L, Gibson BE, Nicole T, Lilleyman JS. Congenital thiopurine methyltransferase deficiency and 6-mercaptopurine toxicity during treatment for acute lymphoblastic leukaemia. Arch Dis Child. 1993;69:577–9.
6. Relling MV, Pui CH, Cheng C, Evans WE. Thiopurine methyltransferase in acute lymphoblastic leukemia. Blood. 2006;107:843–4.
7. Relling MV, Hancock ML, Boyett JM, Pui CH, Evans WE. Prognostic importance of 6-mercaptopurine dose intensity in acute lymphoblastic leukemia. Blood. 1999;93:2817–23.
8. Yang SK, Hong M, Baek J, et al. A common missense variant in NUDT15 confers susceptibility to thiopurine-induced leukopenia. Nat Genet. 2014;46:1017–20.

9. Yang JJ, Landier W, Yang W, et al. Inherited NUDT15 variant is a genetic determinant of mercaptopurine intolerance in children with acute lymphoblastic leukemia. J Clin Oncol. 2015;33:1235–42.
10. Tanaka Y, Kato M, Hasegawa D, et al. Susceptibility to 6-MP toxicity conferred by a NUDT15 variant in Japanese children with acute lymphoblastic leukaemia. Br J Haematol. 2015;171(1):109–15.
11. Moriyama T, Nishii R, Perez-Andreu V, et al. NUDT15 polymorphisms alter thiopurine metabolism and hematopoietic toxicity. Nat Genet. 2016;48:367–73.
12. Tsujimoto S, Osumi T, Uchiyama M, et al. Diplotype analysis of NUDT15 variants and 6-mercaptopurine sensitivity in pediatric lymphoid neoplasms. Leukemia. 2018;32:2710–4.
13. Diouf B, Crews KR, Lew G, et al. Association of an inherited genetic variant with vincristine-related peripheral neuropathy in children with acute lymphoblastic leukemia. JAMA. 2015;313:815–23.
14. Kawedia JD, Kaste SC, Pei D, et al. Pharmacokinetic, pharmacodynamic, and pharmacogenetic determinants of osteonecrosis in children with acute lymphoblastic leukemia. Blood. 2011;117:2340–7. quiz 2556
15. Ben Tanfous M, Sharif-Askari B, Ceppi F, et al. Polymorphisms of asparaginase pathway and asparaginase-related complications in children with acute lymphoblastic leukemia. Clin Cancer Res. 2015;21:329–34.
16. Soh SX, Lim JY, Huang JW, Jiang N, Yeoh AE, Ong ST. Multi-agent chemotherapy overcomes glucocorticoid resistance conferred by a BIM deletion polymorphism in pediatric acute lymphoblastic leukemia. PLoS One. 2014;9:e103435.
17. Tinhofer I, Marschitz I, Henn T, Egle A, Greil R. Expression of functional interleukin-15 receptor and autocrine production of interleukin-15 as mechanisms of tumor propagation in multiple myeloma. Blood. 2000;95:610–8.
18. Cave H, Caye A, Strullu M, et al. Acute lymphoblastic leukemia in the context of RASopathies. Eur J Med Genet. 2016;59:173–8.
19. van der Werff Ten Bosch J, van den Akker M. Genetic predisposition and hematopoietic malignancies in children: primary immunodeficiency. Eur J Med Genet. 2016;59:647–53.
20. Holmfeldt L, Wei L, Diaz-Flores E, et al. The genomic landscape of hypodiploid acute lymphoblastic leukemia. Nat Genet. 2013;45:242–52.
21. Shah S, Schrader KA, Waanders E, et al. A recurrent germline PAX5 mutation confers susceptibility to pre-B cell acute lymphoblastic leukemia. Nat Genet. 2013;45:1226–31.
22. Noetzli L, Lo RW, Lee-Sherick AB, et al. Germline mutations in ETV6 are associated with thrombocytopenia, red cell macrocytosis and predisposition to lymphoblastic leukemia. Nat Genet. 2015;47:535–8.
23. Boutboul D, Kuehn HS, Van de Wyngaert Z, et al. Dominant-negative IKZF1 mutations cause a T, B, and myeloid cell combined immunodeficiency. J Clin Invest. 2018;128:3071–87.
24. Perez-Garcia A, Ambesi-Impiombato A, Hadler M, et al. Genetic loss of SH2B3 in acute lymphoblastic leukemia. Blood. 2013;122:2425–32.
25. Urayama KY, Chokkalingam AP, Manabe A, Mizutani S. Current evidence for an inherited genetic basis of childhood acute lymphoblastic leukemia. Int J Hematol. 2013;97:3–19.
26. Papaemmanuil E, Hosking FJ, Vijayakrishnan J, et al. Loci on 7p12.2, 10q21.2 and 14q11.2 are associated with risk of childhood acute lymphoblastic leukemia. Nat Genet. 2009;41:1006–10.
27. Trevino LR, Yang W, French D, et al. Germline genomic variants associated with childhood acute lymphoblastic leukemia. Nat Genet. 2009;41:1001–5.
28. Migliorini G, Fiege B, Hosking FJ, et al. Variation at 10p12.2 and 10p14 influences risk of childhood B-cell acute lymphoblastic leukemia and phenotype. Blood. 2013;122:3298–307.
29. Sherborne AL, Hosking FJ, Prasad RB, et al. Variation in CDKN2A at 9p21.3 influences childhood acute lymphoblastic leukemia risk. Nat Genet. 2010;42:492–4.
30. Perez-Andreu V, Roberts KG, Harvey RC, et al. Inherited GATA3 variants are associated with Ph-like childhood acute lymphoblastic leukemia and risk of relapse. Nat Genet. 2013;45:1494–8.

31. Xu H, Yang W, Perez-Andreu V, et al. Novel susceptibility variants at 10p12.31-12.2 for childhood acute lymphoblastic leukemia in ethnically diverse populations. J Natl Cancer Inst. 2013;105:733–42.
32. Moriyama T, Metzger ML, Wu G, et al. Germline genetic variation in ETV6 and risk of childhood acute lymphoblastic leukaemia: a systematic genetic study. Lancet Oncol. 2015;16:1659–66.
33. Churchman ML, Qian M, Te Kronnie G, et al. Germline genetic IKZF1 variation and predisposition to childhood acute lymphoblastic leukemia. Cancer Cell. 2018;33(5):937–948.e8.
34. Qian M, Cao X, Devidas M, et al. TP53 Germline variations influence the predisposition and prognosis of B-cell acute lymphoblastic leukemia in children. J Clin Oncol. 2018;36:591–9.
35. Zhang J, Walsh MF, Wu G, et al. Germline mutations in predisposition genes in pediatric cancer. N Engl J Med. 2015;373:2336–46.
36. Porter CC, Druley TE, Erez A, et al. Recommendations for surveillance for children with leukemia-predisposing conditions. Clin Cancer Res. 2017;23:e14–22.

Chapter 4
Immunophenotype of Pediatric ALL

Takao Deguchi

Abstract Flow cytometric immunophenotyping still stands on a significant position as a diagnostic tool against acute lymphoblastic leukemia (ALL). Improvement of flow cytometry (FCM) instrument and increasing availability of monoclonal antibodies and fluorochromes enable multicolor analysis and accurate diagnosis. But molecular diagnostic method also made a big progress especially in genome-wide analysis. The World Health Organization (WHO) classification of tumors of the hematopoietic and lymphoid tissues was first established in 2008, then in 2017 it was revised diagnostic criteria including morphologic, phenotypic, and more genotypic features (Wenzinger et al., Curr Hematol Malig Rep 13:275–288, 2018). Thus, such a recent progress of genomic analysis alters a role of immunophenotyping for pediatric ALL. Currently, entities of pediatric ALL are divided into subtypes by not only immunophenotyping but various cytogenetic abnormalities. Immunophenotyping may become no longer crucial but only exist to decide initial treatment until an identification of cytogenetic abnormalities in the near future. However, recently it turned out that immunophenotypic patterns indicate a specific feature according to each cytogenetic abnormality. To predict cytogenetic abnormality based on specific immunophenotype may fulfill an efficient cytogenetic diagnosis for most of known cytogenetic abnormalities. This chapter indicates the present situation of flow cytometric testing and current application for pediatric ALL, based on a new era of genomic analysis.

Keywords Immunophenotyping · Flow cytometry · Cytogenetics

T. Deguchi (✉)
Division of Cancer Immunodiagnostics, Children's Cancer Center, National Center for Child Health & Development, Setagaya, Tokyo, Japan
e-mail: deguchi-t@ncchd.go.jp

M. Kato (ed.), *Pediatric Acute Lymphoblastic Leukemia*,
https://doi.org/10.1007/978-981-15-0548-5_4

4.1 Diagnostic Criteria of Pediatric ALL According to Immunophenotyping

According to central immunophenotyping study in Japan, 85–90% cases of pediatric ALL belong to B-cell lineage and 10–15% belong to T-cell lineage [1]. Both T- and B-lineage diagnosis basically depends on lineage requirements defined by WHO criteria (Table 4.1) [2]. Generally, in B-lineage ALL, the most important marker for lineage diagnosis is CD19, but it is also important to express CD20, CD22, CD24, CD10, or CD79a. Throughout our experience, CD19 was rarely indicated completely negative expression in case of B-cell lineage ALL [3]. Furthermore, a relapse after chimeric antigen receptor T (CAR-T) cell therapy often indicates loss of CD19 expression [4] on blast cells although those blasts expressed cytoplasmic CD79a and CD22. According to WHO criteria, such relapsed leukemia classified into acute unclassified leukemia (AUL) because B-lineage commitment requires surface CD19 expression. Responding to CD19-negative B-lineage ALL, Japan Children's Cancer Group (JCCG) applied modified criteria. These criteria consist of two elements: one is criterion to decide lineage commitment (Table 4.2), and the other is to define mixed lineage leukemia (Table 4.3) [3]. In these criteria,

Table 4.1 Lineage requirements according to WHO classification

Myeloid lineage
MPO (by flow cytometry, immunohistochemistry, or cytochemistry)
or
Monocytic differentiation (≥2 of the following: non-specific esterase, CD11c, CD14, CD64, lysozyme)
T-cell lineage
Cytoplasmic CD3 (by flow cytometry with antibodies to CD3 epsilon chain Immunohistochemistry using polyclonal anti-CD3 antibody may detect CD3 zeta chain, which is not T-cell-specific)
or
Surface CD3 (rare in mixed-phenotype acute leukemia
B-cell lineage (multiple antigens required)
Strong CD19
with ≥1 of the following strongly expressed: CD79a, cytoplasmic CD22, CD10
or
Weak CD19
with ≥2 of the following strongly expressed: CD79a, cytoplasmic CD22, CD10

Table 4.2 Immunological diagnostic criteria for ALL defined by JCCG

T-ALL	*Pre-B ALL*
CD3 positive and express at least one of CD2, 5, 7, or 8	Express at least two B-lineage markers CD19, 20, 22, or 79a, and cIg μ positive, and Ig κ and Ig λ negative
B-precursor ALL	*Mature B ALL*
Express at least two B-lineage markers CD19, 20, 22, or 79a, and Ig κ and Ig λ negative	Express at least two B-lineage markers CD19, 20, 22, or 79a, and Ig κ or Ig λ positive

Table 4.3 Acute leukemia with aberrant lymphoid or myeloid antigen expression and "true mixed-lineage leukemia" defined by JCCG criteria

Myeloid Ag+ B-lineage ALL (all criteria must be met)	*Lymphoid Ag+ AML (all criteria must be met)*
Leukemic cells are: 1. Express at least two B-lineage markers (CD19, 20, 22, or 79a) 2. CD3− 3. MPO− and express CD13, 15, 33, or 65	Leukemic cells are: 1. MPO+ or express at least two other myeloid markers (CD13, 15, 33, or 65) 2. CD3− and CD79a− 3. Express CD2, 5, 7, 19, 22, or 56
Myeloid Ag+ T-lineage ALL (all criteria must be met)	*True mixed lineage leukemia*
Leukemic cells are: 1. CD3+ and express CD2, 5, 7, or 8 2. CD79a− 3. MPO− and express CD13, 15, 33, or 65	1. MPO+ and meets B-lineage criteria, or 2. MPO+ and meets T-lineage criteria, or 3. Meets B-lineage and T-lineage criteria simultaneously

Table 4.4 Comprehensive immunophenotyping panel in Japan

B-lineage		*T-lineage*		*Non-lineage*	
CD19	*cCD79a*	*cCD3*	CD4	*TdT*	CD38
CD10	*cCD22*	CD7	CD8	HLA-DR	CD58
CD20	Igμ	CD5	CD1a	CD34	CD99
CD22	Igκ	CD2	TCR-αβ	CD56	CRLF2
CD21	Igλ	CD3	TCR-γδ	CD66c	CD27
CD24	*cIgμ*			CD11b	CD244
				7.1	CD44
Granulocytoid		*Monocytoid*		*Megakaryocytoid*	
MPO		CD13		CD41	
CD15		CD14		CD42b	
CD65		CD33		CD61	
CD117		CD36		*Erythroid*	
CD133		CD64		CD235a	
CD123		CD371			

CD19 is one of important B-lineage antigens as well as CD79a, CD22, CD20. According to these modified criteria, most of relapsed cases with a loss of CD19 expression resulted to admit into B-lineage leukemia. In addition, these criteria were also designed to define immunophenotypic diagnosis simply by FCM only. Usually, non-specific esterase was confirmed by cytochemistry, and lysozyme was confirmed by immunohistochemistry, so these testing were independent from FCM. Our modified criteria make it possible to make a diagnosis at a time. To make a precise immunophenotyping, comprehensive diagnostic panel (Table 4.4) and DNA index test have been applied from 2012 in Japan. This panel consists of various markers including B-lymphocyte, T-lymphocyte, granulocyte, monocyte, megakaryocyte, and erythroid markers, as well as non-lineage markers to detect minimal residual disease (MRD) or to speculate specific cytogenetic abnormalities. Then immunological diagnosis is usually done according to the flowchart described

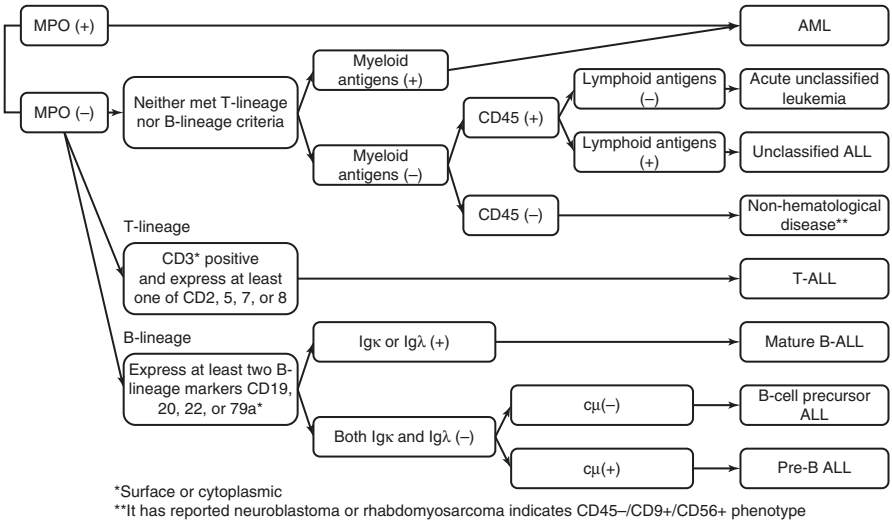


Fig. 4.1 Immunophenotyping flowchart according to JCCG diagnostic criteria

in Fig. 4.1. Currently an analysis by this panel was divided into a lot of sample tubes for FCM, but simultaneous multicolor analysis fulfilled by advance of FCM instrument, such as mass cytometry, may enable to make immunophenotyping by artificial intelligence in near future.

4.2 Immunophenotyping of B-Lineage ALL

Phenotype of leukemia cells usually reflects a normal counterpart such as pro-B, pre-B, or mature B-cells. According to EGIL classification, pro-B ALL express CD19, CD79a, cytoplasmic CD22, and CD34, but lack an expression of CD10. Then, the expression of CD10 antigen (CALLA) defines common ALL. Further, the expression of cytoplasmic heavy mu chain defines the pre-B ALL, and the expression of surface immunoglobulin (Ig) light chains defines mature B-ALL. It is of note that CD34 positive common ALL sometimes express surface or cytoplasmic mu chain [5]. Although typical pre-B ALL usually lack CD34 expression, these cases usually express CD34 and often indicate hyperdiploidy by DNA index analysis. This subgroup used to be called as transitional pre-B ALL [6]; usually surface mu chain is co-expressed with surrogate light chain Vpre-B and λ5 on leukemic cells (Table 4.5) [7]. But currently these differentiation subgroups are not applied to risk stratification in B-cell precursor ALL, because recent clinical stud-

Table 4.5 Lineage marker expression on B-lineage leukemia according to the developmental stage

	CD34	CD19	CD10	sCD22	CD20	sIgμ	cIgμ	sIgκ/λ
Pro-B	+	+	−	±	−	−	−	−
Transitional pre-B	+	+	+	+	−	±	+	−
Common ALL	±	+	+	+	±	−	−	−
Pre-B	−	+	+	+	±	−	+	−
Mature B	−	+	±	+	+	+	+	+

ies indicate immunophenotype did not indicate prognostic impact. Only in case of mature B-ALL, a shortened intensified multi-agent chemotherapy was applied contrary to B-cell precursor ALL. In general, surface Ig light chain expression defines mature B-ALL, but some cases of pre-B ALL also express surface Ig light chain. Mostly, such cases indicate partly positive for surface Ig, positive for TdT, and low expression of CD20. This subgroup indicates quite different clinical features from typical mature B-ALL with *myc* translocation. Some cases have reported to indicate MLL-AF9 translocation [8]. Conversely, high-grade B-cell lymphoma with translocations involving *myc* and *bcl-2* or *bcl-6*, so-called "double-hit lymphoma," surprisingly indicates common ALL phenotype and lacks surface Ig expression [9].

4.3 Immunophenotyping of T-Lineage ALL

T-cell markers are usually thought to be CD1a, CD2, CD3 (surface and cytoplasmic), CD4, CD5, CD7, and CD8. Most of these antigens are ultimately not T-cell specific, so the expression of cytoplasmic CD3 is the most important lineage specific marker. At first, cytoplasmic CD3 and also CD7 are expressed on the most immature T-cell cells, then CD2 and CD5 participate in. In this stage, CD10, CD34, and myeloid antigens (CD13, CD33) can be expressed too. According to EGIL classification, T-ALL are divided into four subsets, pro-T (cyCD3, CD7, TdT), pre-T (cyCD3, CD7, TdT, CD5±), cortical T (cyCD3, CD7, TdT, CD5+, CD1a+, CD4−/8−, or 4+/8+), and mature-T (cyCD3, CD7, TdT−, CD5+, sCD3+, CD4+, or 8+) [5]. Recently, a novel subgroup was characterized as Early T cell precursor (ETP-) ALL by gene-expression profile, which shows characteristic immunophenotype, namely lack of CD1a and CD8 expression, weak or negative CD5 expression, and expression of at least one myeloid and/or stem cell marker [10]. Recently immunophenotypic scoring system for ETP-ALL was reported [11], but ETP-ALL defined by immunophenotyping should be verified to compare with ETP-ALL specified by gene-expression profile.

4.4 Relationship Between Immunophenotyping and Cytogenetic Abnormalities

Recent progress of genome-wide analysis revealed new cytogenetic abnormalities in pediatric ALL. Frequency of cytogenetic subtypes of pediatric ALL consist of 22% of *ETV6-RUNX1*, 20% of hyperdiploid, 9% of *BCR-ABL1*-like, 6% of *MLL* rearrangements, 4% of *TCF3-PBX1*, and so on [12]. Each subtype has peculiar clinical features and prognosis, and several subtypes require different treatment strategy from standard therapy. For example, *ABL1*-class fusion has an indication to use tyrosine kinase inhibitor. So current treatment against pediatric ALL should decide on precise identification of subtype. Multiplex-PCR analysis may help to determine subtypes, but it may be difficult to build rare translocation into multiplex-panel. On the other hand, to apply whole exome-sequencing into all pediatric ALL cases may be still unsatisfactory from an economic point of view. Comprehensive panel for pediatric hematological malignancies in Japan reveals a certain characteristic immunophenotype associated with each cytogenetic abnormality. Brief explanations are described as follows (Table 4.6):

Hyperdiploid is usually presented as common ALL. CD34, CD10, sCD22, and HLA-DR are constitutively positive in most of this subtype and rarely express myeloid antigens (CD13 and/or CD33). CD66c and CD123 are also aberrantly expressed on most of this subtype. A part of this subtype express surface Igμ and/or cytoplasmic Igμ. In case of surface Igμ expression, surrogate light chain, Vpre-B, and λ5 are also expressed. It was named as "transitional pre-B ALL," as described above.

Table 4.6 Relations between genetic subtype and immunophenotyped

Subtype	Immunophenotype	Aberrant antigens
Hyperdiploidy	Common ALL, transitional pre-B	CD66c, CD123, sIgμ
ETV6-RUNX1	Common ALL, pre-B	Myeloid antigens, CD56 (rare)
TCF3-PBX1	Pre-B	
MLL (KMT2A) *MLL-AF4* *MLL-ENL* *MLL-AF9*	 Pro-B Pro-B Pre-B, mature ALL (rare)	 CD15, CD65, 7.1 (NG2), CD133 7.1 (NG2) sIg (rare)
BCR-ABL1	Common ALL	CD66c, myeloid antigens
Ph-like (*Abl*-class, *PDGFR*, *CRLF2*, *JAK*, and *PAX5* fusion)	Common ALL	CD66c, CRLF2 (*CRLF2-P2RY8 or IGH–CRLF2*)
ZNF384	Pro-B, mixed phenotype (rare)	Myeloid antigens, MPO (rare)
MEF2D	Pre-B, common ALL	CD5
IgH-DUX4	Common ALL	CD371

ETV6-RUNX1 is mostly presented as common ALL, but a considerable proportion is also presented as pre-B ALL. CD34 often indicates negative, but most of this subtype can find minor population of CD34-positive cells even in pre-B cell phenotype, although other pre-B subtypes (*TCF3-PBX1* or *MLL-AF9*) consist of only CD34 negative population. This subtype tends to express myeloid antigens (CD13 and/or CD33) and rarely expresses CD56. In addition, CD44 expression tends to be low.

TCF3-PBX1 is mostly presented as pre-B ALL and usually does not express aberrant markers.

MLL rearrangements indicate heterogenous phenotypes, which depend on translocation subtypes. *MLL-AF4* is commonly presented as pro-B ALL, which express CD34, CD19, and HLA-DR but is negative for CD10. Blast cells often express myeloid antigens such as not only CD13 and/or CD33 but CD15/CD65. 7.1 (NG2) or CD133 is often aberrantly expressed, too.

MLL-ENL is also presented as pro-B ALL, and 7.1 (NG2) is also aberrantly expressed. But occasionally CD34 became negative, and expression of myeloid antigens is not frequent compared with *MLL-AF4*.

MLL-AF9 indicates a unique feature, mostly pre-B ALL, and lacks either myeloid or 7.1 antigen expression. This subgroup rarely expresses surface Ig light chain. In such cases, immunophenotypic diagnosis becomes mature ALL according to diagnostic criteria. But most of these cases express only weak or partial surface Ig expression and often indicate low CD20 expression compared with high Ig and CD20 expression on true mature B-ALL.

BCR-ABL1 is mostly presented as common ALL with aberrant CD66c expression [13]. Myeloid antigen (CD13 and CD33) expressions are also often involved. T-lineage antigens such as CD2 and/or CD7 are also expressed.

Ph-like ALL includes *Abl1*-class, *PDGFR*, *CRLF2*, *JAK*, and *PAX5* fusion, usually presented as common ALL. CD66c expression is often observed [13] but sometimes not observed. Myeloid or T-lineage antigen expression is usually rare. In case of *CRLF2* fusion (*CRLF2-P2RY8* or *IGH–CRLF2*), flow cytometric CRLF2 expression becomes very high [14]. It is very useful to specify this cytogenetic abnormality.

ZNF384 fusion is commonly presented as pro-B ALL, which express CD34, CD19, and HLA-DR but are negative for CD10. Blast cells often express myeloid antigens (CD13 and/or CD33) [15]. This subgroup rarely express myeloperoxidase (MPO) [16]. Thus, immunophenotypic diagnosis of these cases becomes true mixed phenotype acute leukemia (MPAL) according to diagnostic criteria. But this type of MPAL is supposed to indicate relatively good prognosis if treated with ALL-oriented therapy.

MEF2D fusion is commonly presented as pre-B ALL, but a considerable proportion is also presented as common ALL. Aberrant phenotype is not so clear, but CD5 expression may be a good suggestion of this subtype [17, 18].

Finally, *IgH-DUX4* fusion is commonly presented as common ALL. Recently CD371 was reported as a diagnostic marker of this subtype [19].

References

1. Wenzinger C, Williams E, Gru AA. Updates in the pathology of precursor lymphoid neoplasms in the revised fourth edition of the WHO classification of Tumors of hematopoietic and lymphoid tissues. Curr Hematol Malig Rep. 2018;13(4):275–88.
2. Horibe K, et al. Incidence and survival rates of hematological malignancies in Japanese children and adolescents (2006–2010): based on registry data from the Japanese Society of Pediatric Hematology. Int J Hematol. 2013;98(1):74–88.
3. Iwamoto S, et al. Flow cytometric analysis of de novo acute lymphoblastic leukemia in childhood: report from the Japanese Pediatric Leukemia/lymphoma study group. Int J Hematol. 2011;94(2):185–92.
4. Sotillo E, et al. Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. Cancer Discov. 2015;5(12):1282–95.
5. Chiaretti S, Zini G, Bassan R. Diagnosis and subclassification of acute lymphoblastic leukemia. Mediterr J Hematol Infect Dis. 2014;6(1):e2014073.
6. Koehler M, et al. Transitional pre-B-cell acute lymphoblastic leukemia of childhood is associated with favorable prognostic clinical features and an excellent outcome: a Pediatric oncology group study. Leukemia. 1993;7(12):2064–8.
7. Tsuganezawa K, et al. Flow cytometric diagnosis of the cell lineage and developmental stage of acute lymphoblastic leukemia by novel monoclonal antibodies specific to human pre-B-cell receptor. Blood. 1998;92(11):4317–24.
8. Tsao L, et al. Mature B-cell acute lymphoblastic leukemia with t(9;11) translocation: a distinct subset of B-cell acute lymphoblastic leukemia. Mod Pathol. 2004;17(7):832–9.
9. Kelemen K, et al. Immunophenotypic and cytogenetic findings of B-lymphoblastic leukemia/lymphoma associated with combined IGH/BCL2 and MYC rearrangement. Cytometry B Clin Cytom. 2017;92(4):310–4.
10. Coustan-Smith E, et al. Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. Lancet Oncol. 2009;10(2):147–56.
11. Inukai T, et al. Clinical significance of early T-cell precursor acute lymphoblastic leukaemia: results of the Tokyo Children's cancer study group study L99-15. Br J Haematol. 2012;156(3):358–65.
12. Mullighan CG. Genomic profiling of B-progenitor acute lymphoblastic leukemia. Best Pract Res Clin Haematol. 2011;24(4):489–503.
13. Kiyokawa N, et al. Significance of CD66c expression in childhood acute lymphoblastic leukemia. Leuk Res. 2014;38(1):42–8.
14. Bugarin C, et al. Fine tuning of surface CRLF2 expression and its associated signaling profile in childhood B-cell precursor acute lymphoblastic leukemia. Haematologica. 2015;100(6):e229–32.
15. Hirabayashi S, et al. ZNF384-related fusion genes define a subgroup of childhood B-cell precursor acute lymphoblastic leukemia with a characteristic immunotype. Haematologica. 2017;102(1):118–29.
16. Alexander TB, et al. The genetic basis and cell of origin of mixed phenotype acute leukaemia. Nature. 2018;562(7727):373–9.
17. Suzuki K, et al. MEF2D-BCL9 fusion gene is associated with high-risk acute B-cell precursor lymphoblastic Leukemia in adolescents. J Clin Oncol. 2016;34(28):3451–9.
18. Ohki K, et al. Clinical and molecular characteristics of MEF2D fusion-positive B-cell precursor acute lymphoblastic leukemia in childhood, including a novel translocation resulting in MEF2D-HNRNPH1 gene fusion. Haematologica. 2019;104(1):128–37.
19. Schinnerl D, et al. CD371 cell surface expression: a unique feature of DUX4-rearranged acute lymphoblastic leukemia. Haematologica. 2019;104(8):e352–5.

Chapter 5
MRD in Pediatric ALL

Motohiro Kato

Abstract Even after microscopic remission after induction therapy, however, in this more than billions of leukemic cells potentially are supposed to escape this microscopic detection. In recent years, technological progress has enabled us to detect residual leukemic cells, which cannot be detected only by means of conventional assessment using microscopic morphology. These residual cells in microscopic remission are "minimal residual diseases (MRD)". PCR and flow cytometry (FCM) are two major methods to detect MRD, and these methods had several advantages/disadvantages. PCR-MRD can detect as low as 0.001% of residual leukemic cells, while the sensitivity of FCM-MRD is 0.01%. On the other hand, FCM-MRD has advantages in terms of simple preparation methods, short duration to obtain results, cheaper cost, and broad applicability. Irrespective of MRD detection methods, time points to be assessed, and threshold, potent impacts on prognosis have been confirmed by numerous clinical studies. MRD assessment is essential to stratify patients according to relapse risk.

Keywords PCR · Flow cytometry · Prognostic factor

5.1 Introduction

Response to chemotherapy is the strongest prognostic indicator in pediatric acute lymphoblastic leukemia (ALL), and several studies confirmed the prognostic importance of the clearance of leukemic blasts in the early phase of treatment. The number of blasts in peripheral blood at day 8, the percentage of residual blasts in bone marrow at day 15, and the achievement of remission at the end of induction are predictors of relapse risk, and most study groups adjust treatment intensity (or reduce) according to early response to treatment [1].

M. Kato (✉)
Department of Transplantation and Cell Therapy, Children's Cancer Center, National Center for Child Health and Development, Setagaya-ku, Tokyo, Japan
e-mail: kato-mt@ncchd.go.jp

M. Kato (ed.), *Pediatric Acute Lymphoblastic Leukemia*,
https://doi.org/10.1007/978-981-15-0548-5_5

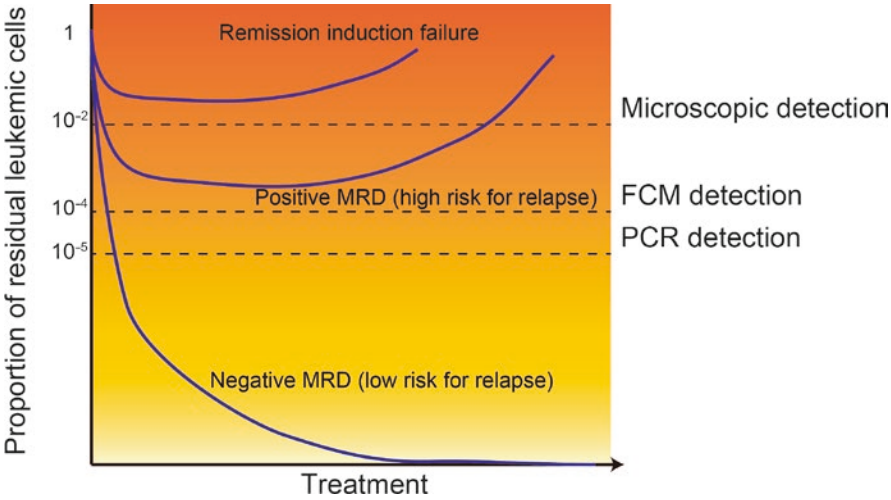


Fig. 5.1 Concept of morphological remission, molecular remission, and MRD

With modern intensive multiagent induction therapy, the vast majority of children with ALL reach complete remission, defined less than 5% of leukemic blasts. However, in this remission status, more than billions of leukemic cells potentially are supposed to escape this microscopic detection. In recent years, technological progress has enabled us to detect residual leukemic cells, which cannot be detected only by means of conventional assessment using microscopic morphology.

These residual cells in microscopic remission are "minimal residual diseases (MRD)" as shown in Fig. 5.1, and numerous studies demonstrated that MRD status was an independent prognostic factor of outcomes of ALL.

5.2 Detection Methods of Minimal Residual Disease

Two major MRD detection techniques have developed into clinical practice: one is PCR-based detection and the other is FCM-based detection. The characteristics of these MRD technologies and advantages/disadvantages are summarized in Table 5.1.

5.2.1 PCR-Based MRD Detection

During lymphocyte differentiation, receptor genes encoding immunoglobulin (Ig) and T-cell receptors (TCR) are rearranged, and this process includes the assembly of Variable (V), Diversity (D), and Joining (J) gene segments. With

Table 5.1 Characteristics of MRD detection methods

	PCR-MRD	FCM-MRD
Sensitivity (detection threshold)	0.1–0.01% (10^{-3} to 10^{-4})	0.01–0.001% (10^{-4} to 10^{-5})
Applicability (% of patients with target)	>90%	>95%
Advantages	Fast Cheaper cost Simple method	Sensitive Well standardized
Disadvantages	Limited standardization	Time-consuming Expensive cost

additional random insertion and/or deletion of nucleotides, each lymphocyte has unique V–(D)–J junctions, providing a wide diversity of these receptors against numerous antigens.

ALL cells are basically monoclonal arising from lymphocytes with unique Ig/TCR sequences; thus each ALL case had unique Ig/TCR, which can be fingerprint of leukemic cells [2]. PCR can detect a small fraction of residual leukemic cells when oligonucleotide primers are designed to amplify individual junctional region sequences of clonal rearrangements, as few as 0.001% of residual leukemic cells as MRD [3]. This ALL specific PCR method was further improved by the introduction of real-time PCR to quantify MRD as a rapid, reliable, and sensitive assay [4, 5]. To standardize and perform quality control of quantitative MRD detection targeting Ig/TCR, the EuroMRD Consortium was established in 2001, consisting of more than 50 PCR-MRD laboratories across more than 20 countries (http://www.euromrd.org).

For PCR-MRD assessment, sequencing of the Ig/TCR region of leukemic cells at diagnosis and design of leukemia specific primers are the first step, which takes 3–5 weeks, and recent studies showed that >90% of ALL cases can be monitored by PCR-MRD [6, 7].

5.2.2 FCM-Based MRD Detection

Most of leukemic lymphoblasts have leukemia-specific immunophenotype. FCM analysis with multi-color immunostainings can also detect MRD by identification of leukemia-specific aberrant immunophenotype, targeting a combination of leukemia-associated surface markers. Recent FCM-MRD panels consist of eight or more markers [8, 9] because of technical innovations of FCM.

Generally, bone marrow (BM) samples for FCM-MRD were processed using bulk erythrocyte lysis protocol with ammonium chloride-based lysing reagent. After lysis and wash steps, cells were resuspended and stained with pre-titrated antibody panels. Because this method cannot eliminate RBCs completely, the enumeration step of nucleated cells is also required [10]. If BM mononuclear cells were obtained by density gradient centrifugation instead of bulk lysis method, more

precise measurements might be achieved to avoid contamination of red cells or cell debris. Acquisition of at least 0.3 million or more cells achieves a sensitivity of 0.01% of MRD.

Previous reports showed that the result of FCM-MRD was highly concordant to that of PCR-MRD [8]. Although PCR-MRD is more sensitive at the level of <0.01%, FCM-MRD has advantages over PCR-MRD in terms of simple preparation methods, short duration to obtain results, cheaper cost, and broad applicability [11]. In most cases, FCM-MRD can be evaluated even without diagnostic immunophenotypic details, but to detect leukemic cells precisely from regenerating BM after chemotherapy, leukemia-specific immunophenotypes should be better to be assessed with leukemic cells at the onset according to comprehensive monoclonal antibody panel for FCM-MRD.

5.3 Clinical Impact of MRD

MRD status during treatment reflects sensitivity of leukemic cells to chemotherapy under host factors. Thus, MRD kinetics strongly influenced on relapse risk of each ALL cases, as shown in several studies including children and adults [2, 12–18].

In 1998, van Dongen et al. demonstrated a strong impact of MRD on a relapse risk by monitoring 240 patients with ALL of the International BFM Study Group (I-BFM-SG) clinical trials. Children with negative MRD at early (end of induction; week 5) and late (before consolidation; week 12) time points were correlated with extremely low relapse rate (2% of relapse rate at 3-year), while children with positive MRD at these time points had five- to ten- fold higher relapse rates [2].

The presence/absence and level of MRD were independent prognostic factor [18], and several studies showed that MRD status affected relapse risks irrespective of disease subtype. The Interfant-99 study showed that infant ALL with positive MRD negative had a relapse rate of 13%, whereas positive MRD cases had a relapse rate of 31% [19]. The European intergroup study of Philadelphia-chromosome positive ALL (EsPhALL) reported that patients with *BCR-ABL1* positive ALL who achieved MRD negativity at end of early consolidation (week 12) had a lower relapse risk compared to those who did not.

MRD levels have significant prognostic impacts, even within high-risk genetic subtypes. A study including 20 children with hypodiploid ALL showed that MRD status at the end of 6-week remission induction were highly curable with intensive chemotherapy alone (without stem cell transplantation) [20]. Following genomic studies including large number (>100) of hypodiploid cases also showed that MRD-stratified therapy could improve the outcome of this high-risk subset [21, 22], while stem cell transplantation failed to improve outcomes of hypodiploid ALL with positive MRD at the end of induction therapy [22]. The advances in cure rate by MRD-directed therapy have extended to *BCR-ABL1*-like ALL, another high-risk genetic subgroup. A study from the St. Jude Children's Research Hospital (SJCRH) showed

that stratification based on MRD could salvage inferior outcome of *BCR-ABL1*-like (Ph-like) ALL [23], although the outcomes of Ph-like ALL in adults remains suboptimal even if they achieved MRD negative remission [24].

By complementary use of PCR-MRD and FCM-MRD, the SJCRH showed that MRD kinetics were able to be assessed for >99% of pediatric ALL [3]. They demonstrated that low MRD at day 19 predicted excellent outcomes for children with high-hyperdiploid or *ETV6-RUNX1*, with less than 4% of relapse risk. Among National Cancer Institute (NCI) standard risk (1–9 years of age and <50,000/μL of leukocyte count at diagnosis) cases, residual MRD ≥1% on day 19 or positive MRD on day 46 were correlated with poor outcome.

Importance of MRD-directed therapy was confirmed by a randomized controlled trial. The UKALL2003 trial showed that ALL with ≥0.01% of MRD at the end of induction therapy could benefit from augmented therapy with asparaginase and methotrexate [25] while treatment reduction was possible for a group defined as low risk by rapid clearance of MRD by the end of induction therapy [26]. The Dutch Childhood Oncology Group also demonstrated that chemotherapy reduction was feasible for ALL with undetectable MRD levels, and intensification of therapy could improve outcomes of ALL with high levels of MRD [14].

5.4 Future of Minimal Residual Disease Detection

Based on the importance of MRD assessment for ALL treatment, many researchers challenged to improve MRD detection methods, mainly using next-generation sequencing technique and/or droplet digital PCR, with higher sensitivity (~0.0001%) and more accurate quantitative detection of residual leukemic cells [27, 28].

Of note, MRD status depends on previous therapy and time points. Threshold dividing positive/negative also differ according to each clinical trial. Thus, the results of MRD should be carefully interpreted, but a meta-analysis showed that MRD kinetics affected outcomes regardless of time points and threshold. Persistent MRD cases had poor outcome by conventional chemotherapy, and experimental regimens should be challenged for this subgroup.

References

1. Kato M, Manabe A. Treatment and biology of pediatric acute lymphoblastic leukemia. Pediatr Int. 2018;60:4–12.
2. van Dongen JJ, Seriu T, Panzer-Grumayer ER, et al. Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. Lancet. 1998;352:1731–8.
3. Pui CH, Pei D, Raimondi SC, et al. Clinical impact of minimal residual disease in children with different subtypes of acute lymphoblastic leukemia treated with response-adapted therapy. Leukemia. 2017;31:333–9.

4. Pongers-Willemse MJ, Verhagen OJ, Tibbe GJ, et al. Real-time quantitative PCR for the detection of minimal residual disease in acute lymphoblastic leukemia using junctional region specific TaqMan probes. Leukemia. 1998;12:2006–14.
5. Bruggemann M, Droese J, Bolz I, et al. Improved assessment of minimal residual disease in B cell malignancies using fluorogenic consensus probes for real-time quantitative PCR. Leukemia. 2000;14:1419–25.
6. van der Velden VH, Cazzaniga G, Schrauder A, et al. Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. Leukemia. 2007;21:604–11.
7. van Dongen JJ, van der Velden VH, Bruggemann M, Orfao A. Minimal residual disease diagnostics in acute lymphoblastic leukemia: need for sensitive, fast, and standardized technologies. Blood. 2015;125:3996–4009.
8. Campana D, Coustan-Smith E. Measurements of treatment response in childhood acute leukemia. Korean J Hematol. 2012;47:245–54.
9. Theunissen P, Mejstrikova E, Sedek L, et al. Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. Blood. 2017;129:347–57.
10. Kalina T, Flores-Montero J, van der Velden VH, et al. EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. Leukemia. 2012;26:1986–2010.
11. Campana D, Coustan-Smith E. Detection of minimal residual disease in acute leukemia by flow cytometry. Cytometry. 1999;38:139–52.
12. 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. 2010;115:3206–14.
13. 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. 2011;118:2077–84.
14. Pieters R, de Groot-Kruseman H, Van der Velden V, et al. Successful therapy reduction and intensification for childhood acute lymphoblastic Leukemia based on minimal residual disease monitoring: study ALL10 from the Dutch childhood oncology group. J Clin Oncol. 2016;34:2591–601.
15. Coustan-Smith E, Behm FG, Sanchez J, et al. Immunological detection of minimal residual disease in children with acute lymphoblastic leukaemia. Lancet. 1998;351:550–4.
16. Berry DA, Zhou S, Higley H, et al. Association of Minimal Residual Disease with Clinical Outcome in Pediatric and adult acute lymphoblastic Leukemia: a meta-analysis. JAMA Oncol. 2017;3:e170580.
17. Eckert C, Henze G, Seeger K, et al. Use of allogeneic hematopoietic stem-cell transplantation based on minimal residual disease response improves outcomes for children with relapsed acute lymphoblastic leukemia in the intermediate-risk group. J Clin Oncol. 2013;31:2736–42.
18. Cave H, van der Werff ten Bosch J, Suciu S, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. European Organization for Research and Treatment of Cancer—childhood Leukemia cooperative group. N Engl J Med. 1998;339:591–8.
19. Van der Velden VH, Corral L, Valsecchi MG, et al. Prognostic significance of minimal residual disease in infants with acute lymphoblastic leukemia treated within the Interfant-99 protocol. Leukemia. 2009;23:1073–9.
20. Mullighan CG, Jeha S, Pei D, et al. Outcome of children with hypodiploid ALL treated with risk-directed therapy based on MRD levels. Blood. 2015;126:2896–9.
21. Pui CH, Rebora P, Schrappe M, et al. Outcome of children with Hypodiploid acute lymphoblastic Leukemia: a retrospective multinational study. J Clin Oncol. 2019;37:770–9.
22. McNeer JL, Devidas M, Dai Y, et al. Hematopoietic stem-cell transplantation does not improve the poor outcome of children with hypodiploid acute lymphoblastic Leukemia: a report from Children's oncology group. J Clin Oncol. 2019;37:780–9.
23. Roberts KG, Pei D, Campana D, et al. Outcomes 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;32:3012–20.

24. Jain N, Roberts KG, Jabbour E, et al. Ph-like acute lymphoblastic leukemia: a high-risk subtype in adults. Blood. 2017;129:572–81.
25. 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 leukaemia (UKALL 2003): a randomised controlled trial. Lancet Oncol. 2014;15:809–18.
26. Vora A, Goulden N, Wade R, et al. Treatment reduction for children and young adults with low-risk acute lymphoblastic leukaemia defined by minimal residual disease (UKALL 2003): a randomised controlled trial. Lancet Oncol. 2013;14:199–209.
27. Faham M, Zheng J, Moorhead M, et al. Deep-sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. Blood. 2012;120:5173–80.
28. Ladetto M, Bruggemann M, Monitillo L, et al. Next-generation sequencing and real-time quantitative PCR for minimal residual disease detection in B-cell disorders. Leukemia. 2014;28:1299–307.

Part II
Treatment of Pediatric ALL

Chapter 6
B-Cell Precursor ALL

Motohiro Kato

Abstract B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is the most common form of pediatric ALL, consisting of >80% of ALL during childhood. In the past ALL was intractable, but now the survival probability is as high as 80–90%. Improved supportive care, treatment stratification based on relapse risk, biological features of leukemic cells, and optimization of treatment regimens by clinical trials have contributed to this dramatic improvement. Treatment regimens typically consist of induction therapy with steroids, vincristine, and asparaginase with or without anthracycline, followed by multiagent consolidation including high-dose/escalating methotrexate and re-induction therapy. After consolidation, less intensive maintenance therapy with thiopurines lasting for 1–2 years is given to maintain event free survival of the patients. The introduction of newly developed agents such as molecular targeted drugs or immunotherapy, and social supports including long-term follow up are required for further reduction of relapse risk without excess toxicity.

Keywords Risk stratification · Steroids · Asparaginase · Methotrexate
Maintenance therapy

6.1 Risk Stratification of B-Cell Precursor ALL

Treatment stratification based on relapse risk, biological features of leukemic cells, and optimization of treatment regimens through nationwide and international collaboration have contributed to dramatic improvement of pediatric ALL [1–3]. Recent clinical trials demonstrated that ALL in children had achieved >80% of event-free survival and 90% of overall survival [2].

M. Kato (✉)
Department of Transplantation and Cell Therapy, Children's Cancer Center, National Center for Child Health and Development, Setagaya-ku, Tokyo, Japan
e-mail: kato-mt@ncchd.go.jp

M. Kato (ed.), *Pediatric Acute Lymphoblastic Leukemia*,
https://doi.org/10.1007/978-981-15-0548-5_6

Intensification for high-risk cases suppresses relapse risk, and reduction of low-risk cases enables to avoid excess acute and late complication without compromising survival probability. For B-cell precursor ALL (BCP-ALL), age and leukocyte count are the two major prognostic factors. As these two universal factors are readily measurable at diagnosis, many clinical trials have adopted them for the purpose of treatment stratification [4, 5]. Excluding infants (<1 year of age), older age and higher leukocyte count were associated with a poor prognosis; thus children of "younger than 10 years" and "a leukocyte count less than 50,000/mm^3" are classified as standard risk by the National Cancer Institute criteria (NCI-SR), while children "aged 10 years or older" or "a leukocyte count 50,000/mm^3 or greater" are classified as high risk (NCI-HR) [6, 7]. Gender had also been recognized as a prognostic factor for BCP-ALL, but in recent clinical studies this difference has diminished, and current clinical trials do not use gender as a prognostic factor although some trials extend the maintenance therapy duration only for boys.

Treatment stratification based on the biological features of leukemic cells is one of the most important contributors to improving the outcome of pediatric BCP-ALL [1, 8]. The major sentinel cytogenetics conferring stratification in pediatric ALL are summarized in Table 6.1. Detailed information regarding leukemia biology is also written in Chap. 3.

Response to chemotherapy is the strongest prognostic indicator in pediatric BCP-ALL [9], and several studies confirmed the prognostic importance of the clearance of leukemic blasts in the early phase of treatment. The number of blasts in peripheral blood at day 8, the percentage of residual blasts in bone marrow at day 15, and the achievement of remission at the end of induction are predictors of relapse risk, and most study groups modify treatment according to these risk indicators.

In recent years, technological progress has enabled us to detect minimal residual diseases (MRD), which cannot be detected only by microscopic morphological assessment. Multiple studies showed that MRD status was significantly associated with relapse risk, and a randomized study by UKALL2003 showed that intensification for residual MRD cases was able to improve EFS [10] while reduction of therapy was possible for a group defined as low risk by MRD status [11]. Detailed information of PCR-MRD is shown in Chap. 5.

Table 6.1 Representative genomic alterations of pediatric BCP-ALL

Genomic alterations	Frequency	Clinical impact as a risk factor
High-hyperdiploid	20–30%	Good prognosis
Hypoploidy	1–3%	Poor prognosis
ETV6-RUNX1	15–25%	Good prognosis
TCF3-PBX1	5–10%	Good (under intensive therapy)
BCR-ABL1	5–8%	Poor, targetable by tyrosine kinase inhibitor
TCF3-HLF	1%	Extremely poor prognosis

6.2 Treatment Backbone of B-Cell Precursor ALL

A schema of a typical treatment for pediatric BCP-ALL is shown in Fig. 6.1. First, induction therapy is given to restore normal blood cell production. More than 95% of pediatric ALL achieved complete remission (usually defined as <5% of blasts in bone marrow) after 4–6 weeks of this regimen. Improved supporting therapy has decreased the mortality rate in induction to 2–3%, but a small fraction of children still suffer severe adverse events including severe infection during the first course of treatment [12].

After induction therapy, consolidation therapy subsequently begins to eradicate residual leukemic cells. Various combinations of cytotoxic agents are used, and high-dose or escalating methotrexate (MTX) plays a very important role in preventing relapses involving the central nervous system (CNS) and in limiting/obviating the need for cranial irradiation [13]. Lastly, maintenance therapy is given after consolidation therapy and typically continues for 1–2 years until the end of therapy.

For children with the extremely high risk of relapse, stem cell transplantation (SCT) is the most potent form of consolidation therapy. Detailed information regarding SCT is shown in Chap. 14.

6.2.1 Induction Therapy

Although a combination of steroids and vincristine achieved remission for >80% of pediatric ALL, steroids, vincristine, and asparaginase with or without anthracycline are generally used as induction therapy. Children's Oncology Group (COG) adopts three-drug induction for NCI-SR, while four-drug induction is used for NCI-HR [4]. The Berlin-Frankfurt-Munster (BFM) [14] group, the St. Jude Children's Research Hospital (SCJRH), and the Japan Pediatric Leukemia/lymphoma Study Group (JPLSG) [5] generally use four-drug induction for all risk groups. Some studies add cyclophosphamide to intensify treatment for high risk cases [15].

Steroids play the most important role in the treatment of ALL. Prednisone/predonisolone (PSL) has been mainly used in traditional ALL therapy, but dexamethasone (DEX) is increasingly adopted in recent clinical trials. Comparing to PSL, DEX has better therapeutic effect with high penetration ability to CNS, while DEX

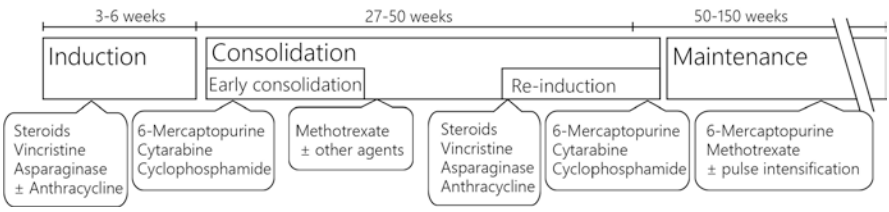


Fig. 6.1 Typical treatment backbone for pediatric BCP-ALL

was associated with more adverse event, such as infection, hyperglycemia, osteonecrosis, and behavioral changes [16]. Several clinical study groups performed prospective trials to compare efficacy and safety between PSL and DEX with various doses (Table 6.2) [17–21]. Eventually, the efficacy depends on doses of each steroid, and a review by Inaba and Pui suggested that in studies using a dose ratio greater than 7, the two drugs showed no difference in efficacy [22].

Asparaginase is also one of the key agents in the treatment of ALL. Three types of asparaginase have been mainly used: native asparaginase derived from *E. coli*, Erwinia-asparaginase derived from *E. chrysanthemi*, and a pegylated form of the *E. coli* asparaginase (PEG-asp). Asparaginase is an enzyme converting asparagine to aspartic acid. Asparagine is not an indispensable amino acid, which can be synthesized by asparagine synthetase in most mammalian cells. Previous studies showed that leukemic cells have low levels of asparagine synthetase, and depletion of extracellular asparagine shows antileukemic effect.

A higher total dose of asparaginase is associated with a better outcome [23] and higher dose is characteristics of pediatric-type intensive therapy [24]; however, adverse events caused by asparaginase, such as pancreatitis, allergic reaction, hyperglycemia, and coagulation disorder, are often severe and occasionally become life-threatening. Re-exposure of asparaginase after thrombotic event can be performed safely [25], but re-exposure for those who once suffered pancreatitis is usually avoided due to a high incidence of more severe pancreatitis, which can be potentially fatal.

Allergic reaction is also an important complication associated with asparaginase. Furthermore, neutralizing antibodies often develop without clinical symptoms and

Table 6.2 Clinical studies comparing prednisone/prednisolone and dexamethasone

Trial name	PSL dose (mg/m^2)	DEX dose (mg/m^2)	Conclusion
AIEOP-BFM ALL 2000 [17]	60	10	All patients; 5year-EFS: 80.8% (PSL) vs. 83.9% (DEX), $p = 0.024$
CCG-1922 [18]	40	6	NCI-SR; 6year-EFS: 77% (PSL) vs. 85% (DEX), $p = 0.02$
COG AALL0232 [19]	60	10	NCI-HR(<10y); 5year-EFS: 82.1% (PSL and Capizzi-MTX) vs. 83.2% (DEX and Capizzi-MTX) vs. 80.8% (PSL and HD-MTX) vs. 91.2% (DEX and HD-MTX), $p = 0.015$ NCI-HR(≥10y); 5year-EFS: 73.9% (PSL) vs. 73.1% (DEX), $p = 0.97$
TCCSG L95-14 [20]	60	8	SR; 8year-EFS: 84.4% (PSL) vs. 81.1 (DEX), $p = 0.22$ Intermediate risk; 8year-EFS: 80.4% (PSL) vs. 84.9 (DEX), $p = 0.63$
EORTC 58951 [21]	60	6	All patients; 8year-EFS: 81.2% (PSL) vs. 81.5 (DEX), $p = 0.73$

PSL prednisone/prednisolone, *DEX* dexamethasone, *NCI* National Cancer Institute, *SR* standard risk, *HR* high risk, *MTX* methotrexate, *HD* high-dose

reduce asparaginase activity while increasing the relapse risk. A previous study demonstrated that 12% of children with ALL suffered this "silent inactivation," and individualized dosing based on asparaginase activity monitoring can improve outcomes [26]. Some reports showed that intramuscular administration could reduce incidence of allergic reaction [27, 28]. For cases with neutralizing antibody, Erwinia-asparaginase can be an alternative, although higher dose is required to provide adequate activity [29] and some cases have cross-activity for Erwinia-asparaginase. PEG-asp is increasingly adopted in recent clinical trials, because PEG-asp has long half-life that can suppress incidence of allergy and inactivation, less than <10%, retaining antileukemic activity [30].

>95% of pediatric ALL can achieve complete remission after the first induction therapy. A retrospective collaborative study demonstrated that "induction failure" was associated poor overall survival, although a certain proportion of BCP-ALL can be cured by chemotherapy only [31].

6.2.2 Consolidation Therapy

After complete remission has been achieved by induction therapy, consolidation therapy is given to eradicate residual leukemic cells. Various combination and doses of cytotoxic agents are used according to each relapse risk. Optimization of treatment component has been tried through prospective studies.

In the typical BFM backbone, combination of 6-mercaptopurine (6-MP), cytarabine, and cyclophosphamide has been used as early consolidation, so called as "IB," followed by high-dose MTX (HD-MTX) therapy with leucovorin rescue. Addition/replacement of another agent to IB/HD-MTX had repeatedly been challenged, which unfortunately failed to achieve significant improvement [32]. On the other hand, the Children's Cancer Group (CCG) 1882 study was conducted to assess efficacy of "augmented BFM" regimen, an intensified standard BFM regimen by additional VCR and asparaginase for NCI-HR cases with slow early responder (bone marrow blasts at day 15 > 25%). The augmented BFM regimen had significantly better outcome, 5-year event-free survival of 75.0%, than the standard BFM regimen (55.0%) [33].

Capizzi MTX is another standard regimen originally adopted in the CCG trials, which is widely used in several trials [10]. It consists of escalating MTX (initial dose of 100 mg/m^2, increasing by 50 mg/m^2 every 10 days) without leucovorin rescue plus asparaginase. Previous studies showed superiority of Capizzi MTX compared to interim maintenance in both NCI-SR [34] and NCI-HR [35]. Comparison of Capizzi-MTX and HD-MTX results in inconsistent results. The COG AALL0232 study for NCI-HR ALL showed superiority of HD-MTX, and 5-year event-free survival was 79.6% for HD-MTX and 75.2% for Capizzi MTX [19]. On the other hand, the COG AALL0434 for T-ALL demonstrated Capizzi MTX was superior to HD-MTX with statistical significance [36].

A similar combination with induction therapy (steroids, vincristine, asparaginase, and anthracycline) termed as "re-induction" or "delayed intensification," is

given at the end of consolidation. The importance of re-induction has been repeatedly confirmed by several randomized trials [37]. Even in the cases with lowest risk for relapse, re-induction therapy is essential to keep event-free survival. The BFM group conducted a prospective randomized study with the Italian group, AIEOP, named AIEOP-BFM 2000 study. In this study, for standard risk group defined by undetectable MRD, they tried to replace standard re-induction (protocol II) with reduced re-induction (protocol III). However, the reduction of intensity failed to maintain disease-free survival (92.3% versus 89.2%) with a statistically significant difference [38]. These experiences underpinned an importance of re-induction therapy for all risk groups, although further optimization should be challenged through clinical trials. Osteonecrosis is often observed after re-induction therapy, especially in older (>10 years) children. Alternate-week administration of DEX significantly reduced an incidence of osteonecrosis [39].

6.2.3 Maintenance Therapy

Maintenance therapy is an essential component in the treatment of pediatric ALL, which generally consists of daily 6-mercaptopurine (6MP) and weekly MTX, with some patients receiving intensified maintenance therapy with pulses of vincristine and steroids. In some regimens, thioguanine, a structural analogue of 6MP, is also used as an alternative for 6MP, although previous studies showed thioguanine is associated with hepatotoxicity.

Generally, sensitivity to 6MP is highly heterogenous in each individual, and actual dose should be adjusted to keep leukocyte count between 1500/mm^3 and 3500/mm^3. Single nucleotide polymorphisms (SNPs) of *TPMT* and *NUDT15* are known to confer the sensitivity to 6MP (or thioguanine), and cases with homozygous variants are extremely sensitive to these thiopurines, and individualized dosing is recommended according to these genotypes [40].

The importance of maintenance therapy has been confirmed by several clinical trials, in which early discontinuation had resulted in inferior event-free survival [41]. The BFM group conducted the BFM81/83 study aiming to shorten the total therapy duration from 24 months to 18 months, but the attempt failed, although statistical difference disappeared by subsequent long-term follow-up [32]. The Tokyo Children's Cancer Study Group (TCCSG) performed the L92-13 trial to shorten maintenance therapy by intensification of consolidation therapy, and treatment of all risk groups was discontinued at 1 year from diagnosis. This challenge turned out to be failure, with significantly worse event-free survival of 59.5% [42]. A meta-analysis by the Childhood ALL collaborative group focusing on maintenance therapy showed that 2 years of total therapy duration had significantly inferior EFS compared with that of 3 years [43].

Accordingly, a sufficient duration of maintenance therapy is essential, and most clinical study groups adopted 2 years or longer as total therapy duration, but the duration varies markedly depending on protocols (Fig. 6.2). On the other hand,

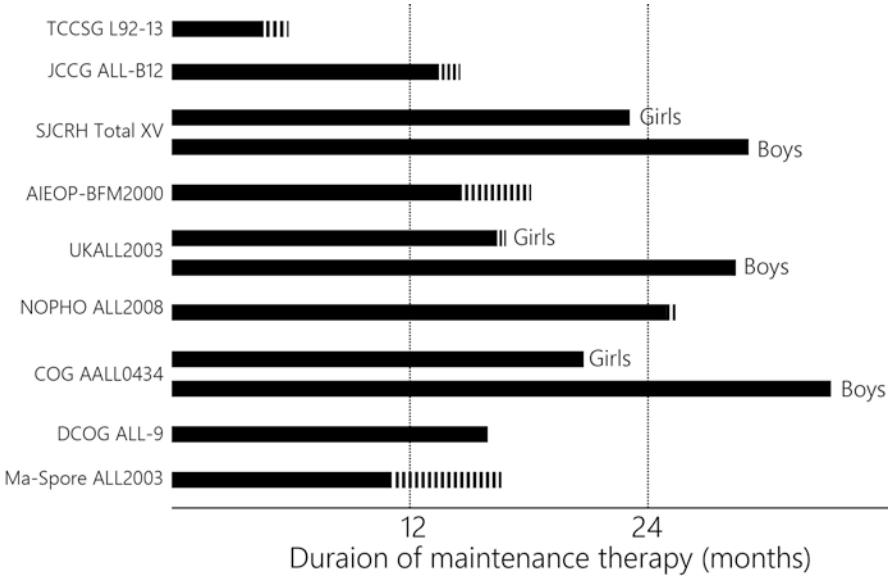


Fig. 6.2 Maintenance therapy duration in representative clinical trials. Duration of maintenance therapy in each clinical trial is shown. Some trial had different duration of maintenance therapy according to risk groups

maintenance therapy is generally less hematotoxic, but infectious complication is not uncommon in maintenance therapy [12]. In addition, 6MP might cause secondary malignant neoplasms (SMN), which has been shown by a Nordic study [44]. Excess duration of therapy may potentially be harmful, and considering the results that a certain faction of cases is curable with short maintenance therapy [45], optimal duration of maintenance therapy for individual cases should be investigated through prospective studies.

In some studies, intensification with addition of VCR/steroids pulses is adopted. The advantage of VCR/steroids pulses is inconsistent according to results [43, 46], even when limited to a specific biological subgroup with *IKZF1* alterations [47, 48]. Thus, efficacy and safety of VCR/steroids pulses is still to be assessed [5].

6.2.4 CNS Directed Therapy

Control of CNS leukemia is another key component of ALL treatment. CNS is a sanctuary site which is protected by the blood-brain barrier from systemically administrated anti-leukemic agents. A clinical study performed by St. Jude Children's Research Hospital showed an effect of cranial irradiation (12 Gy or more) to prevent CNS relapse significantly [49]. In the 1970s, 24 Gy cranial irradiation and intrathecal MTX became the standard for CNS prophylaxis. However,

significant concerns subsequently raised about adverse events of CNS irradiation. Considering these adverse events, prophylactic irradiation is limited or omitted to avoid late neurotoxic complications such as neurocognitive deficits, endocrinopathies, and secondary brain tumors. In recent clinical trials, such as the St. Jude Total XV and the TPOG-ALL-2002, prophylactic irradiation was completely replaced by intensive intrathecal therapy. Incidence of CNS-associated relapse of these studies could be reduced to less than 5% without prophylactic cranial irradiation [13, 50].

6.2.5 Immunotherapy

The survival probability for pediatric ALL has risen to 90% in most of the developed world. However, 10–15% of patients still suffer relapses despite intensive chemotherapy. Recent approaches showed intensity of multiagent chemotherapy hit the ceiling, and further intensification with classical cytotoxic agents for high-risk ALL is practically impossible, leading to unacceptable toxicity without reduction of relapse risk [51]. Immunotherapies such as those using bispecific antibody or chimeric antigen receptor (CAR)-modified T-cells are some of the promising treatments for improving the outcome of refractory/resistant ALL [52, 53].

In CAR-T cells, chimeric antigen receptors specific to a tumor antigen (e.g., CD19) are engineered and expressed on the surface of autologous T-cells, and bispecific antibody with CD3 and CD19 can directory connect endogenous T-cells to BCP-ALL cells. Previous reports demonstrated that both of the immunotherapy could achieve excellent remission rate, up to 90% for relapsed/refractory ALL. Efficacy of these immunotherapy is promising; thus we have to consider how to incorporate these breakthrough into our existing treatment backbone.

References

1. Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. Lancet. 2013;381:1943–55.
2. Pui CH, Yang JJ, Hunger SP, et al. Childhood acute lymphoblastic leukemia: progress through collaboration. J Clin Oncol. 2015;33:2938–48.
3. Kato M, Manabe A. Treatment and biology of pediatric acute lymphoblastic leukemia. Pediatr Int. 2018;60:4–12.
4. Tubergen DG, Gilchrist GS, O'Brien RT, et al. Improved outcome with delayed intensification for children with acute lymphoblastic leukemia and intermediate presenting features: a Childrens cancer group phase III trial. J Clin Oncol. 1993;11:527–37.
5. Koh K, Kato M, Saito AM, et al. Phase II/III study in children and adolescents with newly diagnosed B-cell precursor acute lymphoblastic leukemia: protocol for a nationwide multicenter trial in Japan. Jpn J Clin Oncol. 2018;48:684–91.
6. Schrappe M, Nachman J, Hunger S, et al. Educational symposium on long-term results of large prospective clinical trials for childhood acute lymphoblastic leukemia (1985-2000). Leukemia. 2010;24:253–4.

7. Smith M, Arthur D, Camitta B, et al. Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. J Clin Oncol. 1996;14:18–24.
8. Moorman AV, Ensor HM, Richards SM, et al. Prognostic effect of chromosomal abnormalities in childhood B-cell precursor acute lymphoblastic leukaemia: results from the UK Medical Research Council ALL97/99 randomised trial. Lancet Oncol. 2010;11:429–38.
9. Manabe A, Ohara A, Hasegawa D, et al. Significance of the complete clearance of peripheral blasts after 7 days of prednisolone treatment in children with acute lymphoblastic leukemia: the Tokyo Children's cancer study group study L99-15. Haematologica. 2008;93:1155–60.
10. 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 leukaemia (UKALL 2003): a randomised controlled trial. Lancet Oncol. 2014;15:809–18.
11. Vora A, Goulden N, Wade R, et al. Treatment reduction for children and young adults with low-risk acute lymphoblastic leukaemia defined by minimal residual disease (UKALL 2003): a randomised controlled trial. Lancet Oncol. 2013;14:199–209.
12. O'Connor D, Bate J, Wade R, et al. Infection-related mortality in children with acute lymphoblastic leukemia: a retrospective analysis of infectious deaths on UKALL 2003. Blood. 2014;124(7):1056–61.
13. Pui CH, Campana D, Pei D, et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. N Engl J Med. 2009;360:2730–41.
14. 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. 2010;115:3206–14.
15. Takahashi H, Kajiwara R, Kato M, et al. Treatment outcome of children with acute lymphoblastic leukemia: the Tokyo Children's cancer study group (TCCSG) study L04-16. Int J Hematol. 2018;108:98–108.
16. Schmiegelow K, Attarbaschi A, Barzilai S, et al. Consensus definitions of 14 severe acute toxic effects for childhood lymphoblastic leukaemia treatment: a Delphi consensus. Lancet Oncol. 2016;17:e231–9.
17. Moricke A, Zimmermann M, Valsecchi MG, et al. Dexamethasone vs prednisone in induction treatment of pediatric ALL: results of the randomized trial AIEOP-BFM ALL 2000. Blood. 2016;127:2101–12.
18. Bostrom BC, Sensel MR, Sather HN, et al. Dexamethasone versus prednisone and daily oral versus weekly intravenous mercaptopurine for patients with standard-risk acute lymphoblastic leukemia: a report from the Children's cancer group. Blood. 2003;101:3809–17.
19. Larsen EC, Devidas M, Chen S, et al. Dexamethasone and high-dose methotrexate improve outcome for children and young adults with high-risk B-acute lymphoblastic Leukemia: a report from Children's oncology group study AALL0232. J Clin Oncol. 2016;34:2380–8.
20. Igarashi S, Manabe A, Ohara A, et al. No advantage of dexamethasone over prednisolone for the outcome of standard- and intermediate-risk childhood acute lymphoblastic leukemia in the Tokyo Children's cancer study group L95-14 protocol. J Clin Oncol. 2005;23:6489–98.
21. Domenech C, Suciu S, De Moerloose B, et al. Dexamethasone (6 mg/m^2/day) and prednisolone (60 mg/m^2/day) were equally effective as induction therapy for childhood acute lymphoblastic leukemia in the EORTC CLG 58951 randomized trial. Haematologica. 2014;99:1220–7.
22. Inaba H, Pui CH. Glucocorticoid use in acute lymphoblastic leukaemia. Lancet Oncol. 2010;11:1096–106.
23. Moghrabi A, Levy DE, Asselin B, et al. Results of the Dana-Farber Cancer Institute ALL consortium protocol 95-01 for children with acute lymphoblastic leukemia. Blood. 2007;109:896–904.
24. Ram R, Wolach O, Vidal L, Gafter-Gvili A, Shpilberg O, Raanani P. Adolescents and young adults with acute lymphoblastic leukemia have a better outcome when treated with pediatric-inspired regimens: systematic review and meta-analysis. Am J Hematol. 2012;87:472–8.

25. Grace RF, Dahlberg SE, Neuberg D, et al. The frequency and management of asparaginase-related thrombosis in paediatric and adult patients with acute lymphoblastic leukaemia treated on Dana-Farber Cancer Institute consortium protocols. Br J Haematol. 2011;152:452–9.
26. 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. 2013;31:1202–10.
27. Nesbit M, Chard R, Evans A, Karon M, Hammond GD. Evaluation of intramuscular versus intravenous administration of L-asparaginase in childhood leukemia. Am J Pediatr Hematol Oncol. 1979;1:9–13.
28. Pidaparti M, Bostrom B. Comparison of allergic reactions to pegasparaginase given intravenously versus intramuscularly. Pediatr Blood Cancer. 2012;59:436–9.
29. Duval M, Suciu S, Ferster A, et al. Comparison of *Escherichia coli*-asparaginase with Erwinia-asparaginase in the treatment of childhood lymphoid malignancies: results of a randomized European Organisation for Research and Treatment of Cancer-Children's Leukemia group phase 3 trial. Blood. 2002;99:2734–9.
30. Place AE, Stevenson KE, Vrooman LM, et al. Intravenous pegylated asparaginase versus intramuscular native *Escherichia coli* L-asparaginase in newly diagnosed childhood acute lymphoblastic leukaemia (DFCI 05-001): a randomised, open-label phase 3 trial. Lancet Oncol. 2015;16:1677–90.
31. Schrappe M, Hunger SP, Pui CH, et al. Outcomes after induction failure in childhood acute lymphoblastic leukemia. N Engl J Med. 2012;366:1371–81.
32. Moricke 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. 2010;24:265–84.
33. Nachman J, Sather HN, Gaynon PS, Lukens JN, Wolff L, Trigg ME. Augmented Berlin-Frankfurt-Munster therapy abrogates the adverse prognostic significance of slow early response to induction chemotherapy for children and adolescents with acute lymphoblastic leukemia and unfavorable presenting features: a report from the Children's cancer group. J Clin Oncol. 1997;15:2222–30.
34. Matloub Y, Bostrom BC, Hunger SP, et al. Escalating intravenous methotrexate improves event-free survival in children with standard-risk acute lymphoblastic leukemia: a report from the Children's oncology group. Blood. 2011;118:243–51.
35. Seibel NL, Steinherz PG, Sather HN, et al. Early postinduction intensification therapy improves survival for children and adolescents with high-risk acute lymphoblastic leukemia: a report from the Children's oncology group. Blood. 2008;111:2548–55.
36. Winter SS, Dunsmore KP, Devidas M, et al. Improved survival for children and young adults with T-lineage acute lymphoblastic Leukemia: results from the Children's oncology group AALL0434 methotrexate randomization. J Clin Oncol. 2018;36:2926–34.
37. Gaynon PS, Trigg ME, Heerema NA, et al. Children's cancer group trials in childhood acute lymphoblastic leukemia: 1983–1995. Leukemia. 2000;14:2223–33.
38. Schrappe M, Bleckmann K, Zimmermann M, et al. Reduced-intensity delayed intensification in standard-risk Pediatric acute lymphoblastic Leukemia defined by undetectable minimal residual disease: results of an international randomized trial (AIEOP-BFM ALL 2000). J Clin Oncol. 2018;36:244–53.
39. Mattano LA Jr, Devidas M, Nachman JB, et al. Effect of alternate-week versus continuous dexamethasone scheduling on the risk of osteonecrosis in paediatric patients with acute lymphoblastic leukaemia: results from the CCG-1961 randomised cohort trial. Lancet Oncol. 2012;13:906–15.
40. Relling MV, Schwab M, Whirl-Carrillo M, et al. Clinical Pharmacogenetics implementation consortium guideline for Thiopurine dosing based on TPMT and NUDT15 genotypes: 2018 update. Clin Pharmacol Ther. 2019;105(5):1095–105.
41. Lonsdale D, Gehan EA, Fernbach DJ, Sullivan MP, Lane DM, Ragab AH. Interrupted vs. continued maintenance therapy in childhood acute leukemia. Cancer. 1975;36:341–52.

42. Toyoda Y, Manabe A, Tsuchida M, et al. Six months of maintenance chemotherapy after intensified treatment for acute lymphoblastic leukemia of childhood. J Clin Oncol. 2000;18:1508–16.
43. Childhood ALL Collaborative Group. Duration and intensity of maintenance chemotherapy in acute lymphoblastic leukaemia: overview of 42 trials involving 12 000 randomised children. Lancet. 1996;347:1783–8.
44. Schmiegelow K, Al-Modhwahi I, Andersen MK, et al. Methotrexate/6-mercaptopurine maintenance therapy influences the risk of a second malignant neoplasm after childhood acute lymphoblastic leukemia: results from the NOPHO ALL-92 study. Blood. 2009;113:6077–84.
45. Kato M, Ishimaru S, Seki M, et al. Long-term outcome of 6-month maintenance chemotherapy for acute lymphoblastic leukemia in children. Leukemia. 2017;31:580–4.
46. Conter V, Valsecchi MG, Silvestri D, et al. Pulses of vincristine and dexamethasone in addition to intensive chemotherapy for children with intermediate-risk acute lymphoblastic leukaemia: a multicentre randomised trial. Lancet. 2007;369:123–31.
47. Hinze L, Moricke A, Zimmermann M, et al. Prognostic impact of IKZF1 deletions in association with vincristine-dexamethasone pulses during maintenance treatment of childhood acute lymphoblastic leukemia on trial ALL-BFM 95. Leukemia. 2017;31:1840–2.
48. Clappier E, Grardel N, Bakkus M, et al. IKZF1 deletion is an independent prognostic marker in childhood B-cell precursor acute lymphoblastic leukemia, and distinguishes patients benefiting from pulses during maintenance therapy: results of the EORTC Children's Leukemia group study 58951. Leukemia. 2015;29:2154–61.
49. Aur RJ, Simone JV, Hustu HO, Verzosa MS. A comparative study of central nervous system irradiation and intensive chemotherapy early in remission of childhood acute lymphocytic leukemia. Cancer. 1972;29:381–91.
50. Yeh TC, Liang DC, Hou JY, et al. Treatment of childhood acute lymphoblastic leukemia with delayed first intrathecal therapy and omission of prophylactic cranial irradiation: results of the TPOG-ALL-2002 study. Cancer. 2018;124:4538–47.
51. Burke MJ, Salzer WL, Devidas M, et al. Replacing cyclophosphamide/cytarabine/mercaptopurine with cyclophosphamide/etoposide during consolidation/delayed intensification does not improve outcome for pediatric B-cell acute lymphoblastic leukemia: a report from the COG. Haematologica. 2019;104:986–92.
52. Topp MS, Gokbuget N, Stein AS, et al. Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multicentre, single-arm, phase 2 study. Lancet Oncol. 2015;16:57–66.
53. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med. 2014;371:1507–17.

Chapter 7
Pediatric T-Cell Acute Lymphobastic Leukemia

Atsushi Sato

Abstract T-cell acute lymphoblastic leukemia (T-ALL), which accounts for 7–15% of pediatric ALL, has a distinct biology from B-cell precursor ALL (BCP-ALL). Despite improvements achieved with treatment-intensification strategies, compared with patients with BCP-ALL, the outcomes of patients with T-ALL are inferior. Studies have identified the genetic alterations underpinning T-ALL, defining subgroups with oncogenic transcription factor dysregulation and mutations or deletions leading to aberrant signaling pathway activation. Early T-cell precursor ALL is a recently defined subtype with unique immunophenotypic and genetic features. However, regarding prognostic significance, minimal residual disease (MRD), rather than genetic subgroups, is the most reliable indicator of T-ALL. Recent clinical trials have been designed to incorporate several key interventions—such as those with respect to dexamethasone use in induction, intensive L-asparaginase, high-dose methotrexate, and nelarabine—into MRD-directed treatments. Several studies omit cranial radiotherapy even for patients with central nervous system involvement at diagnosis, thus avoiding long-term adverse events. Progress in knowledge of tumor biology will lead to the development and use of new target therapies directed at genetic alterations, such as ABL1 fusions and aberrant activation of Notch1 or JAK-STAT pathways, via new approaches potentially improving the outcomes of pediatric T-ALL patients.

Keywords T-cell acute lymphoblastic leukemia · Early T-cell precursor ALL
Notch1 · FBXW7 · Dexamethasone · L-Asparaginase · High-dose methotrexate
Nelarabine · Cranial radiotherapy · Minimal residual disease

A. Sato (✉)
Department of Hematology and Oncology, Miyagi Children's Hospital, Sendai, Japan
e-mail: asatoh@miyagi-children.or.jp

M. Kato (ed.), *Pediatric Acute Lymphoblastic Leukemia*,
https://doi.org/10.1007/978-981-15-0548-5_7

7.1 Epidemiology

Acute lymphoblastic leukemia (ALL) is the most common hematological malignancy in children. Most pediatric ALL belongs to the B-cell precursor ALL (BCP-ALL) type, with studies showing that T-cell ALL (T-ALL) accounts for 7–15% of pediatric ALL [1, 2]. In contrast to the peak incidence of BCP-ALL at 2–5 years of age, the incidence of T-ALL gradually increases with age [3]. Therefore, T-ALL has a relatively higher incidence in adolescents and young adults (AYAs) than in children.

7.2 Diagnosis

Because BCP-ALL and T-ALL blasts are morphologically difficult to distinguish, immunophenotypic analyses via flow cytometry is mandatory for diagnosis of T-ALL. The immunological classification criteria for T-ALL are defined by the European Group for the Immunological Characterization of Leukemias (EGIL) [2]. Based on these criteria, T-ALL lymphoblasts commonly express terminal deoxynucleotidyl transferase (TdT) and cytoplasmic CD3 (cCD3), which are essential lineage markers for T-ALL diagnosis. Other T-cell markers, such as CD2, CD4, CD5, CD7, or CD8, are variably co-expressed with TdT and cCD3 according to the stages of T-cell maturation.

Recently, a new T-ALL subgroup, early T-cell precursor ALL (ETP-ALL), has been defined and characterized by its distinct gene expression profile and immunophenotype. ETP-ALL blasts show an absence of CD1a and CD8 expression, weak CD5 expression, and expression of one or more myeloid-associated or stem cell-associated markers [4]. ETP-ALL accounts for approximately 12% of pediatric T-ALL [4, 5] and displays high-risk features [4] (Table 7.1).

Table 7.1 Immunophenotypic classification of T-ALL

	cyCD3	sCD3	CD1a	CD2	CD7	CD5
pro-T	+	−	−	+	+	−
pre-T/Immature	+	−	−	+	+	+
Cortical T	+	±	+	+	+	+
Mature T	+	+	−	+	+	+
Early T-cell precursor	+	−	−	+	+	dim + <75%
			and positive for stem cell or myeloid markers (HLA-DR, CD13, CD33, CD34, or CD117)			

T-ALL T-cell acute lymphoblastic leukemia, *cy* cytoplasmic, *s* surface
Adapted with modification from reference [2]

7.3 Clinical and Biological Characteristics

Compared to patients with BCP-ALL, those with pediatric T-ALL display higher leukocyte counts, older age, and higher incidence of central nervous system (CNS) involvement. Regarding early treatment response, patients with poor response to prednisone (PSL) defined as peripheral blood blasts $\geq 1 \times 10^9$/L after initial treatments with 7 days of PSL and one intrathecal methotrexate (MTX) and patients positive for polymerase chain reaction (PCR)-based minimal residual disease (MRD) (PCR-MRD) at the end of induction are more common in T-ALL than in BCP-ALL [6].

Genetic and cytogenetic features of T-ALL are distinct from those of BCP-ALL. Approximately 70% of pediatric T-ALL with karyotypic abnormalities harbor 46 and eventually, 45 or 47–49 chromosomes, while in BCP-ALL, high hyperdiploidy or hypodiploidy can be observed [7]. Chromosomal translocations commonly implicated in T-ALL involve T-cell receptor (TCR) genes at 14q11 (TCR α and δ) and 7q34 (TCR β), juxtaposing to transcription factor genes, such as TAL1, TAL2, LMO1, LMO2, TLX1, TLX3, HOXA, and MYB, often resulting in increased expression of these transcription factors. Based on dysregulated expression of transcription factor genes and on gene expression profiling data reported in recent studies, T-ALL in children and AYAs have been classified into several subgroups, including TAL1, TLX1, TLX3, HOXA, LMO2, NKX2-1, and others [2, 8, 9]. The association between subgroups and genetic alterations is reported, with the LMO2-LYL1-overexpressing group mostly linked to ETP-ALL [9]. Well-known fusion genes not related with TCR are t(5;14)(q35;q32) (BCL11B-TLX3), del(1)(p33;p33) (SIL-TAL1), and t(10;11)(p12;q14) (PICALM-MLLT10) that are observed in 20% [10], 12% [11], and 8% [12] of children with T-ALL, respectively. Notably, although ABL1 fusion genes—such as NUP214-ABL1, EML1-ABL1, BCR-ABL1, and ETV6-ABL1—are not common, they are clinically important, as their proteins are sensitive to tyrosine kinase inhibitors (TKIs). NUP214-ABL1 is the most frequent ABL1 fusion gene, observed in 6% of T-ALL [13].

Genes frequently mutated and/or deleted in T-ALL have been also reported. Among them, cell cycle-related deletions, including CDKN2A/B, and Notch pathway-related mutations, including NOTCH1 and FBXW7, are commonly seen in pediatric T-ALL. Deletion of tumor-suppressor genes CDKN2A and CDKN2B, located in 9q21 chromosome, are reportedly found in 72% and 62% of pediatric T-ALL, respectively [14]. Notch1 signaling is critical for T-cell differentiation. Notch1, located at 9q34, is a transmembrane receptor. Ligand binding leads to Notch1 cleavage, resulting in the release of the intracellular domain of Notch (ICN), which acts as a transcription factor complex. As ICN degradation is induced by FBXW7, located at 4q32 and dependent on ICN PEST domain, both activating Notch1 mutations and inactivating FBXW7 mutations cause ligand-independent Notch1 activation. Notch1 and FBXW7 mutations are observed in approximately

30–60% and 15–30% of pediatric ALL, respectively [15–17]. PI3K-AKT-mTOR, JAK-STAT, and RAS signaling pathways are also abnormally activated in T-ALL and have been recently identified in 29%, 25%, and 14% of patients, respectively [9]. This aberrant signaling pathway activation constitutes another T-ALL subgroup. PI3K-AKT-mTOR pathway activation commonly occurs from PTEN inactivation through a variety of mechanisms, including mutations or deletions [18].

7.4 Treatment

In most clinical trials of pediatric ALL, patients with BCP-ALL and T-ALL have been treated with the same regimens. Although treatment intensification has improved the outcomes of patients with T-ALL, event-free survival (EFS) rates of these patients were inferior to those with BCP-ALL [2, 18], ranging from 70% to 80% (Table 7.2) [3, 15, 19–29]. Clinical trials have shown that several key therapeutic interventions used in ALL, such as dexamethasone (DEX), high-dose MTX (HD-MTX), and L-asparaginase (ASNase), were important in T-ALL.

Firstly, DEX was associated with a higher anti-leukemic effect than PSL, despite higher incidence of several adverse effects including infections, induction death, and osteonecrosis [30]. Associazione Italiana di Ematologia e Oncologia Pediatrica and Berlin-Frankfurt-Münster (AIEOP-BFM) ALL 2000 trial randomized patients for induction treatment with PSL (60 mg/m^2 per day) or DEX (10 mg/m^2 per day) for 3 weeks and showed that children with T-ALL and good response to PSL on induction have superior overall survival (OS) (DEX, 91.4% ± 2.4%; PSL, 82.6% ± 3.2%; $P = 0.036$) [30]. These results suggest that DEX may be beneficial for the subgroup of patients with pediatric T-ALL.

As T-ALL blasts show lower concentrations of the MTX active metabolite polyglutamate compared with BCP-ALL blasts [31], higher doses of MTX are required for the treatment of T-ALL. Therefore, high-dose (HD)-MTX is considered to be an effective therapy in T-ALL, and four doses of 5 g/m^2 MTX have been used in BFM trials since the landmark ALL-BFM 86 trial [32]. In the Pediatric Oncology Group (POG) 9404 trial for T-ALL, patients were randomly assigned to receive or not receive four doses of 5 g/m^2 MTX to evaluate HD-MTX efficacy. Results showed that HD-MTX significantly improved EFS and decreased CNS relapse in children with T-ALL [33]. However, the AALL0434 study compared Capizzi-style escalating intravenous MTX without leucovorin rescue plus two doses of PEG-ASNase with HD-MTX with leucovorin rescue, resulting in superior disease-free survival rates in the Capizzi MTX arm (91.5% versus 85.3% in the HD-MTX arm, $P = 0.005$) [20]. As AALL0434 did not perform a strict comparison between the two MTX schedules, further investigation may be required to clarify the appropriate MTX dose and its integration into multidrug chemotherapy regimens.

As lymphoblasts lack or have low levels of asparagine synthetase, depleting asparagine by ASNase leads to reduced protein synthesis and leukemic cell death. In POG 8704 study, pediatric T-ALL patients were randomized to receive or not

Table 7.2 Selected clinical trials in children, adolescents, and young adults with T-ALL ($n > 50$)

Trial	Patients (n)	Age range (years)	Inclusion period	Induction steroid	Prophylactic CRT		Therapeutic CRT for CNS3	5-year EFS, % (SE or 95%CI)	5-year OS, % (SE or 95%CI)
					Indication				
AIEOP-BFM ALL 2000 [19]	464	1–18	2000–2006	PSL vs. DEX	BFM	All patients	Yes	75.9%(2.0) at 7 years	80.7% (1.9) at 7 years
					AIEOP	HR and non-HR with WBC $\geq 100 \times 10^9$/L	Yes		
COG AALL0434 [20]	1189	1–31	2007–2014	PSL		IR, HR	Yes	83.8% (81.2–86.4)	89.5% (87.4–91.7)
DCOG ALL-10 [21]	116	1–18	2004–2012	PSL		HR with ≥4 years	Yes: HR with ≥4 years)	80.0% (3.7)	84.4% (3.4)
NOPHO ALL2008 [3]	231	1–45	2008–2014	DEX		No	No	74% (3)	–
St. Jude Total Therapy XV [22]	76	1–18	2000–2007	PSL		No	No	78.4% (7.8)	87.6% (6.3)
UKALL 2003 [23, 24]	388	1-24	2003–2011	DEX		No	Yes[a]	81.2% (77.3–85.1)	86.4% (82.9–89.9)
EORTC-CLG 58951 [25]	296	0–17	1998–2008	PSL vs. DEX		No	No	PSL:76.7% (3.5) DEX:71.3%(3.8) at 8 years	PSL:82.1% (3.2) DEX:74.2% (3.8) at 8 years
DFCI 05-001 [26]	69	1–18	2005–2010	PSL		All patients	Yes	87% (76–93)	91% (81–96)
FRALLE 2000 T [27]	405	1–14	2000–2010	PSL		SR: ≥4 years with WBC $\geq 100 \times 10^9$/L HR: ≥4 years	Yes	PSL 72.8%[b]	76.6%

(continued)

Table 7.2 (continued)

Trial	Patients (*n*)	Age range (years)	Inclusion period	Induction steroid	Prophylactic CRT		Therapeutic CRT for CNS3	5-year EFS, % (SE or 95%CI)	5-year OS, % (SE or 95%CI)
					Indication				
TCCSG L04-16 [28]	116	1–17	2005–2013	PSL		PGR	Yes	62.0% (4.6)	71.9% (4.3)
JACLS ALL-T97 [15, 29]	72	1–15	1997–2001	PSL, DEX		WBC $\geq 50 \times 10^9$/L	Yes	70.7% (5.5) at 10 years	80.2% (4.9) at 10 years

T-ALL T-cell acute lymphoblastic leukaemia, *CRT* cranial radiotherapy, *DFCI* Dana Farber Cancer Institute, *DFS* disease-free survival, *EFS* event-free survival, *OS* overall survival, *FRALLE* French Acute Lymphoblastic Leukemia Group, *BFM* Berlin-Frankfurt-Münster, *AIEOP* Associazione Italiana di Ematologia e Oncologia Pediatrica, *PSL* predonisole, *DEX* dexamethasone, *SR* standard risk, *HR* high risk, *IR* intermediate risk, *COG* Children's Oncology Group, *DCOG* Dutch Children's Oncology Group, *NOPHO* Nordic Society of Paediatric Haematology and Oncology, *UKALL* Medical Research Council UK ALL, *EORTC-CLG* European Organizention for Research and Treatment of Cancer-Children's Leukemia Group, *JACLS* Japan Association of Childhood Leukemia Study, *TCCSG* Tokyo Children's Cancer Study Group, *SE* standard deviation, *CI* confidence interval, *PGR* predonisone good response, *CNS3* overt central nervous system involvement at diagnosis

[a]Since September 2009, CRT was restricted to patients with persistent blasts in cerebrospinal fluid after two courses of intrathecal therapies

[b]Disease-free survival

receive intensive ASNase 25,000 IU/m^2 given weekly for 20 weeks after achieving a complete remission (CR). As the intensive asparagine regimen was significantly superior to control (4-year continuous CR rate of 67.9% versus 54.5%, $P = 0.002$) [34], intensive ASNase treatment became an integral component of T-ALL treatment. Therefore, when clinical hypersensitivity or silent inactivation of ASNase is observed in these patients, ASNase preparations should be switched as recommended by guidelines, to prevent the decrease in survival rates [35].

Nelarabine (NEL), a prodrug of the deoxyguanosine analog 9-β-D-arabino furanosylguanine (ara-G), has been shown to be preferentially cytotoxic to T lymphoblasts through accumulation of ara-GTP, formed by ara-G phosphorylation [36]. In Children's Oncology Group (COG) phase II study of NEL in children and adolescents with recurrent or refractory T-ALL, 650 mg/m^2 NEL daily for 5 days were administered every 3 weeks, with response rates of 55% and 27% in first and second or greater relapse, respectively. However, attention to neurologic adverse effects should be paid during NEL treatment, as 18% of patients in this study developed grade 3 or higher neurologic events [37]. Thus, NEL was incorporated into the first-line treatment of T-ALL. COG further conducted the AALL00P2 study, which adds NEL to a modified BFM-86 regimen and result in a 5-year EFS of 69% in patients with slow early response, with no neurotoxicity increase [38]. In the subsequent COG AALL0434 study, patients were randomized to receive or not the same NEL doses incorporated into a COG-augmented BFM-based regimen [39]. In Japan, children and AYAs with newly diagnosed T-ALL are treated with ongoing ALL-T11 protocol, which incorporates NEL into BFM backbone chemotherapy when patients are classified as high- or very high-risk.

As T-immunophenotype is a risk factor for CNS relapse in pediatric ALL, CNS-directed therapy is an essential component of T-ALL treatment. Although most clinical trials have included cranial radiotherapy (CRT) in pediatric T-ALL treatment [40], this modality has been increasingly reduced in recent years due to its high risk of long-term adverse effects. In St. Jude total therapy XV, DCOG ALL9, and EORTC58881 and 58,951 studies, CRT was omitted even for patients with CNS involvement at diagnosis [40, 41]. CRT omission is supported by the result of a recent meta-analysis of pediatric ALL showing that it may not be necessary in current chemotherapy [42].

Finally, stem cell transplantation (SCT) is an encouraging treatment option for T-ALL in relapse. In first CR, as SCT demonstrated to improve survival rates of very high-risk pediatric T-ALL patients [43], patients with relapse high-risk features currently receive SCT according to treatment protocols.

7.5 Prognostic Factors

Survival rates of T-ALL patients after relapse are dismal, as evidenced by 5-year OS rates of 23% [44] and 3-year EFS rates of 20% reported [45]. Therefore, it is relevant to clarify prognostic factors, which allow identification of patients with an

increased risk of relapse. In pediatric T-ALL, well-known ALL prognostic factors as leukocyte count and age at diagnosis have been shown to be of little relevance [19, 34].

Conversely, some genetic subgroups have been associated with distinct clinical prognosis [7]. TLX1(HOX11) and TLX3(HOX11L2) overexpression has been either associated with good and poor prognosis, respectively [11, 46], or with an absence of clinical significance [47]. Prognostic significance associated with SIL-TAL1 is also controversial [11, 46]. In contrast, patients with PICALM-MLLT10 show poor prognosis [47]. Patients with Notch1/FBXW7 mutations have been reported to have good clinical outcomes in some studies [15, 17, 48] but no clinical benefit in others [16, 41], suggesting that clinical significance of genetic aberrations may depend on the treatment protocol used. Recently, new recurrent fusion genes involving SPI1—accounting for 3.9% of pediatric T-ALL—have been identified. Patients harboring SPI1 fusion genes showed significantly poor outcomes [49]. SPI1 fusion is clinically important to predict survival of pediatric T-ALL.

According to early reports, patients with ETP-ALL have poor prognosis [4, 50]. However, despite poor early response to conventional induction treatments, recent clinical trials have shown that ETP-ALL has no apparent survival impact [5]. Consequently, an ETP-ALL diagnosis may be insufficient for allogeneic SCT indication.

In contrast to the mostly incomplete significance of genetic alterations as outcome predictors, studies have shown the prognostic relevance of MRD [19, 51]. MRD analyses revealed that slower blast clearance in T-ALL compared with BCP-ALL and MRD levels within 3 months of diagnosis are more predictive of outcomes in T-ALL than in BCP-ALL [6]. Thus, the AIEOP-BFM-ALL 2000 study conducted PCR-MRD-based stratification of pediatric T-ALL and showed that MRD at the end of induction and consolidation protocol IB (day 78) clearly predicted patients' outcomes. In this protocol, patients with negative or high ($\geq 10^{-3}$) MRD at day 78 had excellent and poor outcomes and a 7-year cumulative incidence of relapse of 8.5% or 44.7%, respectively [19]. MRD measured by not only PCR but also flow cytometry has been revealed to be reliable predictors of outcome for pediatric T-ALL and is now widely used for risk stratification in clinical trials (Table 7.3).

7.6 Future Directions

Remarkable results are being achieved for patients with BCP-ALL with the use of new therapeutic modalities including chimeric antigen receptor T-cell (CAR-T) therapy, the bi-specific anti-CD19/CD3 chimeric antibody blinatumomab, and the anti-CD22 immunoconjugate inotuzumab ozogamicin. However, these new immunotherapy strategies could not be established in T-ALL patients yet.

One attractive target in T-ALL is the Notch1 signaling pathway. γ-secretase cleavage is essential for Notch1 activation. Therefore, the efficacy of γ-secretase inhibitors has been examined for T-lineage malignancies [55].

Table 7.3 Outcomes based on MRD level in selected clinical trials for children, adolescents, and young adults with T-ALL

Trial	MRD assay	MRD time point	MRD categorization	EFS, (SE or 95%CI)		*P* value	Cumulative Incidence of Relapse, (SE or 95% CI)		*P* value
AIEOP-BFM ALL 2000 [19]	PCR	Day 78	NEG				7-year	8.5% (1.9)	<0.001
			$<10^{-3}$					26.3% (3.7)	
			10^{-3}					33.0% (6.2)	
			$>10^{-3}$					44.7% (8.1)	
COG AALL0434 [52]	FCM	Day 29	$\leq 10^{-4}$	5-year	89.0%	0.0001			
			$>10^{-4}$		76.3%				
NOPHO ALL2008 [51]	PCR	Day 29	$<10^{-4}$				5-year	6.6% (1.0–12)	0.003[c]
			$\geq 10^{-4}$, $<10^{-3}$					0.0%	
			$\geq 10^{-3}$					24.6% (10–40)	
UKALL 2003 [23]	PCR	Day 29	NEG	5-year	90.3%	0.001			
			$<10^{-3}$		89.6%				
			$\geq 10^{-3}$		71.5%				
FRALLE 2000 T [27]	PCR	Day 35	$<10^{-4}$	5-year	86.4%[b]	<0.0001	5-year	13.6%	0.0007
			$\geq 10^{-4}$		58.4%[b]			37.6%	
St. Jude Total Therapy XV [53]	FCM[a]	Day 19	$<10^{-4}$	10-year	84.6% (64.0–93.9)	0.072	10-year	7.7% (0.0–18.2)	0.122
			$\geq 10^{-4}$, $<10^{-2}$		80.0% (58.4–91.1)			20.0% (4.0–36.0)	
			$\geq 10^{-2}$		55.0% (32.4–72.9)			31.3% (11.4–51.1)	
		Day 46	$<10^{-4}$	10-year	78.7% (64.9–87.6)	0.245	10-year	15.5% (5.5–25.5)	0.163
			$\geq 10^{-4}$, $<10^{-2}$		66.7% (37.5–84.6)			20.0% (0.0–41.1)	
			$\geq 10^{-2}$		57.1% (17.2–83.7)			42.9% (2.4–83.3)	

T-ALL T-cell acute lymphoblastic leukaemia, *yr* year, *EFS* event-free survival, *FRALLE* French Acute Lymphoblastic Leukemia Group, *BFM* Berlin-Frankfurt-Münster, *AIEOP* Associazione Italiana di Ematologia e Oncologia Pediatrica, *COG* Children's Oncology Group, *NOPHO* Nordic Society of Paediatric Haematology and Oncology, *UKALL* Medical Research Council UK ALL, *SE* standard deviation, *CI* confidence interval, *MRD* minimal residual disease, *PCR* polymerase chain reaction, *FCM* flow cytometry, *NEG* negative

[a]PCR-MRD was used for only two out of 76 patients [54]

[b]Disease-free survival

[c]Comparison of $\geq 10^{-3}$ with $\geq 10^{-4}$, $<10^{-3}$

Actionable T-ALL targets have been recently recognized. ABL1 fusions are observed in pediatric T-ALL, suggesting that TKIs are effective in these patients [9, 13]. Mutations activating the JAK-STAT signaling pathway are also present in 25% of pediatric T-ALL and approximately half of ETP-ALL patients [9]. As the

JAK inhibitor ruxolitinib has proven effective in patient-derived murine xenograft ETP-ALL models [56], it may also be promising for ETP-ALL patients and others with these mutations.

7.7 Conclusion

Recent progress in tumor biology and treatment has improved outcomes in pediatric T-ALL. Discovery of new biological evidences will lead to the potential use of new target therapies. New approaches will further improve the outcomes of patients with pediatric ALL, especially high-risk patients.

References

1. Hunger SP, Mullighan CG. Acute lymphoblastic Leukemia in children. New Eng J Med. 2015;373:1541–52.
2. Teachey DT, Pui C. Comparative features and outcomes between paediatric T-cell and B-cell acute lymphoblastic leukaemia. Lancet Oncol. 2019;20:e142–54.
3. Toft N, Birgens H, Abrahamsson J, et al. Results of NOPHO ALL2008 treatment for patients aged 1-45 years with acute lymphoblastic leukemia. Leukemia. 2018;32:606–15.
4. Coustan-Smith E, Mullighan CG, Onciu M, et al. Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. Lancet Oncol. 2009;10:147–56.
5. Conter V, Valsecchi MG, Buldini B, et al. Early T-cell precursor acute lymphoblastic leukaemia in children treated in AIEOP centres with AIEOP-BFM protocols: a retrospective analysis. Lancet Haematol. 2016;3:e80–6.
6. Willemse MJ, Seriu T, Hettinger K, et al. Detection of minimal residual disease identifies differences in treatment response between T-ALL and precursor B-ALL. Blood. 2002;99:4386–93.
7. Karrman K, Johansson B. Pediatric T-cell acute lymphoblastic leukemia. Genes Chromosomes Cancer. 2017;56:89–116.
8. Girardi T, Vicente C, Cools J, et al. The genetics and molecular biology of T-ALL. Blood. 2017;129:1113–23.
9. Liu Y, Easton J, Shao Y, et al. The genomic landscape of pediatric and young adult T-lineage acute lymphoblastic leukemia. Nat Genet. 2017;49:1211–8.
10. Bernard OA, Busson-LeConiat M, Ballerini P, et al. A new recurrent and specific cryptic translocation, t(5;14)(q35;q32), is associated with expression of the Hox11L2 gene in T acute lymphoblastic leukemia. Leukemia. 2001;15:1495–504.
11. Cavé H, Suciu S, Preudhomme C, et al. Clinical significance of HOX11L2 expression linked to t(5;14)(q35;q32), of HOX11 expression, and of SIL-TAL fusion in childhood T-cell malignancies: results of EORTC studies 58881 and 58951. Blood. 2004;103:442–50.
12. Asnafi V, Radford-Weiss I, Dastugue N, et al. CALM-AF10 is a common fusion transcript in T-ALL and is specific to the TCRγδ lineage. Blood. 2003;102:1000–6.
13. Hagemeijer A, Graux C. ABL1 rearrangements in T-cell acute lymphoblastic leukemia. Genes Chromosomes Cancer. 2010;49:299–308.
14. Karrman K, Castor A, Behrendtz M, et al. Deep sequencing and SNP array analyses of pediatric T-cell acute lymphoblastic leukemia reveal NOTCH1 mutations in minor subclones and a high incidence of uniparental isodisomies affecting CDKN2A. J Hematol Oncol. 2015;8:42.
15. Park M, Taki T, Oda M, et al. FBXW7 and NOTCH1 mutations in childhood T cell acute lymphoblastic leukaemia and T cell non-Hodgkin lymphoma. Br J Haematol. 2009;145:198–206.

16. Zuurbier L, Homminga I, Calvert V, et al. NOTCH1 and/or FBXW7 mutations predict for initial good prednisone response but not for improved outcome in pediatric T-cell acute lymphoblastic leukemia patients treated on DCOG or COALL protocols. Leukemia. 2010;24:2014–22.
17. Jenkinson S, Koo K, Mansour MR, et al. Impact of NOTCH1/FBXW7 mutations on outcome in pediatric T-cell acute lymphoblastic leukemia patients treated on the MRC UKALL 2003 trial. Leukemia. 2013;27:41–7.
18. Raetz EA, Teachey DT. T-cell acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program. 2016;2016:580–5588.
19. 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. 2011;118:2077–84.
20. Winter SS, Dunsmore KP, Devidas M, et al. Improved survival for children and young adults with T-lineage acute lymphoblastic Leukemia: results from the Children's oncology group AALL0434 methotrexate randomization. J Clin Oncol. 2018;36:2926–34.
21. Pieters R, de Groot-Kruseman H, Van der Velden V, et al. Successful therapy reduction and intensification for childhood acute lymphoblastic Leukemia based on minimal residual disease monitoring: study ALL10 from the Dutch childhood oncology group. J Clin Oncol. 2016;34:2591–601.
22. Pui C, Campana D, Pei D, et al. Treating childhood acute lymphoblastic Leukemia without cranial irradiation. New Eng J Med. 2009;360:2730–41.
23. Patrick K, Wade R, Goulden N, et al. Improved outcome for children and young people with T-acute lymphoblastic leukaemia: results of the UKALL 2003 trial. Blood. 2014;124:3702. Meeting Abstract
24. Hough R, Rowntree C, Goulden N, et al. Efficacy and toxicity of a paediatric protocol in teenagers and young adults with Philadelphia chromosome negative acute lymphoblastic leukaemia: results from UKALL 2003. Br J Haematol. 2016;172:439–51.
25. Domenech C, Suciu S, De Moerloose B, et al. Dexamethasone (6 mg/m^2/day) and prednisolone (60 mg/m^2/day) were equally effective as induction therapy for childhood acute lymphoblastic leukemia in the EORTC CLG 58951 randomized trial. Haematologica. 2014;99:1220–7.
26. Place AE, Stevenson KE, Vrooman LM, et al. Intravenous pegylated asparaginase versus intramuscular native *Escherichia coli* l-asparaginase in newly diagnosed childhood acute lymphoblastic leukaemia (DFCI 05-001): a randomised, open-label phase 3 trial. Lancet Oncol. 2015;16:1677–90.
27. Petit A, Trinquand A, Chevret S, et al. Oncogenetic mutations combined with MRD improve outcome prediction in pediatric T-cell acute lymphoblastic leukemia. Blood. 2018;131: 289–300.
28. Takahashi H, Kajiwara R, Kato M, et al. Treatment outcome of children with acute lymphoblastic leukemia: the Tokyo Children's cancer study group (TCCSG) study L04-16. Int J Hematol. 2018;108:98–108.
29. Kobayashi R, Takimoto T, Nakazawa A, et al. Inferior outcomes of stage III T lymphoblastic lymphoma relative to stage IV lymphoma and T-acute lymphoblastic leukemia: long-term comparison of outcomes in the JACLS NHL T-98 and ALL T-97 protocols. Int J Hematol. 2014;99:743–9.
30. Möricke A, Zimmermann M, Valsecchi MG, et al. Dexamethasone vs prednisone in induction treatment of pediatric ALL: results of the randomized trial AIEOP-BFM ALL 2000. Blood. 2016;127:2101–12.
31. Synold TW, Relling MV, Boyett JM, et al. Blast cell methotrexate-polyglutamate accumulation in vivo differs by lineage, ploidy, and methotrexate dose in acute lymphoblastic leukemia. J Clin Invest. 1994;94:1996–2001.
32. Schrappe M, Reiter A, Zimmermann M, et al. Long-term results of four consecutive trials in childhood ALL performed by the ALL-BFM study group from 1981 to 1995. Berlin-Frankfurt-Munster. Leukemia. 2000;14:2205–22.
33. Asselin BL, Devidas M, Wang C, et al. Effectiveness of high-dose methotrexate in T-cell lymphoblastic leukemia and advanced-stage lymphoblastic lymphoma: a randomized study by the Children's oncology group (POG 9404). Blood. 2011;118:874–83.

34. Amylon MD, Shuster J, Pullen J, et al. Intensive high-dose asparaginase consolidation improves survival for pediatric patients with T cell acute lymphoblastic leukemia and advanced stage lymphoblastic lymphoma: a Pediatric oncology group study. Leukemia. 1999;13:335–42.
35. van der Sluis IM, Vrooman LM, Pieters R, et al. Consensus expert recommendations for identification and management of asparaginase hypersensitivity and silent inactivation. Haematologica. 2016;101:279–85.
36. Rodriguez CO, Stellrecht CM, Gandhi V. Mechanisms for T-cell selective cytotoxicity of arabinosylguanine. Blood. 2003;102:1842–8.
37. Berg SL, Blaney SM, Devidas M, et al. Phase II study of Nelarabine (compound 506U78) in children and young adults with refractory T-cell malignancies: a report from the Children's oncology group. J Clin Oncol. 2005;23:3376–82.
38. Dunsmore KP, Devidas M, Linda SB, et al. Pilot study of Nelarabine in combination with intensive chemotherapy in high-risk T-cell acute lymphoblastic Leukemia: a report from the Children's oncology group. J Clin Oncol. 2012;30:2753–9.
39. Winter SS, Dunsmore KP, Devidas M, et al. Safe integration of nelarabine into intensive chemotherapy in newly diagnosed T-cell acute lymphoblastic leukemia: Children's oncology group study AALL0434. Pediatr Blood Cancer. 2015;62:1176–83.
40. Vora A, Andreano A, Pui C, et al. Influence of cranial radiotherapy on outcome in children with acute lymphoblastic Leukemia treated with contemporary therapy. J Clin Oncol. 2016;34:919–26.
41. Clappier E, Collette S, Grardel N, et al. NOTCH1 and FBXW7 mutations have a favorable impact on early response to treatment, but not on outcome, in children with T-cell acute lymphoblastic leukemia (T-ALL) treated on EORTC trials 58881 and 58951. Leukemia. 2010;24:2023–31.
42. Kelly MJ, Trikalinos TA, Dahabreh IJ, et al. Cranial radiation for Pediatric T-lineage acute lymphoblastic Leukemia: a systematic review and meta-analysis. Am J Hematol. 2014;89:992–7.
43. Schrauder A, Reiter A, Gadner H, et al. Superiority of allogeneic hematopoietic stem-cell transplantation compared with chemotherapy alone in high-risk childhood T-cell acute lymphoblastic Leukemia: results from ALL-BFM 90 and 95. J Clin Oncol. 2006;24:5742–9.
44. Nguyen K, Devidas M, Cheng S, et al. Factors influencing survival after relapse from acute lymphoblastic leukemia: a Children's oncology group study. Leukemia. 2008;22:2142–50.
45. Paganin M, Zecca M, Fabbri G, et al. Minimal residual disease is an important predictive factor of outcome in children with relapsed 'high-risk' acute lymphoblastic leukemia. Leukemia. 2008;22:2193–200.
46. Ballerini P, Landman-Parker J, Cayuela JM, et al. Impact of genotype on survival of children with T-cell acute lymphoblastic leukemia treated according to the French protocol FRALLE-93: the effect of *TLX3/HOX11L2* gene expression on outcome. Haematologica. 2008;93:1658–65.
47. van Grotel M, Meijerink JP, van Wering ER, et al. Prognostic significance of molecular-cytogenetic abnormalities in pediatric T-ALL is not explained by immunophenotypic differences. Leukemia. 2008;22:124–31.
48. Kox C, Zimmermann M, Stanulla M, et al. The favorable effect of activating NOTCH1 receptor mutations on long-term outcome in T-ALL patients treated on the ALL-BFM 2000 protocol can be separated from FBXW7 loss of function. Leukemia. 2010;24:2005–13.
49. Seki M, Kimura S, Isobe T, et al. Recurrent SPI1 (PU.1) fusions in high-risk pediatric T cell acute lymphoblastic leukemia. Nat Genet. 2017;49:1274–81.
50. Inukai T, Kiyokawa N, Campana D, et al. Clinical significance of early T-cell precursor acute lymphoblastic leukaemia: results of the Tokyo Children's cancer study group study L99-15. Br J Haematol. 2012;156:358–65.
51. Modvig S, Madsen HO, Siitonen SM, et al. Minimal residual disease quantification by flow cytometry provides reliable risk stratification in T-cell acute lymphoblastic leukemia. Leukemia. 2019;33(6):1324–36. https://doi.org/10.1038/s41375-018-0307-6.

52. Wood BL, Winter SS, Dunsmore KP, et al. T-lymphoblastic Leukemia (T-ALL) shows excellent outcome, lack of significance of the early Thymic precursor (ETP) Immunophenotype, and validation of the prognostic value of end-induction minimal residual disease (MRD) in Children's oncology group (COG) study AALL0434. Blood. 2014;124:1. meeting abstract
53. Pui C, Pei D, Raimondi SC, et al. Clinical impact of minimal residual disease in children with different subtypes of acute lymphoblastic leukemia treated with response-adapted therapy. Leukemia. 2017;31:333–9.
54. Pui C, Pei D, Coustan-Smith E, et al. Clinical utility of sequential minimal residual disease measurements in the context of risk-based therapy in childhood acute lymphoblastic leukaemia: a prospective study. Lancet Oncol. 2015;16:465–74.
55. Papayannidis C, DeAngelo DJ, Stock W, et al. A phase 1 study of the novel gamma-secretase inhibitor PF-03084014 in patients with T-cell acute lymphoblastic leukemia and T-cell lymphoblastic lymphoma. Blood Cancer J. 2015;5:e350.
56. Maude SL, Dolai S, Delgado-Martin C, et al. Efficacy of JAK/STAT pathway inhibition in murine xenograft models of early T-cell precursor (ETP) acute lymphoblastic leukemia. Blood. 2015;125:1759–67.

Chapter 8
Mature B-Cell Acute Lymphoblastic Leukemia

Reiji Fukano

Abstract Mature B-cell acute lymphoblastic leukemia (ALL), which is known as Burkitt leukemia, is included in Burkitt lymphoma according to the WHO classification. Approximately 80% of mature B-cell ALL is associated with t(8;14)(q24;q32). The Lymphoma Malignancy B and Berlin–Frankfurt–Munster studies have showed that intensive multiagent chemotherapy improves the outcome of mature B-cell ALL, and that the long-term event-free survival rate of advanced stage mature B-cell non-Hodgkin lymphoma (B-NHL), including mature B-cell leukemia, is 80–85%. However, the prognosis of relapsed or refractory B-NHL is poor. The short-term overall survival of patients with relapsed or refractory B-NHL has been reported to be approximately 20–30%, even though hematopoietic stem cell transplantation has been adopted. The efficacy of rituximab, an anti-CD20 antibody, has been established for adults with B-NHL. Recently, the safety and efficacy of rituximab for pediatric B-NHL has also been reported. Thus, it is expected that rituximab combined with chemotherapy will be established as the standard treatment for high-risk pediatric patients with B-NHL as well as for adults with B-NHL, and this therapy is believed to improve the outcome of mature B-cell leukemia.

Keywords Mature B-cell acute lymphoblastic leukemia · Burkitt leukemia
Burkitt lymphoma · Mature B-cell non-Hodgkin lymphoma · Rituximab

R. Fukano (✉)
Department of Pediatrics, Yamaguchi University Graduate School of Medicine, Yamaguchi, Japan
e-mail: fukano.r@yamaguchi-u.ac.jp

M. Kato (ed.), *Pediatric Acute Lymphoblastic Leukemia*,
https://doi.org/10.1007/978-981-15-0548-5_8

8.1 Epidemiology

Mature B-cell acute lymphoblastic leukemia (ALL) is defined as positive for surface immunoglobulin (usually immunoglobulin M) according to the European Group for the Immunological Characterization of Leukemias (EGIL) classification [1]. Mature B-cell ALL is rare and accounts for only 1–2% of childhood ALL [2]. In the WHO classification, mature B-cell ALL is included in Burkitt lymphoma and not as a part of ALL [3]. Childhood non-Hodgkin lymphoma (NHL), including Burkitt lymphoma, is staged according to the St. Jude classification system described by Murphy (Table 8.1) [4, 5]. Burkitt lymphoma accounts for approximately 40% of the childhood NHL, and bone marrow involvement is observed in approximately 20% of the patients with Burkitt lymphoma [6, 7]. Mature B-cell ALL is included in stage IV of Burkitt lymphoma.

8.2 Pathology/Biology

The blast cells of mature B-cell ALL tend to show the French–American–British (FAB) L3 type of ALL (Burkitt leukemia) features during morphological examination (Fig. 8.1). The blast is large and homogenous with basophilia in the cytoplasm [8]. Approximately 80% of Burkitt lymphoma is associated with chromosomal translocation t(8;14)(q24;q32), which involves overexpression of the *cMYC* gene [9–11]. Less commonly, t(2;8)(p12;q24) and t(8;22)(q24;q11), which are also involved with the *cMYC* gene, are associated with Burkitt lymphoma. Studies have shown that rearranged 8q24 is associated with additional chromosomal aberrations of +1q, +7q, and del(13q) [12–14].

Table 8.1 Staging system according to St. Jude classification for childhood with non-Hodgkin Lymphoma [4, 5]

Stage	Criteria for extent of disease
I	Single tumor (extranodal) or single anatomic area (nodal) outside mediastinum or abdomen
II	Single tumor (extranodal) with regional nodal involvement
	Two or more nodal areas, same side of diaphragm
	Two single (extranodal) tumors with or without regional node involvement on the same side of the diaphragm
	A primary gastrointestinal tract tumor with or without involvement of associated mesenteric nodes only, grossly completely resected
III	Two single tumors (extranodal) on opposite sides of the diaphragm
	Two or more nodal areas above and below the diaphragm
	All the primary intrathoracic tumors
	All extensive primary intraabdominal disease, unresectable
	All paraspinal or epidural tumors, regardless of other tumor site(s)
IV	Any of the above with initial CNS and/or bone marrow involvement

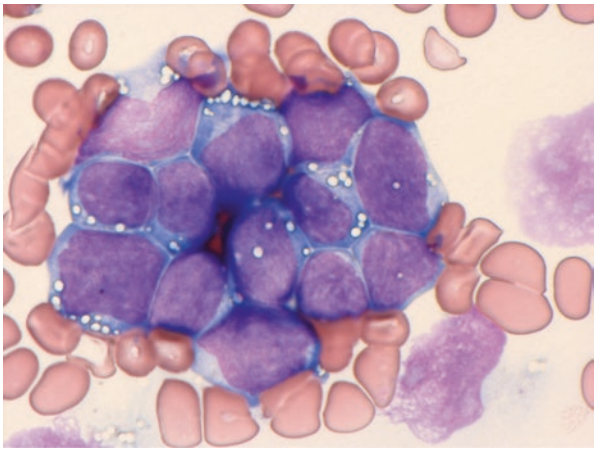


Fig. 8.1 The blast is large and homogenous, and the cytoplasm showed basophilia

8.3 Clinical Presentation

Patients with mature B-cell ALL often have abdominal masses [15]. Burkitt lymphoma/leukemia is highly aggressive; therefore, the risk of tumor lysis syndrome is high because of the rapidly growing lymphoma or bulky tumors. High-risk patients should be offered prophylaxis for tumor lysis syndrome, such as hydration and administration of allopurinol or rasburicase.

8.4 Treatment

8.4.1 Standard Treatments

The standard treatments for mature B-cell ALL differ from the conventional therapy for childhood ALL, which is typically a B-cell precursor ALL. Because mature B-cell ALL is included in the advanced stage of B-NHL, mature B-cell ALL should be treated with advanced B-NHL treatment, which is a short, intensive multiagent chemotherapy [16, 17]. The French Society of Pediatrics Oncology conducted clinical trials for B-NHL, and the regimens were based on the combinations of vincristine, cyclophosphamide, prednisolone, doxorubicin, cytarabine, and high-dose methotrexate [17–20]. In the Lymphoma Malignancy B (LMB) study, patients with B-NHL were classified into three groups (Table 8.2), and patients with mature B-cell ALL were included in group C. The treatment of group C in the LMB 89 study consisted of eight courses of chemotherapy, which comprised four courses of intensive chemotherapy based on 8 mg/m^2 of methotrexate ± continuous infusion or high dose of cytarabine and four courses of maintenance chemotherapy. The 5-year overall survival rate of group C was 84%, and central nervous system (CNS)

Table 8.2 The definitions of risk groups according to LMB and BFM studies

LMB study [17–20]	
A	Complete resection of stage I and abdominal stage II
B	Unresected stage I, nonabdominal stage II, any stage III or IV, L3ALL CNS negative (with <70% of blasts in BM)
C	CNS involvement (+), L3ALL (with ≥70% of blasts in BM)
BFM study [22–25]	
R1	Complete resection of stage I and stage II
R2	Unresected stage I and stage II, stage III with LDH <500 U/L
R3	Stage III with LDH ≥500 U/L, <1000 U/L Stage IV or B-AL with CNS involvement (−)
R4	Stage III, IV, or B-AL with LDH ≥1000 U/L, CNS involvement (+)

LMB Lymphoma Malignancy B, *BFM* Berlin–Frankfurt–Munster, *ALL* acute lymphoblastic leukemia, *BM* bone marrow, *CNS* central nervous system, *LDH* Lactate Dehydrogenase, *AL* acute leukemia

involvement was a prognostic factor in the multivariate analysis in group C [19]. Following the randomized FAB/LMB 96 study, the intensity of treatment for group C was reduced by decreasing the doses of cytarabine and etoposide. Consequently, the 4-year event-free survival (EFS) after reduced therapy was lower than that after standard therapy (80% versus 90%, respectively) [21].

The Berlin–Frankfurt–Munster (BFM) group conducted a clinical trial for B-NHL. In the BFM study, the B-NHL patients were classified into four groups (Table 8.2). The patients with mature B-cell ALL were included in the R3 and R4 groups. The treatment was based on dexamethasone, cyclophosphamide, vincristine, vindesine, ifosfamide, cytarabine, etoposide, and high-dose methotrexate [22–25]. In the BFM 95 study, the patients of R3 and R4 received five and six courses of intensive chemotherapy, respectively. The 3-year EFS of the R3 and R4 groups were 85% and 81%, respectively [24].

Because the intensity of the treatment for advanced Burkitt lymphoma is high, acute toxicities, including infection and mucositis, can become a problem during the chemotherapy. Supportive care is important, but it should not extend the treatment intervals due to the toxicities, because the chemotherapy should be administered on schedule as much as possible. Radiation does not improve the outcome of B-NHL—even for CNS diseases. The treatment for CNS involvement in B-NHL has been established by intensifying the intrathecal administration and systemic high-dose methotrexate without using CNS radiation [21, 26, 27]. Both the LMB and BFM studies completely omitted craniospinal irradiation, and the outcome was not affected by the omission of craniospinal irradiation [20, 22, 24].

The blast cells of B-NHL highly express CD20 [28]; therefore, the efficacy of rituximab, an anti-CD20 antibody, is expected to be high for the treatment of pediatric B-NHL. In adults, rituximab is currently the standard treatment for B-NHL, and rituximab combined with chemotherapy such as cyclophosphamide, doxorubi-

cin, vincristine, and prednisone regimen has been reported to be highly effective [29–31]. In pediatric B-NHL, the BFM group and the Children's Oncology Group (COG) group evaluated the efficacy and safety of rituximab in monotherapy and in combination with multiagent chemotherapy [32–34].

Recently, the international randomized phase III trial compared the standard LMB chemotherapy with the standard LMB chemotherapy + rituximab in pediatric high-risk patients with B-NHL (stage III with high levels of lactate dehydrogenase, stage IV, and mature B-cell leukemia). Six doses of rituximab were administered. The one-year EFS rates of the patients receiving rituximab and that of those not receiving rituximab were 94% and 82%, respectively, and the efficacy of rituximab in addition to standard chemotherapy was suggested even though the median follow-up was short (11.5 months) [35]. It is expected that rituximab combination chemotherapy will be established as the standard treatment for patients newly diagnosed with high-risk B-NHL, including mature B-cell lymphoma, in the near future.

8.4.2 Relapsed or Refractory Disease

For patients that are newly diagnosed with mature B-cell ALL, the standard treatments lead to excellent outcomes. However, the prognosis of relapsed or refractory B-NHL is poor. The short-term overall survival of patients with relapsed or refractory B-NHL is approximately 20–30%. Although hematopoietic stem cell transplantation (HSCT) has been established as one of the curative treatments in relapsed or refractory hematological malignancies, the overall survival rate of HSCT for relapsed or refractory B-NHL has been reported to be 20–30% [36–39]. It is still a matter of debate whether autologous HSCT or allogeneic HSCT should be adopted. The European Lymphoma Bone Marrow Transplantation Registry reported that the 5-year EFS of relapsed or refractory B-NHL was 39%, and patients with chemorefractory B-NHL did not have a chance to survive [40]. According to a retrospective analysis of the Center for International Blood and Marrow Transplant Research, the 5-year EFS rates of relapsed or refractory Burkitt lymphoma were 27% for autologous HSCT and 31% for allogeneic HSCT [41]. An elevated level of initial lactate dehydrogenase is suggested as a risk factor for decreased survival rates after salvage therapy [37, 42].

The efficacy of rituximab was evaluated for relapsed disease as well as for newly diagnosed disease. The COG reported a study of rituximab combined with ifosfamide, carboplatin, and etoposide chemotherapy in children with relapsed or refractory B-NHL, including mature B-cell ALL [43]. Among 14 patients with Burkitt lymphoma and mature B-cell leukemia, four showed complete responses, five showed a partial response, one showed stable disease, and four had progressive disease. Nonresponding patients for rituximab-ifosfamide-carboplatin-etoposide showed poor outcome with no survivors and very short survival.

References

1. Bene MC, et al. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia. 1995;9(10):1783–6.
2. Crist WM, et al. Immunologic markers in childhood acute lymphocytic leukemia. Semin Oncol. 1985;12(2):105–21.
3. Swerdlow SH, Harris NL. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2017.
4. Murphy SB. Classification, staging and end results of treatment of childhood non-Hodgkin's lymphomas: dissimilarities from lymphomas in adults. Semin Oncol. 1980;7(3):332–9.
5. Murphy SB, et al. Non-Hodgkin's lymphomas of childhood: an analysis of the histology, staging, and response to treatment of 338 cases at a single institution. J Clin Oncol. 1989;7(2):186–93.
6. Sandlund JT. Should adolescents with NHL be treated as old children or young adults? Hematology Am Soc Hematol Educ Program. 2007:297–303.
7. Hochberg J, et al. Adolescent non-Hodgkin lymphoma and Hodgkin lymphoma: state of the science. Br J Haematol. 2009;144(1):24–40.
8. Bennett JM, et al. Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. Br J Haematol. 1976;33(4):451–8.
9. Berger R, Bernheim A. Cytogenetic studies on Burkitt's lymphoma-leukemia. Cancer Genet Cytogenet. 1982;7(3):231–44.
10. Croce CM, Nowell PC. Molecular basis of human B cell neoplasia. Blood. 1985;65(1):1–7.
11. Allday MJ. How does Epstein-Barr virus (EBV) complement the activation of Myc in the pathogenesis of Burkitt's lymphoma? Semin Cancer Biol. 2009;19(6):366–76.
12. Lai JL, et al. Cytogenetic studies in 30 patients with Burkitt's lymphoma or L3 acute lymphoblastic leukemia with special reference to additional chromosome abnormalities. Ann Genet. 1989;32(1):26–32.
13. Johansson B, Mertens F, Mitelman F. Cytogenetic evolution patterns in non-Hodgkin's lymphoma. Blood. 1995;86(10):3905–14.
14. Poirel HA, et al. Specific cytogenetic abnormalities are associated with a significantly inferior outcome in children and adolescents with mature B-cell non-Hodgkin's lymphoma: results of the FAB/LMB 96 international study. Leukemia. 2009;23(2):323–31.
15. Magrath IT, Ziegler JL. Bone marrow involvement in Burkitt's lymphoma and its relationship to acute B-cell leukemia. Leuk Res. 1980;4(1):33–59.
16. Murphy SB, et al. Results of treatment of advanced-stage Burkitt's lymphoma and B cell (SIg+) acute lymphoblastic leukemia with high-dose fractionated cyclophosphamide and coordinated high-dose methotrexate and cytarabine. J Clin Oncol. 1986;4(12):1732–9.
17. Patte C, et al. High survival rate in advanced-stage B-cell lymphomas and leukemias without CNS involvement with a short intensive polychemotherapy: results from the French Pediatric Oncology Society of a randomized trial of 216 children. J Clin Oncol. 1991;9(1):123–32.
18. Patte C, et al. Improved survival rate in children with stage III and IV B cell non-Hodgkin's lymphoma and leukemia using multi-agent chemotherapy: results of a study of 114 children from the French Pediatric Oncology Society. J Clin Oncol. 1986;4(8):1219–26.
19. Patte C, et al. The Societe Francaise d'Oncologie Pediatrique LMB89 protocol: highly effective multiagent chemotherapy tailored to the tumor burden and initial response in 561 unselected children with B-cell lymphomas and L3 leukemia. Blood. 2001;97(11):3370–9.
20. Patte C, et al. Results of the randomized international FAB/LMB96 trial for intermediate risk B-cell non-Hodgkin lymphoma in children and adolescents: it is possible to reduce treatment for the early responding patients. Blood. 2007;109(7):2773–80.
21. Cairo MS, et al. Results of a randomized international study of high-risk central nervous system B non-Hodgkin lymphoma and B acute lymphoblastic leukemia in children and adolescents. Blood. 2007;109(7):2736–43.

22. Reiter A, et al. Improved treatment results in childhood B-cell neoplasms with tailored intensification of therapy: A report of the Berlin-Frankfurt-Munster Group Trial NHL-BFM 90. Blood. 1999;94(10):3294–306.
23. Seidemann K, et al. Primary mediastinal large B-cell lymphoma with sclerosis in pediatric and adolescent patients: treatment and results from three therapeutic studies of the Berlin-Frankfurt-Munster Group. J Clin Oncol. 2003;21(9):1782–9.
24. Woessmann W, et al. The impact of the methotrexate administration schedule and dose in the treatment of children and adolescents with B-cell neoplasms: a report of the BFM Group Study NHL-BFM95. Blood. 2005;105(3):948–58.
25. Salzburg J, et al. Prevalence, clinical pattern, and outcome of CNS involvement in childhood and adolescent non-Hodgkin's lymphoma differ by non-Hodgkin's lymphoma subtype: a Berlin-Frankfurt-Munster Group Report. J Clin Oncol. 2007;25(25):3915–22.
26. Murphy SB, Hustu HO. A randomized trial of combined modality therapy of childhood non-Hodgkin's lymphoma. Cancer. 1980;45(4):630–7.
27. Link MP, et al. Results of treatment of childhood localized non-Hodgkin's lymphoma with combination chemotherapy with or without radiotherapy. N Engl J Med. 1990;322(17):1169–74.
28. Perkins SL, et al. B-Cell non-Hodgkin's lymphoma in children and adolescents: surface antigen expression and clinical implications for future targeted bioimmune therapy: a children's cancer group report. Clin Adv Hematol Oncol. 2003;1(5):314–7.
29. Czuczman MS, et al. Treatment of patients with low-grade B-cell lymphoma with the combination of chimeric anti-CD20 monoclonal antibody and CHOP chemotherapy. J Clin Oncol. 1999;17(1):268–76.
30. Coiffier B, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med. 2002;346(4):235–42.
31. Pfreundschuh M, et al. CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomised controlled trial by the MabThera International Trial (MInT) Group. Lancet Oncol. 2006;7(5):379–91.
32. Goldman S, et al. Rituximab with chemotherapy in children and adolescents with central nervous system and/or bone marrow-positive Burkitt lymphoma/leukaemia: a Children's Oncology Group Report. Br J Haematol. 2014;167(3):394–401.
33. Meinhardt A, et al. Phase II window study on rituximab in newly diagnosed pediatric mature B-cell non-Hodgkin's lymphoma and Burkitt leukemia. J Clin Oncol. 2010;28(19):3115–21.
34. Goldman S, et al. Rituximab and FAB/LMB 96 chemotherapy in children with Stage III/IV B-cell non-Hodgkin lymphoma: a Children's Oncology Group report. Leukemia. 2013;27(5):1174–7.
35. Goldman S, et al. Preliminary results of a reduced burden of therapy trial by incorporation of rituximab and intrathecal liposomal cytarabine in children, adolescents and young adults with intermediate (FAB Group B) and high risk (FAB Group C) mature B-cell lymphoma/leukemia. J Clin Oncol. 2016;34(15):10534.
36. Philip T, et al. Curability of relapsed childhood B-cell non-Hodgkin's lymphoma after intensive first line therapy: a report from the Societe Francaise d'Oncologie Pediatrique. Blood. 1993;81(8):2003–6.
37. Jourdain A, et al. Outcome of and prognostic factors for relapse in children and adolescents with mature B-cell lymphoma and leukemia treated in three consecutive prospective "Lymphomes Malins B" protocols. A Societe Francaise des Cancers de l'Enfant study. Haematologica. 2015;100(6):810–7.
38. Anoop P, et al. Outcome of childhood relapsed or refractory mature B-cell non-Hodgkin lymphoma and acute lymphoblastic leukemia. Leuk Lymphoma. 2012;53(10):1882–8.
39. Fujita N, et al. The role of hematopoietic stem cell transplantation with relapsed or primary refractory childhood B-cell non-Hodgkin lymphoma and mature B-cell leukemia: a retrospective analysis of enrolled cases in Japan. Pediatr Blood Cancer. 2008;51(2):188–92.

40. Ladenstein R, et al. High-dose chemotherapy with autologous bone marrow rescue in children with poor-risk Burkitt's lymphoma: a report from the European Lymphoma Bone Marrow Transplantation Registry. Blood. 1997;90(8):2921–30.
41. Gross TG, et al. Hematopoietic stem cell transplantation for refractory or recurrent non-Hodgkin lymphoma in children and adolescents. Biol Blood Marrow Transplant. 2010;16(2):223–30.
42. Cairo M, et al. Overall survival of children and adolescents with mature B cell non-Hodgkin lymphoma who had refractory or relapsed disease during or after treatment with FAB/LMB 96: A report from the FAB/LMB 96 study group. Br J Haematol. 2018;182(6):859–69.
43. Griffin TC, et al. A study of rituximab and ifosfamide, carboplatin, and etoposide chemotherapy in children with recurrent/refractory B-cell (CD20+) non-Hodgkin lymphoma and mature B-cell acute lymphoblastic leukemia: a report from the Children's Oncology Group. Pediatr Blood Cancer. 2009;52(2):177–81.

Chapter 9
Infant ALL

Daisuke Tomizawa

Abstract Acute lymphoblastic leukemia (ALL) in infants (<1 year old) accounts for less than 5% of childhood ALL, but demonstrate as very aggressive form of ALL with *KMT2A* gene rearrangement (MLL-r ALL) in 70–80% of the patients. Outcome of infants with MLL-r ALL is poor with <50% event-free survival rate even with intensive chemotherapy with or without hematopoietic stem cell transplantation. Introduction of novel therapies through international collaboration is necessary for further improvement in outcome.

Keywords Infant · Acute lymphoblastic leukemia · KMT2A · MLL

9.1 Introduction

ALL in infants younger than 1-year old accounts for less than 5% of childhood ALL and is positioned as a special entity both biologically and clinically. Among this age group, acute leukemia, neuroblastoma, and brain tumors occur with similar frequency, in contrast to the children over 1-year old in which acute leukemia predominates. As for acute leukemia, frequency of ALL and AML is almost equal in infants. In addition, there is a female predominance in infant ALL and most present with B-lineage phenotype.

Infant ALL comprises two distinct subtypes; ALL with rearrangements of *histone lysine methyltransferase 2A* gene (*KMT2A*, also known as *mixed lineage leukemia* [*MLL*] gene) which accounts for 70–80% of infant ALL, and ALL with germline *KMT2A* gene. *KMT2A* gene rearrangement occurs as a result of balanced chromosomal translocations involving 11q23 locus, which results in fusion of the N terminus of the *KMT2A* gene with the C terminus of a partner gene. Among the 94 known *KMT2A* partner genes nowadays, *AFF1* (known as *AF4*) comprises approximately 50% of the infant ALL cases followed by *MLLT1* (*ENL*) and *MLLT2* (*AF9*) [1].

D. Tomizawa (✉)
Division of Leukemia and Lymphoma, Children's Cancer Center, National Center for Child Health and Development, Tokyo, Japan
e-mail: tomizawa-d@ncchd.go.jp

M. Kato (ed.), *Pediatric Acute Lymphoblastic Leukemia*,
https://doi.org/10.1007/978-981-15-0548-5_9

Table 9.1 Outcome of infant ALL in recent clinical trials

Study group	Study acronym & inclusion time	No. of patients (MLL-r/ MLL-g)	No. of patients treated with HSCT in 1CR	CR (%)	EFS (%)	OS (%)	Source
JILSG	MLL96/98 (1995–2001)	102 (80/22)	49	94.1	4-year 50.9 ± 4.9	4-year 60.5 ± 4.8	Kosaka et al. [18] Isoyama et al. [17] Nagayama et al. [6] Tomizawa et al. [41]
JPLSG	MLL03 (2004–2009)	62 (62/–)	44	80.6	4-year 43.2 ± 6.3	4-year 67.2 ± 6.0	Koh et al. [19]
Interfant	Interfant-99 (1999–2005)	482 (314/82)	37	94	4-year 47.0 ± 2.6	4-year 55.3 ± 2.7	Pieters et al. [5]
	Interfant-06 (2005–2016)	651 (476/167)	76	92	6-year 46.1 ± 2.1	6-year 58.2 ± 2.0	Pieters et al. [12]
COG	COG P9407 (2001–2006)	147 (100/35)	0	91.8	5-year 42.3 ± 6.0	5-year 52.9 ± 6.5	Dreyer et al. [15]

COG Children's Oncology Group, *JILSG* Japan Infant Leukemia Study Group, *JPLSG* Japanese Pediatric Leukemia/Lymphoma Study Group
CR complete remission, *EFS* event-free survival, *HSCT* hematopoietic stem cell transplantation, *MLL-g* germline *KMT2A* gene, *MLL-r* rearranged *KMT2A* gene, *OS* overall survival

Infants with *KMT2A*-rearranged ALL (MLL-r ALL) usually present with high leukocyte count (WBC) and frequent involvement of extramedullary sites, such as central nervous system (CNS) and/or skin (leukemia cutis). Additionally, majority of MLL-r ALL has an immature CD10-negative B-cell precursor phenotype and is frequently associated with co-expression of myeloid-specific antigens, suggesting that infant MLL-r ALL originates from very immature lymphoid progenitors [2]. In fact, MLL-r leukemia in infants could present as an acute leukemia with ambiguous lineage (mixed phenotype acute leukemia [MPAL] or acute undifferentiated leukemia [AUL]) [3]. Also "lineage switch" from ALL to AML (usually, acute monocytic leukemia) is occasionally observed [4].

Prognosis of infants with MLL-r ALL is extremely poor with <50% event-free survival (EFS) rate in published clinical trials worldwide (Table 9.1) [5]. On the other hand, EFS rate of infants with germline *KMT2A* (MLL-g) ALL is >70%, relatively similar to that of older children with ALL [6, 7].

9.2 Risk Stratifications in Infant ALL

There are three major cooperative study groups worldwide conducting infant ALL-specific clinical trials: Interfant (mainly based on European countries), Children's Oncology Group (COG, mainly based on North America), and the Japan Children's

Table 9.2 Risk stratification of infant ALL in major clinical trials

Risk group	Interfant-06	COG AALL0631	JPLSG MLL-10
High risk (HR)	MLL-r and either • Age < 6 mo and WBC >300 K • Age < 6mo and PPR	MLL-r and age < 3mo	MLL-r and either • Age < 6 mo • CNS-3
Intermediate risk (IR)	MLL-r without HR features[a]	MLL-r without HR features	MLL-r without HR features
Low risk (LR)	MLL-g	MLL-g	MLL-g

CNS-3 5/μL or higher cells in cerebrospinal fluid at diagnosis, *COG* Children's Oncology Group, *JPLSG* Japanese Pediatric Leukemia/Lymphoma Study Group, *MLL-g* germline *KMT2A* gene, *MLL-r KMT2A* gene rearrangement, *mo* months old, *MRD* minimal residual disease, *PPR* poor prednisolone response, *WBC* leukocyte count

[a]IR patients with MRD 10^{-4} or higher before re-induction phase are allocated to stem cell transplantation

Cancer Group (JCCG). The Japanese infant ALL trials were formerly conducted by the Japanese Infant Leukemia Cooperative Study Group (JILSG, 1995–2002) and the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG, 2003–2013). Risk stratifications used in the recently completed clinical trials conducted by each study group is shown in Table 9.2.

As *KMT2A* gene status is the most significant prognostic factor in infant ALL, all three groups consider MLL-g ALL as low-risk (LR) group and MLL-r ALL as either high-risk (HR) or intermediate-risk (IR) group. Among the infants with MLL-r ALL, young age at diagnosis is most predictive of relapse, although cutoff age used is somewhat different. High WBC at diagnosis and CNS disease at diagnosis is also prognostic, but are strongly associated with young age.

In vivo treatment response at the early phase of the treatment, such as "prednisolone response" evaluating residual leukemic blasts in peripheral blood following 7 days of prednisolone monotherapy with single intrathecal methotrexate injection, is widely used for risk stratification in pediatric ALL protocols [8]. Nearly 30% of the infants with ALL fall into prednisolone poor responders (≥1000 blasts/μL), while it is less than 10% in older children with B-lineage ALL. In recent years, measurement of minimal (or measurable) residual disease (MRD) using flow cytometry detecting aberrant combinations of leukemic cell-surface antigen or PCR amplification targeting leukemic clone-specific rearrangement of immunoglobulin (Ig) or T-cell receptor (TCR) genes has become a main stratification tool in pediatric ALL protocols. These techniques can detect submicroscopic levels of residual leukemia with sensitivity of 0.01% in flow-MRD and 0.01–0.001% in PCR-MRD. MRD is highly predictive of relapse risk also in infant ALL, therefore, should be used in future risk stratifications. However, there are several cautions especially when using Ig/TCR PCR-MRD in infant MLL-r ALL. It has been reported that only half of the Ig/TCR targets in infant ALL cases reached a quantitative range of at least 10^{-4} and that approximately 10% of infant ALL samples resulted in underestimation of actual MRD load [9]. This phenomenon is due to less frequency of Ig/TCR rearrangements and oligoclonality of infant MLL-r ALL cells. In that sense, use of *KMT2A* breakpoint as a PCR target might be preferable because it should be present in total leukemic clone [10].

9.3 Treatment of Infant MLL-r ALL

9.3.1 Chemotherapy

Based on in vitro drug sensitivity experiments of infant ALL cells showing high sensitivity to cytarabine while showing high resistance against major key ALL drugs, prednisolone and asparaginase, a "hybrid chemotherapy" incorporating AML-oriented drugs (e.g., cytarabine, anthracyclines, etoposide) to ALL chemotherapy backbone is generally used to treat infants with ALL [11]. Currently, all the major study groups adopt an identical induction therapy based on Interfant-99, adding cytarabine to typical 4-drug pediatric ALL induction, which enables more than 90% of the patients to obtain complete remission [5].

A problem of infant ALL chemotherapy lies on post-induction phase, because nearly half of the patients in remission eventually relapse in relatively early phase of the treatment (usually, 4–5 months after achieving remission). A failure of improving the outcome of infants with ALL by intensifying delayed intensification phase with "VIMARAM (combination of high-dose cytarabine, high-dose methotrexate and others)" in the Interfant-99 study led the Interfant group to intensify early intensification phase with two courses of AML-oriented chemotherapy (ADE [cytarabine, daunomycin and etoposide] followed by MAE [cytarabine, mitoxantrone, and etoposide]) comparing with single course of ALL-oriented chemotherapy "IB (cyclophosphamide, 6-mercaputopurine and cytarabine)" in the Interfant-06 study, but again it showed no improvement in survival rate [5, 12]. So far, it is unlikely that further improvement could be achieved by intensifying post-induction chemotherapy using conventional drugs.

Another issue on chemotherapy for infant ALL is that most of the drugs currently used lack pharmacokinetic (PK) data on this age group [13]. PK in infants is influenced by many age-specific factors: higher percentage of total and extracellular body water content than older children or adults, higher unbound active fraction of drugs because of lower affinity of drugs to serum protein, lower P450 enzyme activity, lower tubular and glomerular function, and lower bodyweight to body surface area ratio. Currently, each cooperative study group is adjusting the dose according to age of the patients based on anecdotal evidence as shown in Table 9.3.

9.3.2 Hematopoietic Stem Cell Transplantation

There is a controversy over the role of allogeneic hematopoietic stem cell transplantation (HSCT) as a curative option for infants with ALL. A retrospective analysis of children and young adults with MLL-r ALL treated by 11 cooperative groups and single institutions in the United States and Europe demonstrated worse disease-free survival (DFS) and overall survival (OS) in infants with t(4;11) ALL who underwent any HSCT compared to those who underwent chemotherapy alone [14]. Combined analysis of the studies Children's Cancer Group (CCG) 1953 and Pediatric Oncology Group (POG) 9407 showed a 5-year EFS rate of 48.8% in

Table 9.3 Dose adjustment of chemotherapeutic drugs for infants with ALL

Interfant-06[a]	COG AALL0631[a]	JPLSG MLL-10[b]
Calculate dose based on body surface area: • <6 mo: 2/3 of the calculated dose • 6–12 mo: 3/4 of the calculated dose • >12 mo: full dose	Calculate dose based on body surface area: • ≥7 days to <6 mo: 11% dose reduction • <7 days: additional 25% reduction	Calculate dose based on body surface area: • <2 mo: 2/3 of the calculated dose • 2 to <4 mo: 3/4 of the calculated dose • ≥4 mo: full dose

COG Children's Oncology Group, *JPLSG* Japanese Pediatric Leukemia/Lymphoma Study Group, *mo* months old

[a]Except intrathecals (age-adjusted)

[b]Except vincristine (dose based on body weight), corticosteroids (dose based on body surface area), and intrathecals (age-adjusted)

infants who received HSCT vs. 48.7% in infants who received chemotherapy alone ($P = 0.60$) [15]. Given these negative results on benefit of HSCT, the recent COG infant ALL studies have entirely eliminated an indication of HSCT for infants with ALL in first remission. On the other hand, analysis of the Interfant-99 study demonstrated a benefit of HSCT in a high-risk subset of infants with ALL in terms of DFS (59.0% versus 22.7%, $P = 0.01$) and OS (66.0% versus 19.3%, $P = 0.001$) rates, therefore, indication of HSCT is restricted but allocated to infants with HR group or with high MRD before re-induction phase in the Intrerfant-06 study [16]. The Japanese infant ALL studies in the late 1990s (MLL96 and MLL98 by JILSG) have shown the potential benefit of HSCT in an early phase before a relapse occurs [17, 18]. To prospectively evaluate this hypothesis, JPLSG MLL03 study was conducted, but nearly 50% of the infants who underwent HSCT still relapsed and ended up with 4-year EFS of 43.2% [19]. In the recently completed MLL-10 study (umin.ac.jp, UMIN000004801), HSCT was restricted to the HR cases only.

A retrospective study on 132 infants with MLL-r ALL using nationwide registry data in Japan has demonstrated no difference in relapse, non-relapse mortality and OS regarding donor type (related versus unrelated versus cord blood), and conditioning (busulfan [BU]-based vs. total body irradiation [TBI]-based myeloablative regimen) [20]. Currently, unrelated cord blood transplantation using BU-based myeloablative conditioning (e.g., BU, etoposide and cyclophosphamide) is a preferred method for transplanting infants with MLL-r ALL because of donor availability and risk of late effects associated with TBI. However, cautions are needed because BU-based conditioning is associated with risk of sinusoidal obstruction syndrome and/or pulmonary artery hypertension and with HSCT-related late effects as well [21].

9.3.3 Novel Therapies

It is obvious that further improvement in the outcome of infant ALL is unlikely to be achieved without novel therapeutic approach. Additionally, international collaboration is important given the rarity of the disease. Development of several novel

therapies with effort on international collaboration among the Interfant, COG, and JCCG is currently underway.

9.3.3.1 Nucleoside Analogues

Infant MLL-r ALL cells are highly sensitive to purine nucleoside analog. Clofarabine is a second-generation purine nucleoside analog and showed highest in vitro activity among all the nucleoside analogs as well as synergistic cytotoxicity in combination with cytarabine. Additionally, clofarabine induces demethylation of the promoter region of a tumor suppressor gene *FHIT* (*fragile histidine triad*) which is often hyper-methylated in infant MLL-r ALL [22]. Efficacy and safety of clofarabine/cytarabine combination is currently tested in the JCCG infant ALL trial MLL-17 (jrct.niph.go.jp, jRCTs041190043).

9.3.3.2 FLT3 Inhibitors

Gene-expression profile studies have shown a unique pattern of infant MLL-r ALL. One of the highly expressed is *fms-related tyrosine kinase 3* (*FLT3*) gene and was associated with poor prognosis [23, 24]. COG has evaluated the role of FLT3 inhibitor lestaurtinib for infants with newly diagnosed MLL-r ALL in combination with post-induction chemotherapy in the AALL0631 trial, but could not show any improvement in the outcome. Midostaurin, a multi-kinase inhibitor including FLT3, was tested against 13 children with relapsed or refractory MLL-r ALL in a European phase 1/2 single-agent study, but the response rate was modest [25].

9.3.3.3 Epigenetic Agents

Recent genomic studies have revealed that infant MLL-r leukemia cells are characterized by aberrant methylated genomic state with very few cooperating gene alterations [26]. Its leukemogenesis is driven by leukemia-specific histone modifications such as H3K79 dimethylation induced via DOT1L recruitment by KMT2A fusion proteins, which leads to site-specific hyper-methylation and to aberrant transcription of leukemogenic genes [27]. Thus, epigenetic modifiers such as hypomethylating agents (e.g., azacytidine, decitabine) and/or histone deacetylase inhibitors (e.g., vorinostat, panobinostat) are attractive targeting agents for infant MLL-r ALL. Pilot studies testing azacytidine-combined chemotherapy are ongoing in the United States (COG AALL15P1; clinicaltrials.gov, NCT02828358) and in Japan (AZA-MLL-P16; jrct.niph.go.jp, jRCTs031180063). In the early clinical trial, testing single-agent DOT1L inhibitor pinometostat for adults and children with MLL-r leukemia, clinical activity was unfortunately modest [28].

9.3.3.4 BCL-2 Inhibitors

BCL-2 family proteins regulate the intrinsic apoptosis pathway by integrating diverse prosurvival or proapoptotic intracellular signals. Recent studies revealed that *KMT2A* rearrangement directly induces BCL-2 overexpression in ALL cells by promoting DOT1L-mediated H3K79 methylation at the *BCL2* locus [29]. BCL-2 inhibitor venetoclax has shown potent in vitro and in vivo single-agent activity against MLL-r ALL and synergized with standard ALL induction chemotherapy in a xenograft model [30].

9.3.3.5 Immunotherapies

Immunotherapies targeting CD19 and/or CD22 are emerging as attractive therapeutic options for high-risk B-lineage ALL [31–33]. A pilot study of blinatumomab, a CD19/CD3 bi-specific T-cell engager (BiTE), combined with the Interfant chemotherapy backbone is currently underway (clinicaltrialsregister.eu, 2016-004674-17). Inotuzumab ozogamicin, a CD22-targeting immunoconjugate of calicheamicin, is also drawing attention as a promising agent for B-lineage ALL, but low levels of CD22 expression in MLL-r ALL might be a limitation for its use in infants. Ultimately, chimeric antigen receptor (CAR) T-cell therapy is expected as a curative option. However, generating autologous CAR-T cells is not easy in heavily pretreated infants because of the low number of host T-cells. Recently, two successful infant cases with relapsed MLL-r ALL, who received third-party CD19 CAR-T cells, were reported. This "off-the-shelf" allogeneic CAR-T was manufactured by disrupting TCR alpha and CD52 to avoid rejection and graft-versus-host disease (GVHD) by a gene-editing technique [34]. In the future, identification of more potent target will be important for immunotherapy in infant MLL-r ALL, because CD19 is not uniformly expressed in their leukemic cells and both CD19-negative relapse and lineage switch to CD19-negative myeloid leukemia are reported as a result of immune escape [35, 36].

9.4 Treatment of Infant MLL-g ALL

Unlike infants with MLL-r ALL, majority of infants with MLL-g ALL could be cured with combination of conventional chemotherapy. However, it is unclear whether they could be treated with the identical chemotherapy regimen as for older childhood counterparts or requires infant-specific chemotherapy regimen. Clinically, infants with MLL-g ALL are diagnosed at older age (majority are over 6 months old) and with lower WBC and demonstrates with higher percentage of good prednisolone response compared to infants with MLL-r ALL. The reported EFS rate is

67–95.5% [6, 7]. Biologically, frequency of favorable cytogenetics such as *ETV6-RUNX1* or high hyperdiploidy is much less and have fewer genetic alterations compared to older children with ALL. Reports by the Interfant group showed high expression levels of *MEIS-1* and were associated with unfavorable prognosis [37]. So far, infants with MLL-g ALL should be treated with chemotherapy specifically designed for infant ALL.

9.5 Treatment of Relapsed Infant ALL

Report on relapsed infant ALL is very few, and there is no standardized approach for these patients. Outcome of infants with relapsed ALL is very poor with approximately 20% OS rate. However, irrespective of previous history of HSCT, the study from Japan showed 50% chance of survival if a remission was achieved [38]. Interestingly, data from the Interfant group demonstrates that outcome of patients with MLL-g ALL is also dismal once they relapse [39]. Given the dismal outcome of relapsed patients, novel therapeutic options should be offered if available.

9.6 Acute and Late Toxicities on Infant ALL Treatment

Given the vulnerability of infants to cytotoxic agents, toxicity management is extremely important. Particularly during the remission induction phase, infants are at high risk of tumor lysis syndrome and intracranial hemorrhage because of the high leukemic burden in MLL-r ALL, together with a risk of severe infection [40]. To prevent sever tumor lysis syndrome, use of rasburicase is mandatory, and exchange transfusion should be considered for patients presenting with very high WBC (e.g., ≥500 K/μL).

Infants are also at high risk of developing late toxicities especially for those who underwent HSCT. Various late effects are observed among the infant ALL survivors with HSCT history such as chronic GVHD, hypothyroidism, skin abnormalities, ophthalmologic complications, pulmonary complications, dental abnormalities, and neurocognitive problems. In particular, growth retardation is very common [41, 42]. Generally, severe late effects are not commonly observed among the survivors who underwent chemotherapy only. But recently, there has been a series of case reports on fatal secondary T-cell immunodeficiency soon after the completion of COG AALL0631 chemotherapy [43]. It is not clear whether this phenomenon is derived from age-related, therapy-related, or disease-related factors, but close monitoring on immune function should be considered for follow-up of infant ALL patients.

References

1. Meyer C, Burmeister T, Gröger D, Tsaur G, Fechina L, Renneville A, et al. The MLL recombinome of acute leukemias in 2017. Leukemia. 2017;92:3793.
2. Tomizawa D. Recent progress in the treatment of infant acute lymphoblastic leukemia. Pediatr Int. 2015;57(5):811–9.
3. Alexander TB, Gu Z, Iacobucci I, Dickerson K, Choi JK, Xu B, et al. The genetic basis and cell of origin of mixed phenotype acute leukaemia. Nature. 2018;562(7727):373–9.
4. Sakaki H, Kanegane H, Nomura K, Goi K, Sugita K, Miura M, et al. Early lineage switch in an infant acute lymphoblastic leukemia. Int J Hematol. 2009;90(5):653–5.
5. Pieters R, Schrappe M, de Lorenzo P, Hann I, De Rossi G, Felice M, et al. A treatment protocol for infants younger than 1 year with acute lymphoblastic leukaemia (Interfant-99): an observational study and a multicentre randomised trial. Lancet. 2007;370(9583):240–50.
6. Nagayama J, Tomizawa D, Koh K, Nagatoshi Y, Hotta N, Kishimoto T, et al. Infants with acute lymphoblastic leukemia and a germline MLL gene are highly curable with use of chemotherapy alone: results from the Japan Infant Leukemia Study Group. Blood. 2006;107(12):4663–5.
7. Moorman AV, Pieters R, Dreyer ZE, Heerema NA, Carroll AJ, Hunger SP, et al. Cytogenetics and outcome of infants with acute lymphoblastic leukemia and absence of MLL rearrangements. Leukemia. 2014;28(2):428–30.
8. Dördelmann M, Reiter A, Borkhardt A, Ludwig WD, Götz N, Viehmann S, et al. Prednisone response is the strongest predictor of treatment outcome in infant acute lymphoblastic leukemia. Blood. 1999;94(4):1209–17.
9. Van der Velden VHJ, Corral L, Valsecchi MG, Jansen MWJC, Cazzaniga G, Schrappe M, et al. Prognostic significance of minimal residual disease in infants with acute lymphoblastic leukemia treated within the Interfant-99 protocol. Leukemia. 2009;23(6):1073–9.
10. Jansen MWJC, Corral L, Van der Velden VHJ, Panzer-Grümayer R, Schrappe M, Schrauder A, et al. Immunobiological diversity in infant acute lymphoblastic leukemia is related to the occurrence and type of MLL gene rearrangement. Leukemia. 2007;21(4):633–41.
11. Pieters R, Boer den ML, Durian M, Janka G, Schmiegelow K, Kaspers GJL, et al. Relation between age, immunophenotype and in vitro drug resistance in 395 children with acute lymphoblastic leukemia–implications for treatment of infants. Leukemia. 1998;12(9):1344–8.
12. Pieters R, de Lorenzo P, Ancliffe P, Aversa LA, Brethon B, Biondi A, et al. Outcome of infants younger than 1 year with acute lymphoblastic leukemia treated with the interfant-06 protocol; results from an International Phase III Randomised Study. J Clin Oncol. 2019;37(25):2246–56.
13. Balis FM, Womer RB, Berg S, Winick N, Adamson PC, Fox E, et al. Dosing anticancer drugs in infants: Current approach and recommendations from the Children“s Oncology Group”s Chemotherapy Standardization Task Force. Pediatr Blood Cancer. 2017;14(9):e26636.
14. Pui C-H, Gaynon PS, Boyett JM, Chessells JM, Baruchel A, Kamps W, et al. Outcome of treatment in childhood acute lymphoblastic leukaemia with rearrangements of the 11q23 chromosomal region. Lancet. 2002;359(9321):1909–15.
15. Dreyer ZE, Dinndorf PA, Camitta B, Sather H, La MK, Devidas M, et al. Analysis of the role of hematopoietic stem-cell transplantation in infants with acute lymphoblastic leukemia in first remission and MLL gene rearrangements: a report from the Children's Oncology Group. J Clin Oncol. 2011;29(2):214–22.
16. Mann G, Attarbaschi A, Schrappe M, de Lorenzo P, Peters C, Hann I, et al. Improved outcome with hematopoietic stem cell transplantation in a poor prognostic subgroup of infants with mixed-lineage-leukemia (MLL)-rearranged acute lymphoblastic leukemia: results from the Interfant-99 Study. Blood. 2010;116(15):2644–50.
17. Isoyama K, Eguchi M, Hibi S, Kinukawa N, Ohkawa H, Kawasaki H, et al. Risk-directed treatment of infant acute lymphoblastic leukaemia based on early assessment of MLL gene

status: results of the Japan Infant Leukaemia Study (MLL96). Br J Haematol. 2002;118(4): 999–1010.
18. Kosaka Y, Koh K, Kinukawa N, Wakazono Y, Isoyama K, Oda T, et al. Infant acute lymphoblastic leukemia with MLL gene rearrangements: outcome following intensive chemotherapy and hematopoietic stem cell transplantation. Blood. 2004;104(12):3527–34.
19. Koh K, Tomizawa D, Moriya Saito A, Watanabe T, Miyamura T, Hirayama M, et al. Early use of allogeneic hematopoietic stem cell transplantation for infants with MLL gene-rearrangement-positive acute lymphoblastic leukemia. Leukemia. 2015;29(2):290–6.
20. Kato M, Hasegawa D, Koh K, Kato K, Takita J, Inagaki J, et al. Allogeneic haematopoietic stem cell transplantation for infant acute lymphoblastic leukaemia with KMT2A (MLL) rearrangements: a retrospective study from the paediatric acute lymphoblastic leukaemia working group of the Japan Society for Haematopoietic Cell Transplantation. Br J Haematol. 2015;168(4):564–70.
21. Kawashima N, Ikoma M, Sekiya Y, Narita A, Yoshida N, Matsumoto K, et al. Successful treatment of pulmonary hypertension with beraprost and sildenafil after cord blood transplantation for infantile leukemia. Int J Hematol. 2013;97(1):147–50.
22. Stumpel DJPM, Schneider P, Pieters R, Stam RW. The potential of clofarabine in MLL-rearranged infant acute lymphoblastic leukaemia. Eur J Cancer. 2015;51(14):2008–21.
23. Armstrong SA, Staunton JE, Silverman LB, Pieters R, Boer Den ML, Minden MD, et al. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. Nat Genet. 2002;30(1):41–7.
24. Stam RW, Schneider P, de Lorenzo P, Valsecchi MG, Boer Den ML, Pieters R. Prognostic significance of high-level FLT3 expression in MLL-rearranged infant acute lymphoblastic leukemia. Blood. 2007;110(7):2774–5.
25. Zwaan CM, Söderhäll S, Brethon B, Luciani M, Rizzari C, Stam RW, et al. A phase 1/2, open-label, dose-escalation study of midostaurin in children with relapsed or refractory acute leukaemia. Br J Haematol. 2018;4:263.
26. Andersson AK, Ma J, Wang J, Chen X, Gedman AL, Dang J, et al. The landscape of somatic mutations in infant MLL-rearranged acute lymphoblastic leukemias. Nat Genet. 2015;47(4):330–7.
27. Krivtsov AV, Armstrong SA. MLL translocations, histone modifications and leukaemia stem-cell development. Nat Rev Cancer. 2007;7(11):823–33.
28. Stein EM, Garcia-Manero G, Rizzieri DA, Tibes R, Berdeja JG, Savona MR, et al. The DOT1L inhibitor pinometostat reduces H3K79 methylation and has modest clinical activity in adult acute leukemia. Blood. 2018;131(24):2661–9.
29. Benito JM, Godfrey L, Kojima K, Hogdal L, Wunderlich M, Geng H, et al. MLL-rearranged acute lymphoblastic leukemias activate BCL-2 through H3K79 methylation and are sensitive to the BCL-2-specific antagonist ABT-199. Cell Rep. 2015;13(12):2715–27.
30. Khaw SL, Suryani S, Evans K, Richmond J, Robbins A, Kurmasheva RT, et al. Venetoclax responses of pediatric ALL xenografts reveal sensitivity of MLL-rearranged leukemia. Blood. 2016;128(10):1382–95.
31. Stackelberg von A, Locatelli F, Zugmaier G, Handgretinger R, Trippett TM, Rizzari C, et al. Phase I/Phase II study of blinatumomab in pediatric patients with relapsed/refractory acute lymphoblastic leukemia. J Clin Oncol. 2016;34(36):4381–9.
32. Bhojwani D, Sposto R, Shah NN, Rodriguez V, Yuan C, Stetler-Stevenson M, et al. Inotuzumab ozogamicin in pediatric patients with relapsed/refractory acute lymphoblastic leukemia. Leukemia. 2018;14:e205.
33. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med. 2018;378(5):439–48.
34. Qasim W, Zhan H, Samarasinghe S, Adams S, Amrolia P, Stafford S, et al. Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. Sci Transl Med. 2017;9(374):eaaj2013.

35. Gardner R, Wu D, Cherian S, Fang M, Hanafi L-A, Finney O, et al. Acquisition of a CD19-negative myeloid phenotype allows immune escape of MLL-rearranged B-ALL from CD19 CAR-T-cell therapy. Blood. 2016;127(20):2406–10.
36. Zoghbi A, Stadt Zur U, Winkler B, Müller I, Escherich G. Lineage switch under blinatumomab treatment of relapsed common acute lymphoblastic leukemia without MLL rearrangement. Pediatr Blood Cancer. 2017;95(6):e26594.
37. van der Linden MH, Boer JM, Schneider P, Willekes M, Seslija L, de Lorenzo P, et al. Clinical and molecular genetic characterization of wild-type MLL infant acute lymphoblastic leukemia identifies few recurrent abnormalities. Haematologica. 2016;101(3):e95–9.
38. Tomizawa D, Koh K, Hirayama M, Miyamura T, Hatanaka M, Saikawa Y, et al. Outcome of recurrent or refractory acute lymphoblastic leukemia in infants with MLL gene rearrangements: A report from the Japan Infant Leukemia Study Group. Pediatr Blood Cancer. 2009;52(7):808–13.
39. Driessen EMC, de Lorenzo P, Campbell M, Felice M, Ferster A, Hann I, et al. Outcome of relapsed infant acute lymphoblastic leukemia treated on the interfant-99 protocol. Leukemia. 2016;30(5):1184–7.
40. Salzer WL, Jones TL, Devidas M, Hilden JM, Winick N, Hunger S, et al. Modifications to induction therapy decrease risk of early death in infants with acute lymphoblastic leukemia treated on Children's Oncology Group P9407. Pediatr Blood Cancer. 2012;59(5):834–9.
41. Tomizawa D, Koh K, Sato T, Kinukawa N, Isoyama K, Kosaka Y, et al. Outcome of risk-based therapy for infant acute lymphoblastic leukemia with or without an MLL gene rearrangement, with emphasis on late effects: a final report of two consecutive studies, MLL96 and MLL98, of the Japan Infant Leukemia Study Group. Leukemia. 2007;21(11):2258–63.
42. Gandemer V, Bonneau J, Oudin C, Berbis J, Bertrand Y, Tabone M-D, et al. Late effects in survivors of infantile acute leukemia: a study of the L.E.A program. Blood Cancer J. 2017;7(1):e518.
43. Geerlinks AV, Issekutz T, Wahlstrom JT, Sullivan KE, Cowan MJ, Dvorak CC, et al. Severe, persistent, and fatal T-cell immunodeficiency following therapy for infantile leukemia. Pediatr Blood Cancer. 2016;63(11):2046–9.

Chapter 10
Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia

Yuichi Kodama and Hiroyuki Shimada

Abstract In the treatment of childhood Ph+ALL, TKI-combined chemotherapy has become the standard option, and HSCT in first remission is no longer an absolute indication. However, pediatric Ph+ALL is still a refractory leukemia, and 5-year EFS in children without HSCT in first remission was only 55–60% because of treatment-related death and post-chemotherapy relapse. Further improvement in clinical outcomes may require intensified targeted therapy in combination with low-intensity chemotherapy and/or immune antibody therapy with reduced toxicity. Continuing to address these challenges in prospective clinical studies will change childhood Ph+ALL from refractory leukemia to more manageable leukemia in the future.

Keywords Philadelphia chromosome · BCR-ABL1 · Tyrosine kinase inhibitor imatinib · Dasatinib · Ponatinib

10.1 Introduction

Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ALL) is caused by t(9;22)(q34;q11) translocation and represents 3–5% of all childhood ALL [1]. The BCR-ABL1 chimeric protein produced by the translocation has strong tyrosine kinase activity and activates downstream molecules such as RAS, PI3K, etc., leading to cell proliferation. In clinical studies, before the introduction of imatinib, a tyrosine kinase inhibitor (TKI), allogeneic hematopoietic stem cell

Y. Kodama
Department of Pediatrics, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

H. Shimada (✉)
Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan
e-mail: hshimada@a5.keio.jp

M. Kato (ed.), *Pediatric Acute Lymphoblastic Leukemia*,
https://doi.org/10.1007/978-981-15-0548-5_10

transplantation (HSCT) is associated with better outcomes compared to chemotherapy alone, and HSCT in first remission was considered the standard treatment for Ph+ALL. However, the appearance of TKIs has dramatically changed the treatment outcome of Ph+ALL.

10.2 Mechanism of Action of TKIs

10.2.1 Imatinib

Imatinib is a first-generation TKI that competitively binds to the ATP-binding site of the kinase domain (KD) of BCR-ABL1, thereby inhibiting the phosphorylation of tyrosine on the downstream signaling molecules and inducing inhibition and apoptosis of leukemic cell proliferation. Imatinib inhibits not only ABL1 but also KIT and platelet-derived growth factor receptor (PDGFR). Point mutations in the KD of BCR-ABL1 alter the conformation of ABL1 and cause resistance to imatinib.

10.2.2 Dasatinib

Dasatinib is a second-generation TKI with a chemical structure completely different from imatinib and has 325 times the BCR-ABL1 inhibitory activity of imatinib in vitro. Dasatinib potently inhibits not only imatinib-resistant BCR-ABL1 mutants except for T315I, F317L, and V299L but also SRC family kinase, KIT, EphA2 receptor, and PDGFR. In addition, compared with imatinib, which has a low blood–brain barrier permeability, dasatinib is known to be effective against central nervous system leukemias. These actions are clinically effective against refractory or recurrent Ph+ALL treated with imatinib.

10.2.3 Ponatinib

Ponatinib is a third-generation TKI theoretically developed by molecular design drug discovery based on the interaction analysis of receptor protein and ligand and is not only effective against T315I mutation in BCR-ABL1, which is resistant to other TKIs, but also has activity against other BCR-ABL1 mutations. Ponatinib also inhibits kinase activity in vitro against wild-type and various mutants of SRC family kinases and the receptor tyrosine kinases, including RET, FLT3, KIT, FGFR, PDGFR, VEGFR, and EPH.

10.3 Outcomes of Treatment for Ph+ALL Before and After Introduction of TKI

10.3.1 Treatment for Ph+ALL Before the TKI Era

In the clinical studies for children with Ph+ALL before the TKI era, the 7-year event-free survival (EFS) was 32.0% and the 7-year overall survival (OS) was 44.9%. Patients who received HSCT in first remission from an HLA-matched related or unrelated donor demonstrated fewer relapses or deaths in remission at 5 years than patients who received chemotherapy alone (Hazard ratio 0.32, 95% CI 0.20–0.52) [2]. Therefore, HSCT in first remission was the standard option for Ph+ALL before the TKI era, and the outcomes were not satisfactory compared with Ph-negative ALL.

10.3.2 Imatinib-Combined Chemotherapy

Imatinib is the first TKI used for Ph+ALL. Initially evaluated as a single agent for blastic phase CML and relapsed/refractory Ph+ALL, TKIs were used in combination with chemotherapy because of their low remission rate and increased susceptibility to resistance. Among the adult clinical studies, the study of imatinib plus Hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, and dexamethasone) at MD Anderson Cancer Center (MDACC) had a 5-year OS of 43%, with a significantly improved outcome compared with Hyper-CVAD without imatinib (5-year OS 7%). Although most of adult clinical studies have used transplantation in first remission, the MDACC clinical study showed only a 30% transplantation rate in first remission, demonstrating the usefulness of imatinib in combination with Hyper-CVAD [3].

Clinical studies of imatinib-combined chemotherapy in children with Ph+ALL were conducted by the Children's Oncology group (COG), the European Intergroup Study on Post Induction Treatment of Philadelphia Positive Acute Lymphoblastic Leukaemia (EsPhALL), and the Japan Pediatric Leukemia and Lymphoma Study Group (JPLSG). The COG evaluated the efficacy and safety of imatinib-combined chemotherapy in the AALL0031 study. The 5-year disease-free survival (DFS) (70%) of the non-HSCT group with long-term continuous imatinib at 340 mg/m^2 was not significantly different from that of the HSCT group from related (65%) or unrelated (59%) donor, indicating that the superiority of HSCT in first remission for children with Ph+ALL was waived [4, 5]. In the EsPhALL2004 study, the good-risk patients were randomized to imatinib group versus no imatinib group, and all poor-risk patients received imatinib. A dose of 300 mg/m^2 of imatinib was adopted. Although imatinib was administered intermittently after induction for a short period of time, 126 days, 4-year DFS (75%) in the good-risk, imatinib group was significantly better than that (56%) in the good-risk, no imatinib group, when analyzed as

treated. In the poor-risk, imatinib group, 4-year EFS was 53.5% and 4-year overall survival (OS) was 63.5%, indicating that these outcomes were significantly better than the historical studies without imatinib [6]. However, 77% of overall patients in this study had HSCT in first remission. In the subsequent EsPhALL2010 study, the protocol was amended so that all patients received imatinib continuously from day 15 of induction based on the outcomes of the COG AALL0031 study, and HSCT was limited only to patients with poor minimal residual disease (MRD) response [7]. Although EFS and OS were similar between the two studies, the use of HSCT in first remission, which is associated with long-term toxicities, was reduced to 38% in the EsPhALL2010 study (Table 10.1). On the other hand, 15% of patients treated with chemotherapy alone died in continuous complete remission, most of which were due to infectious complications. The treatment-related mortality in this study is higher than that observed in the conventional high-risk chemotherapy or the EsPhALL2004 study, with imatinib intermittently dosed for a shorter period, suggesting that imatinib given early and continuously combined with intensive chemotherapy may increase toxicity. These trials by the COG and EsPhALL groups showed that chemotherapy with imatinib significantly improved outcomes compared with treatment without imatinib and that HSCT in first remission can be avoided in a certain population.

In Japan, the JPLSG conducted the Ph+ALL04 study, which was a clinical trial of induction, intensifications, and reinduction therapy followed by a 2 week-phase of imatinib 340 mg/m^2 alone, with a subsequent HSCT in all patients in first remission prior to 30 weeks at which relapse often occurs. 4-year EFS was 54.1%, and 4-year OS was 78.1%, resulting in a favorable OS [8]. In a retrospective analysis of patients with induction failure or relapse in the Ph+ALL04 study, many patients achieved complete remission with chemotherapy such as Hyper-CVAD combined with imatinib (286–340 mg/m^2) and were salvaged with a subsequent HSCT [9]. Chemotherapy combined with imatinib has been shown to be effective for patients with induction failure or relapse who have not fully used imatinib.

10.3.3 Dasatinib-Combined Chemotherapy

Many clinical studies of dasatinib combined with chemotherapy have been conducted in adults with newly diagnosed Ph+ALL. In MDACC, the complete remission rate with dasatinib combined with Hyper-CVAD was 94%, and 2-year OS was 64%. This outcome was similar to that of imatinib plus Hyper-CVAD, but the transplant rate in first remission was reduced from 30% to 10% [10]. In the Italian GIMEMA LAL 1205 study, adults with newly diagnosed Ph+ALL received induction therapy with dasatinib (80 mg/m^2) for 84 days combined with prednisone for the first 32 days and achieved complete hematological remissions in 92.5% on day 22 and 100% on day 57. No deaths occurred during the dasatinib induction treatment [11]. Induction treatment with dasatinib plus steroids may also be safe and effective in children.

Table 10.1 Clinical trials in children using TKIs in combination with chemotherapy for Philadelphia chromosome-positive acute lymphoblastic leukemia

	N	TKI				Prophylactic CRT	EFS (%, 95% CI)	OS (%, 95% CI)	Indication for HSCT	HSCT rate
		Type	Dose	Time of initiation	Administration method					
JPLSG Ph+ALL04	42	Imatinib	340 mg/m^2	After reinduction therapy	2 weeks monotherapy	−	4y, 54.1 (46.3–61.9)	4y, 78.1 (71.6–84.6)	All patients	100%
COG AALL0031	91	Imatinib	340 mg/m^2	After induction therapy	Intermittent (Cohort 1–4) Continuous (Cohort 5)	+	5y, 58 (52–64)[a]	5y, 70 (64–76)	Having HLA-matched-related donor	37%
COG AALL0622	60	Dasatinib	60 mg/m^2	Day 15 induction therapy	Intermittent (Cohort 1) Continuous (Cohort 2)	−	5y, 60 (53–67)	5y, 86 (81–91)	Standard-risk patients with HLA-matched-related donor and all high-risk patients[b]	32%
EsPhALL 2004	178	Imatinib	300 mg/m^2	After induction therapy	Intermittent (Good-risk, imatinib group, and poor risk) No imatinib (Good-risk, no imatinib group)	+	4y, 61.9 (52.2–69.8)	4y, 72.1 (64.5–79.7)	Good-risk with HLA-matched-related donor and all poor-risk patients[c]	77%
EsPhALL 2010	155	Imatinib	300 mg/m^2	Day 15 induction therapy	Continuous	+	5y, 57.0 (48.5–64.6)	5y, 71.8 (63.5–78.5)	Ig/TCR MRD at the end of phase IB was $\geq 5 \times 10^{-4}$ or positivity at any detectable MRD level at the end of Block HR3	38%

TKI tyrosine kinase inhibitor, *CRT* cranial radiation therapy, *EFS* event free survival, *OS* overall survival, *HSCT* hematopoietic stem cell transplantation, *HLA* human leukocyte antigen, *MRD* minimal residual disease

[a]Induction failure patients were excluded ($N = 81$)

[b]Patients were stratified as high risk (HR) if flow MRD levels were ≥1% at end of induction and/or ≥0.01% at end of consolidation 2

[c]Good-risk patients were those who had both early response and complete remission at the end of induction

In children with newly diagnosed Ph+ALL, the COG conducted the AALL0622 study, which used dasatinib (60 mg/m^2) continuously from day 15 of induction therapy with the same backbone as the AALL0031 study. There were no treatment-related deaths, and no grade 3 or higher pleural effusion or cardiotoxicity was observed. Dasatinib combined with intensive chemotherapy was well tolerated in children. Despite early favorable MRD response, 5-year EFS was 60%, similar to that of the AALL0031 study although induction-failure patients were excluded for the analysis of EFS in AALL0311 (Table 10.1). However, as cranial radiation was focused on patients with central nervous system leukemia in AALL0622 and the transplant rate was reduced from 37% to 32%, dasatinib was considered to contribute to reducing late complications (Table 10.1). Furthermore, Slayton et al. note in this paper that most children with Ph+ALL should not undergo transplantation in first remission, because of the favorable 5-year OS of 88% in patients receiving dasatinib and chemotherapy alone [12].

10.3.4 Ponatinib-Combined Chemotherapy

The combination of ponatinib and Hyper-CVAD in adults with newly diagnosed Ph+ALL has been reported by MDACC. Sasaki et al. compared outcomes of Hyper-CVAD with ponatinib and that with dasatinib in an analysis using propensity scores. 3-year OS in Hyper-CVAD with ponatinib was 83%, which was significantly higher than 3-year OS of 56% in Hyper-CVAD with dasatinib [13]. The use of ponatinib as a frontline TKI combined with chemotherapy may further improve clinical outcomes. Clinical trials of ponatinib have not yet been conducted in children, and there is only information from case reports using ponatinib [14]. Recently, a safety trial of ponatinib, the JPLSG PedPona19 study, in children younger than 15 years has begun in Japan.

References

1. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. N Engl J Med. 2006;354:166–78.
2. Aricò M, Schrappe M, Hunger SP, Carroll WL, Conter V, Galimberti S, et al. Clinical outcome of children with newly diagnosed Philadelphia chromosome–positive acute lymphoblastic leukemia treated between 1995 and 2005. J Clin Oncol. 2010;28:4755–61.
3. Daver N, Thomas D, Ravandi F, et al. Final report of a phase II study of imatinib mesylate with hyper-CVAD for the front-line treatment of adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia. Haematologica. 2015;100:653–61.
4. Schultz KR, Bowman WP, Aledo A, Slayton WB, Sather H, Devidas M, Wang C, Davies SM, Gaynon PS, Trigg M, Rutledge R, Burden L, Jorstad D, Carroll A, Heerema NA, Winick N, Borowitz MJ, Hunger SP, Carroll WL, Camitta B. Improved early event-free survival with imatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia: a children's oncology group study. J Clin Oncol. 2009;27:5175–81.

5. 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. 2014;28:1467–71.
6. Biondi A, Schrappe M, De Lorenzo P, et al. Imatinib after induction for treatment of children and adolescents with Philadelphia-chromosome-positive acute lymphoblastic leukaemia (EsPhALL): a randomised, open-label, intergroup study. Lancet Oncol. 2012;13:936–45.
7. Biondi A, Gandemer V, De Lorenzo P, Cario G, Campbell M, Castor A, Pieters R, Baruchel A, Vora A, Leoni V, Stary J, Escherich G, Li CK, Cazzaniga G, Cavé H, Bradtke J, Conter V, Saha V, Schrappe M, Grazia Valsecchi M. Imatinib treatment of paediatric Philadelphia chromosome-positive acute lymphoblastic leukaemia (EsPhALL2010): a prospective, intergroup, open-label, single-arm clinical trial. Lancet Haematol. 2018;5:e641–52.
8. Manabe A, Kawasaki H, Shimada H, et al. Imatinib use immediately before stem cell transplantation in children with Philadelphia chromosome-positive acute lymphoblastic leukemia: Results from Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) Study Ph(+) ALL04. Cancer Med. 2015;4:682–9.
9. Kodama Y, Manabe A, Kawasaki H, et al. Salvage therapy for children with relapsed or refractory Philadelphia chromosome-positive acute lymphoblastic leukemia. Pediatr Blood Cancer. 2017;64:pbc.26423.
10. Ravandi F, O'Brien S, Thomas D, et al. First report of phase 2 study of dasatinib with hyper-CVAD for the frontline treatment of patients with Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia. Blood. 2010;116:2070–7.
11. Foà R, Vitale A, Vignetti M, et al. Dasatinib as first-line treatment for adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia. Blood. 2011;118:6521–8.
12. Slayton WB, Schultz KR, Kairalla JA, et al. Dasatinib plus intensive chemotherapy in children, adolescents, and young adults with Philadelphia chromosome-positive acute lymphoblastic leukemia: results of Children's Oncology Group Trial AALL0622. J Clin Oncol. 2018;36:2306–14.
13. Sasaki K, Jabbour EJ, Ravandi F, et al. Hyper-CVAD plus ponatinib versus hyper-CVAD plus dasatinib as frontline therapy for patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: a propensity score analysis. Cancer. 2016;122:3650–6.
14. Yamamoto M, Hori T, Igarashi K, Shimada H, Tsutsumi H. Response to ponatinib before hematopoietic stem cell transplantation in a child with relapsed Philadelphia chromosome-positive acute lymphoblastic leukemia. Pediatr Int. 2018;60:85–7.

Chapter 11
Acute Lymphoblastic Leukemia in Down Syndrome

Yasuhiro Okamoto

Abstract Improvement in the outcomes associated with acute lymphoblastic leukemia (ALL) in Down syndrome (DS-ALL) has been delayed. However, as the clinical characteristics of DS-ALL, including leukemogenesis, are elucidated, strategies for improving the outcomes are being considered and implemented. As host side problems, infectious complications and complication deaths due to immunodeficiency and mucosal disorders are problematic. Close control of infection using prophylactic antibiotics and intravenous immunoglobulin replacement should assist in overcoming these problems. DS-ALL is commonly associated with a poor prognosis for gene abnormality, similar to Ph-like ALL. While selecting appropriate treatment for DS-ALL, minimal residual disease (MRD) is determined to assess the disease status and calculate the risk. DS-ALL is considered as a good indication for immunotherapy, such as inotuzumab ozogamicin, blinatumomab, and chimeric antigen receptor T cell therapy, because of less adverse events than those of anticancer drugs. CRLF2-JAK abnormalities are frequently observed in DS-ALL, and specific therapies targeting them are also being developed. As mentioned above, it is thought that improvements in the treatment of DS-ALL outcomes will lead to similar improvements in other ALL. This review will point out the current and future direction of DS-ALL to clinicians treating DS-ALL and those doing research on DS-ALL.

Keywords Infectious complication · Immunodeficiency · Mucosal disorder Ph-like ALL · Minimal residual disease · CRLF2 · Methotrexate

Y. Okamoto (✉)
Department of Pediatrics, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan
e-mail: okamoto@m2.kufm.kagoshima-u.ac.jp

M. Kato (ed.), *Pediatric Acute Lymphoblastic Leukemia*,
https://doi.org/10.1007/978-981-15-0548-5_11

11.1 Introduction

Treatment outcomes for acute lymphoblastic leukemia (ALL) in children have been improved by risk-based treatment and improvements in supportive care. However, improvement in outcomes associated with Down syndrome (DS) ALL (DS-ALL) has been delayed. This chapter describes the clinical problems associated with DS-ALL, the factors that make DS prone to develop into ALL, and how to treat DS-ALL in the future, and it is expected to be useful to clinicians who treat DS-ALL and those who do research on DS-ALL.

11.2 Clinical Practice for the Treatment of DS-ALL

11.2.1 Treatment Results

Retrospective research findings gathered from clinical research groups around the world were reported in 2014 from the Ponte di Legno group. The 5- to 10-year event-free survival rate for DS-ALL cases was 50–70%, which was worse compared to 63–88% for non-DS-ALL cases [1]. This study was very important as it was the first to clarify the reasons why DS-ALL cases had inferior outcomes. Poor prognosis factors associated with DS-ALL were classified into two: those related to ALL cells and those to the host (Fig. 11.1). As was similar to non-DS-ALL cases, DS can develop into ALL among those with various genetic backgrounds; however, as factors related to ALL cells, ETV6-RUNX1 abnormalities and hyperdiploidy, which are good prognostic factors, are uncommon. Conversely, there are more treatment-resistant ALL with so-called Ph-like ALL features with CRLF2 abnormalities. In other words, a high proportion of ALL with resistance to treatment accounts for poor outcomes for DS-ALL cases. Host problems include a high frequency and severity of treatment complications. Infectious complications require treatment to be discontinued temporarily, sometimes resulting in death. Unfortunately, despite the need for chemotherapy for DS-ALL, it is not possible to increase the treatment intensity as a result of complications. As a result, the therapeutic strength is reduced.

Although t(9;22)/BCR-ABL1 is rare among DS-ALL cases, there are reports of cases with t(9;22)/BCR-ABL1 where dasatinib was effective as well as among non-DS-ALL cases [2]. DS-ALL has a poor prognosis once it recurs [3], and the results of hematopoietic cell transplantation are not satisfactory [4, 5]. Recently, there was a report on the use of inotuzumab ozogamicin [6] or blinatumomab [7]. DS cases tend to be excluded from clinical trials; however, several trials have been opened to include DS-ALL patients in a relapse study of blinatumomab by the International BFM Study Group (NCT01802814) and in the Children's Cancer Group (COG) phase 2 study of inotuzumab ozogamicin in relapsed or refractory B-ALL

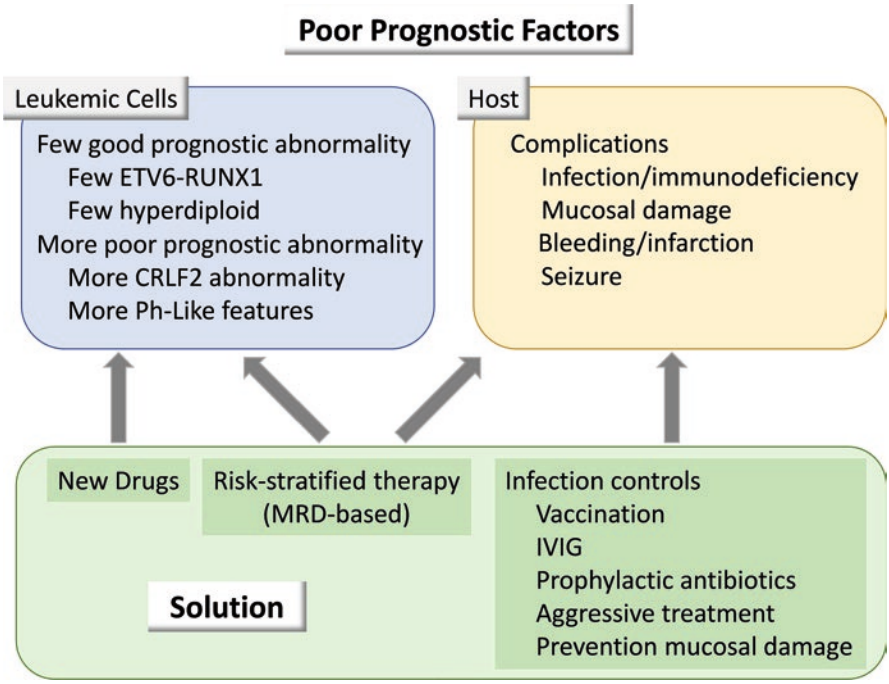


Fig. 11.1 Poor prognostic factors and their solutions. Poor prognosis factors have been classified into two: one relating to leukemia cells and the other to the host's immune system. Recommended solutions are geared toward the development of new drugs, treatments based on MRD, and infection control

(NCT02981628). Chimeric antigen receptor T (CAR-T) cells should be a particularly attractive treatment option for children with DS since this therapy could be more efficacious and less toxic [8].

11.2.2 Features of DS-ALL

DS-ALL is a heterogeneous disease with various genetic backgrounds aside from 21 trisomy. Overall, t(9;22)/BCR-ABL1, MLL abnormality, hyperdiploid, and t(12;21)/ETV6-RUNX1 account for 0.7%, 0.5%, 9%, and 8.3% of DS-ALL, respectively [1, 2, 9]. These DS-ALL cases are less frequent than those in non-DS, and cases in which no chromosomal abnormality is found other than trisomy 21 should have a high frequency of CRLF2 abnormalities. When Japanese DS-ALL cases were examined, 21% were abnormal according to the JAK-STAT system including CRLF2, 33% were abnormal according to the RAS system, and 45% were abnormal based on other systems [10]. DS cases have a 150-fold incidence of AML compared

to non-DS cases. Although it is rare, it has been reported that ALL has developed after AML [11–13]. The incidence of ALL after AML seems similar between DS and non-DS cases, and the prognosis seems to be comparable [11].

11.2.3 Treatment Complications

In the Ponte di Legno study, mortality from complications was 7.7% among DS-ALL cases, which was significantly higher than the 2.3% reported among non-DS-ALL cases [1]. Characteristically, the mortality rate during induction therapy was as high as 2.8% and the mortality rate during remission was as high as 4.9%. Of the deaths attributable to complications, 75% has been reported to result from infections [1, 14]. Various types of immunodeficiency have been reported in DS, whose wide range of abnormalities resembles combined immunodeficiency. Mucosal damage occurs in 52% of DS-ALL cases, and mucosal damage also occurs by intrathecal injection of methotrexate (MTX) [14]. In the COG AALL0232 and AALL0932 studies, the frequency of deaths resulting from infection due to mucosal damage during induction was relatively high compared to other studies [15]. Reducing the mortality rate of these comorbid infections is important in the treatment of DS-ALL (Fig. 11.1). It is recommended that prophylactic antibiotics, immunoglobulin replacement, and active treatment for infections be administered [16]. Prevention of mucosal disorders is also important. Usually, a high-dose of MTX is used at 2–5 g/m^2 in non-DS-ALL cases; however, it is recommended to reduce the dose to 0.5–1.0 g/m^2 among DS-ALL cases. In addition, although not performed for non-DS-ALL cases, leucovorin rescue is performed after an intrathecal injection of MTX among DS-ALL cases; as a result, mortality decreased in the COG and Dana-Farber Cancer Institute (DFCI) studies [14, 15].

11.3 Basic Science in DS-ALL

11.3.1 Mechanisms Involved in the Development of ALL

The frequency of DS-ALL is reported to be 1.1–3.2% [1, 17, 18]. As the incidence of ALL is 20 times higher in DS than in non-DS cases, and the frequency of the addition of chromosome 21 is highest in ALL cases with hyperdiploidy, it is believed that chromosome 21 is responsible for the development of leukemia. In 2014, researchers in DFCI tripled 31 mouse genes that are homologs of the human 21q22 region in mouse models to cause B precursor cells to self-amplify and cause maturation failure [19]. Furthermore, overexpression of HMGN1 in this 21q22 region accelerated B cell proliferation and the onset of B cell ALL by BCR-ABL [19]. In 2015, Thompson et al. revealed that DYRK1A on chromosome 21 controls the transition

from G0 mitosis by T283-mediated cyclin D3 degradation [20]. HMGN1 and DYRK1A on chromosome 21 are considered to be the reason for high incidence of ALL in DS cases. Prior to their discoveries, it was reported that the fusion of P2RY8-CRLF2 causes the overexpression of CRLF2, and the activation of the downstream JAK-STAT system is one of the mechanisms involved in ALL onset [21]. The fusion of P2RY8-CRLF2 occurs in only 7% of non-DS-ALL cases; however, it is frequently observed in 29–53% of DS-ALL cases [10, 21]. The reason why the frequency of CRLF2 abnormality is high in DS cases has yet to be clarified. Studies involving the analysis of fetal liver hematopoietic microenvironment have shown that DS is prone to cell cycle arrest early in B cell development. An increase in proliferation due to an abnormality in CRLF2 may compensate for the arrest of this cell cycle; additionally, it may be easy to select a CRLF2 abnormal cell [16]. In summary, the pathogenesis of ALL in DS cases is assumed to be that in Fig. 11.2. Recently Potter et al. reported that CRLF2 rearrangements were observed in both early and late events in DS cases using single-cell analysis [22].

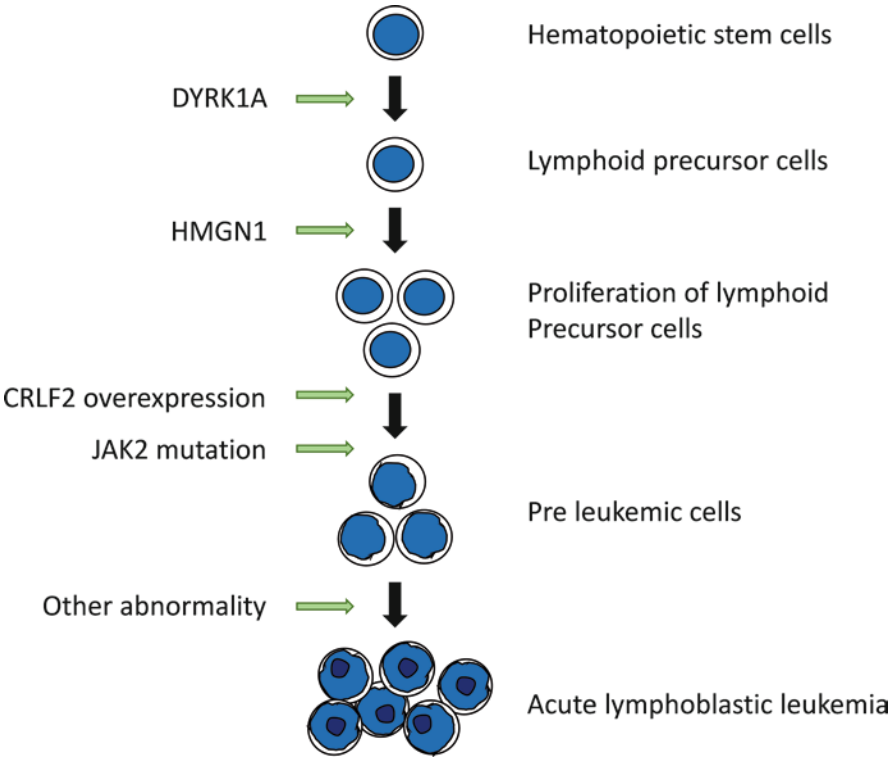


Fig. 11.2 Leukemogenesis in Down syndrome. Leukemogenesis in Down syndrome is illustrated based on published data. Several factors related to trisomy 21 (DYRK1A and HMGN1), as well as other factors including the CRLF2-JAK pathway, play a major role in the development of acute lymphoblastic leukemia in Down syndrome

11.3.2 CRLF 2

11.3.2.1 Function of CRLF2

CRLF2 is a receptor expressed on Th2 cells, macrophages, dendritic cells, etc., and forms a dimer with IL7RA upon binding of its ligand thymic interstitial lymphopoietin (TSLP). By dimerization, JAK1 and JAK2 are phosphorylated and signals are transmitted downstream. Downstream are the STAT pathway and the PI3K-AKT-mTOR pathway, which drives the expression of target genes. The physiological functions of CRLF2 are activation of dendritic cells and the inflammatory response of Th2 cells, as well as proliferation and homeostasis of T cells. In DS-ALL, 30–50% have JAK2 mutations in addition to CRLF2 overexpression, and 40–60% have other kinase mutations. A mutation in CRLF2 is found in 10% of DS-ALL cases which overexpress CRLF2. Point mutations in CRLF2 which result in overexpression of CRLF2 in 9% of DS-ALL cases have also been reported. In either case, the overexpression of CRLF2 or of mutations in JAK2 results in cell activation regardless of the signal.

11.3.2.2 CRLF2 as a Therapeutic Target

In the United States, a phase II trial (NCT 02723994) of ruxolitinib, which is an inhibitor of JAK2, was initiated in August 2016 for ALL cases with CRLF2 mutations or JNK pathway mutations. DS-ALL cases were excluded from this study. Schwartzman et al. examined the distribution of CRLF2 and JAK mutations at the onset and relapse of DS-ALL. JAK2 abnormalities are relatively infrequent at the time of relapse and cannot be regarded as abnormalities that cause relapse. JAK2 mutations are necessary for ALL with CRLF2 abnormalities; however, the data suggest that it is more likely for these cases to relapse if the JAK2 mutation is absent. Therefore, it is possible that the effect of JAK inhibitors on relapsed DS-ALL will be limited among these cases.

11.4 New Treatment

11.4.1 Optimization of Treatment

There are two possible ways to improve the outcomes of DS-ALL (Fig. 11.1). One is to select the optimal treatment intensity for DS-ALL, which is heterogeneous and prone to complications. Treatment response should be assessed by MRD, and appropriate treatment intensity should improve outcomes. In fact, in the recent DFCI 00-001/05-001 study, although there were only 38 cases of DS-ALL, PCR-MRD was used to optimize treatment, and the outcomes for DS-ALL were reported to be similar to those for non-DS-ALL cases [14].

11.4.2 New Drugs

It is reported that besides ruxolinitib, givinostat, which inhibits the JAK-STAT system as an HDAC inhibitor, and gedatolisib, which inhibits both PI3K and mTOR, are useful in mouse models as therapeutic agents targeting CRLF2 abnormalities. These drugs do not target DS-ALL alone. They may have an effect on DS-ALL cases in addition to other ALL cases with JAK-STAT abnormalities secondary to CRLF2 abnormalities. In addition, CAR-T therapy for the TSLP receptor has also been reported to be successful in mouse models. CAR-T therapy has been reported to be equally as effective in DS cases, with similar toxicity to that observed in non-DS-ALL cases; thus, it may be unnecessary to exclude DS-ALL cases from future studies [23].

11.4.3 Clinical Research

It is reasonable to conduct clinical trials that treat DS-ALL as an independent disease; however, DS-ALL is a heterogeneous disease and host-side problems are common. An international clinical trial (DS-ALL 2016, NCT03286634) has started, which has been joined by several Asian clinical trial groups including the Japan Children's Cancer Group (JCCG). In this study, the MTX dose was reduced to 0.5 g/m^2 and the risk-optimized treatment with flow cytometric MRD was adopted.

In conclusion, although treatment results for DS-ALL cases were poor, various measures are expected to improve treatment results. DS-ALL cases should be treated under specialized infection control at specialized facilities. DS-ALL develops relatively in the elderly. While solving various problems associated with Down syndrome, developing ALL further creates a very difficult situation. However, medical advances as described in this chapter will allow more DS-ALL children to survive in the future.

References

1. 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. 2014;123(1):70–7.
2. Hirabayashi S, Hasegawa D, Yamamoto K, et al. Dasatinib and low-intensity chemotherapy for Philadelphia chromosome-positive acute lymphoblastic leukemia in a child with down syndrome. Pediatr Blood Cancer. 2019;66(5):e27612.
3. Meyr F, Escherich G, Mann G, et al. Outcomes of treatment for relapsed acute lymphoblastic leukaemia in children with down syndrome. Br J Haematol. 2013;162(1):98–106.
4. Hitzler JK, He W, Doyle J, et al. Outcome of transplantation for acute lymphoblastic leukemia in children with down syndrome. Pediatr Blood Cancer. 2014;61(6):1126–8.

5. Goto H, Kaneko T, Shioda Y, et al. Hematopoietic stem cell transplantation for patients with acute lymphoblastic leukemia and down syndrome. Pediatr Blood Cancer. 2015;62(1):148–52.
6. Murillo L, Dapena JL, Velasco P, de Heredia CD. Use of inotuzumab-ozogamicin in a child with down syndrome and refractory B-cell precursor acute lymphoblastic leukemia. Pediatr Blood Cancer. 2019;66(4):e27562.
7. A W, MA K, AC X. Blinatumomab activity in a patient with down syndrome B-precursor acute lymphoblastic leukemia. Pediatr Blood Cancer. 2018;65:2.
8. Rabin K, Izraeli S, Hijiya N, Hitzler J. Need for new thinking: treatment of relapsed leukemia in children with down syndrome. Pediatr Blood Cancer. 2019;66(6):e27644.
9. Tamaura M, Iwasaki F, Yokosuka T, Fukuda K, Hamonoue S, Goto H. Philadelphia chromosome-positive acute lymphoblastic leukemia and down syndrome. Pediatr Int. 2016;58(8):794–7.
10. Hanada I, Terui K, Ikeda F, et al. Gene alterations involving the CRLF2-JAK pathway and recurrent gene deletions in down syndrome-associated acute lymphoblastic leukemia in Japan. Genes Chromosomes Cancer. 2014;53(11):902–10.
11. Murphy BR, Roth M, Kolb EA, Alonzo T, Gerbing R, Wells RJ. Development of acute lymphoblastic leukemia following treatment for acute myeloid leukemia in children with down syndrome: A case report and retrospective review of Children's oncology group acute myeloid leukemia trials. Pediatr Blood Cancer. 2019;66:e27700.
12. Tomizawa D, Endo A, Kajiwara M, et al. Acute lymphoblastic leukemia in patients with down syndrome with a previous history of acute myeloid leukemia. Pediatr Blood Cancer. 2017;64(8):e26411.
13. Hellebostad M, Carpenter E, Hasle H, Mitchell C, Vyas P. GATA1 mutation analysis demonstrates two distinct primary leukemias in a child with down syndrome; implications for leukemogenesis. J Pediatr Hematol Oncol. 2005;27(7):408–9.
14. Athale UH, Puligandla M, Stevenson K, et al. Outcome of children and adolescents with down syndrome treated on Dana-Farber Cancer Institute acute lymphoblastic leukemia consortium protocols 00-001 and 05-001. Pediatr Blood Cancer. 2018;65:e27256.
15. Maloney KW, Wood B, Whitlock JA, et al. Event free (EFS) and overall survival (OS) for children with down syndrome (DS) and B-lymphoblastic leukemia in Children's Oncology Group (COG) trials AALL0232 and AALL0331. Pediatr Blood Cancer. 2014;61:S1–4.
16. Izraeli S, Vora A, Zwaan CM, Whitlock J. How I treat ALL in Down's syndrome: pathobiology and management. Blood. 2014;123(1):35–40.
17. Goto H, Inukai T, Inoue H, et al. Acute lymphoblastic leukemia and down syndrome: the collaborative study of the Tokyo Children's cancer study group and the Kyushu Yamaguchi Children's cancer study group. Int J Hematol. 2011;93(2):192–8.
18. Whitlock JA, Sather HN, Gaynon P, et al. Clinical characteristics and outcome of children with down syndrome and acute lymphoblastic leukemia: a Children's Cancer Group study. Blood. 2005;106(13):4043–9.
19. Lane AA, Chapuy B, Lin CY, et al. Triplication of a 21q22 region contributes to B cell transformation through HMGN1 overexpression and loss of histone H3 Lys27 trimethylation. Nat Genet. 2014;46(6):618–23.
20. Thompson BJ, Bhansali R, Diebold L, et al. DYRK1A controls the transition from proliferation to quiescence during lymphoid development by destabilizing Cyclin D3. J Exp Med. 2015;212(6):953–70.
21. Mullighan CG, Collins-Underwood JR, Phillips LA, et al. Rearrangement of CRLF2 in B-progenitor- and down syndrome-associated acute lymphoblastic leukemia. Nat Genet. 2009;41(11):1243–16.
22. Potter N, Jones L, Blair H, et al. Single-cell analysis identifies CRLF2 rearrangements as both early and late events in down syndrome and non-down syndrome acute lymphoblastic leukaemia. Leukemia. 2019;33(4):893–904.
23. Laetsch TW, Maude SL, Grupp S, et al. CTL019 therapy appears safe and effective in pediatric patients with down syndrome with relapsed/refractory (r/r) acute lymphoblastic leukemia. Blood. 2017;130:1280.

Chapter 12
Adolescents and Young Adults with Acute Lymphoblastic Leukemia

Etsuko Yamazaki

Abstract Acute lymphoblastic leukemia (ALL) in adolescents and young adults (AYAs) is a relatively new concept for patients between the ages 15 and 39 years, who have unique pathophysiology and require specific clinical care. The results of many clinical studies demonstrated that treatment with the pediatric protocol has better disease-free survival and overall survival compared to treatment with the adult protocol for AYA-ALL. Survival of AYA-ALL was greatly improved to 70% due to pediatric regimens from 30% by adult regimen. There are two types of strategies for adapting pediatric regimen for AYA-ALL: one is pediatric-inspired regimen and the other is non-modified pediatric regimens. It is difficult to determine which of these two strategies should be recommended. New knowledge of specific genetical features of AYA-ALL will provide new strategies for targetable ALL, particularly Philadelphia-like ALL. Novel immunotherapies are approved for refractory and relapsed ALL. The firstline introduction of immunotherapy in BCP-ALL, kinase inhibitors in Ph-like ALL will further improve the outcome of AYA-ALL. Appropriate and long-term follow up by multidisciplinary care teams is needed to further improve survival and quality of life for survivors of AYA-ALL.

Keywords AYA-ALL · Genetical feature · Pediatric-inspired regimen · Fully pediatric regimen · Immunotherapy · Long-term follow up

E. Yamazaki (✉)
Clinical Laboratory Department, Yokohama City University Hospital, Yokohama, Kanagawa, Japan
e-mail: etsukoy@yokohama-cu.ac.jp

M. Kato (ed.), *Pediatric Acute Lymphoblastic Leukemia*,
https://doi.org/10.1007/978-981-15-0548-5_12

12.1 Introduction

There was no precise definition of adolescents and young adults (AYAs) in the early 2000s. The National Cancer Institute Adolescence and Young Adult Oncology Progress Review Group considered the issue and defined as individuals diagnosed with cancer from age 15 to 39 years in 2006. Approximately 60% of leukemia in AYAs is acute lymphoblastic leukemia (ALL) with peak occurrence between 15 and 20 years old, and 40% is acute myeloid leukemia (AML).

Children, age younger than 15 years, diagnosed with ALL have an excellent prognosis, with cure rates exceeding 85% [1]. However, the outcome of ALL worsen with age, treatment of adults with ALL has been much less successful, with overall survival (OS) rates of only 30%–40%, despite equivalent complete remission (CR) rates of 90% [2, 3]. A period analysis of ALL patients between 2000 and 2004 in the United States showed 5-year relative survival rates of 80.7% in patients aged 10–14 years, 61.1% in patients aged 15–19 years, 44.8% in patients aged 20–29 years, and 34.3% in patients aged 30–44 years [4, 5]. The results of EUROCARE-5 were similar to that of the outcomes of 4617 AYAs with ALL compared with 15,089 children diagnosed in 2000–2007 in Europe. A remarkable decline with age in 5-year relative survival was seen: 85.8% in patients of age 0–14 years, 62.2% in those of age 15–19 years, and 52.8% in those of age 20–39 years [6].

The age-related decline in survival is partly explained by an increasing of high-risk factors and a decreasing of good prognostic factors, especially genetic alterations. AYAs present with higher risk biologic features (T cell, unfavorable cytogenetics including Philadelphia chromosome (Ph1) positive [BCR–ABL1–positive]) or an aggressive "Ph-like" ALL characterized by a gene-expression profile similar to that of Ph1 positive ALL [7].

12.2 Higher Risk Biologic Features in AYA-ALL

12.2.1 Genetical Features of AYA-ALL

Previous genetical analyses of B cell precursor (BCP) ALL have greatly improved and revealed the pathogenesis and prognostic impact of many molecular rearrangements in BCP-ALL [8]. High hyperdiploidy and the cryptic t(12;21) encoding *ETV6-RUNX1* are associated with a favorable outcome. Hypodiploidy with less than 44 chromosomes, *KMT2A* (also called *MLL*) rearrangement, Ph1 positive, Ph-like (*BCR-ABL1*-like) ALL, *CRLF2* rearrangement, intrachromosomal amplification of chromosome 21 are associated with high-risk clinical features or a poor outcome.

High hyperdiploidy (51–67 chromosomes) is present in 25–30% of childhood BCP-ALL patients but accounts for 5–10% of AYAs and adults [9, 10]. The *ETV6-*

RUNX1 gene fusion observed in ~25% of cases of childhood ALL [1], but in <5% of cases of AYAs and adults [11].

Low-hypodiploidy (30–39 chromosome) is very rare in children (<1%) but increases with age, accounting for 5–10% of AYAs and adults [10]. *KMT2A* rearrangements are the most common in infants aged <1 year. It is less common in older children and then becomes increasingly common with age into adulthood. Intrachromosomal amplification of chromosome 21, which is newly categorized in WHO classification 2017 and is associated with a relatively poor prognosis, is more common in older children. The prevalence of Ph1 positive ALL is 2–4% of childhood BCP-ALL but increase to 6% of AYAs and at least 25% of adults [1, 12]. Ph1-positive ALL used to be considered to have the worst prognosis in ALL, but therapy with tyrosine kinase inhibitors has been significantly improved outcome.

Ph-like ALL, which is a provisional entity in WHO classification 2017, lacks the *BCR-ABL1* translocation but shows a pattern of gene expression very similar to that of Ph1 positive ALL [13]. Ph-like ALL shows various types of chromosomal rearrangements, involving many different genes and various partners [14]. These include rearrangements of *CRLF2*, fusions involving ABL-class genes, rearrangements of *JAK2* or *EPOR*, alterations activating *JAK-STAT* or *Ras* signaling pathways, and other less common fusions. The incidence of Ph-like ALL increases with age, comprising 10%–15% of children and over 20% of adults and peaking at 25%–30% in AYAs [14–16]. These cases are characterized by high levels of post-induction minimal residual disease (MRD) and overall poor outcomes [17, 18]. Alterations of IKZF1, which encodes the lymphoid transcription factor Ikaros, are common in Ph1 positive and Ph-like ALL. These alterations are also associated with a poor outcome [13, 14]. Ph-like ALL include specific targetable rearrangements; ABL-class fusions or JAK mutations/translocations. Tyrosine kinase inhibitors for patients with ABL-class fusions and JAK inhibitors for patients with JAK mutations or translocation are new strategies in clinical studies [19].

Myocyte enhancer factor 2D (MEF2D) ALL is associated with older age, aberrant immunophenotype (CD10 negative, CD38 positive), and poor outcome [20, 21]. It occurs in ~4%–7% of patients, mostly AYAs.

The zinc finger protein 384 (ZNF384)-rearranged ALL are found in BCP-ALL with aberrant expression of the myeloid markers CD13 and/or CD33. These fusions represent 7–12% of AYAs and older patients, and the prognosis may depend on the fusion partner [22].

Iacobucci and Mullighan provided a figure of age distribution of ALL subtypes in their review article [23].

12.2.2 Other Characteristic Risk of AYA-ALL

Historically, T-cell ALL (T-ALL) in childhood has had inferior survival compared with BCP-ALL [24]. The proportion of T-ALL is higher in the AYA than in children or older adults. Patients with T-ALL often present with high-risk clinical features.

Among T-ALL, early T-cell precursor (ETP) ALL, which is provisional entity in WHO classification 2017, accounting for approximately 10–13% of cases of T-ALL in children and for 5–10% of T-ALL in adults. Prognosis of patients with ETP-ALL under adequate treatment appears to be same as other patients with T-ALL.

The Nordic Society of Pediatric Hematology and Oncology (NOPHO) ALL2008 trial were performed for 1509 patients aged 1–45 years with Ph negative ALL. They stratified into three risk groups using leukemia characteristics at diagnosis (high white blood cell, central nervous system involvement, T or B phenotype, cytogenetics) and MRD after induction therapy on days 15 and 29, and after consolidation therapy on day 79, but not age. A risk group distribution for six age groups of 1–4, 5–9, 10–14, 15–17, 18–25, and 26–45 years apparently shifted to higher risk with aging. (Fig. 12.1) Older patients more often had T-ALL, KMT2A rearrangements and higher day 29 MRD for BCP-ALL, but not T-ALL [25].

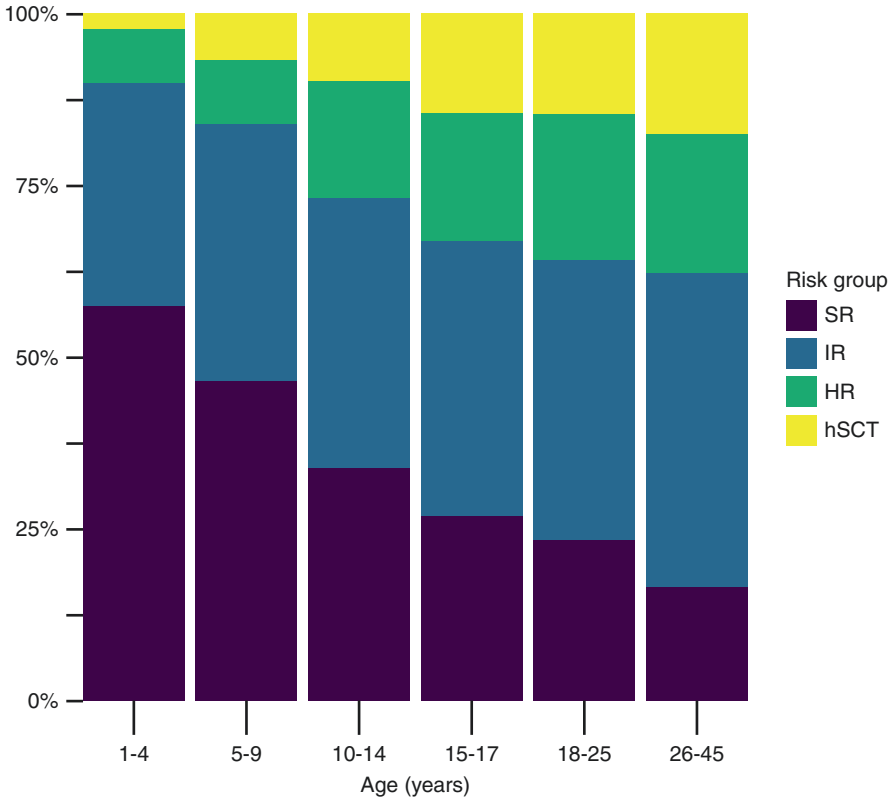


Fig. 12.1 Risk group distribution for six age groups in NOPHO ALL2008: 1–4, 5–9, 10–14, 15–17, 18–25, and 26–45 years. Standard risk (SR), intermediate risk (IR), high risk (HR), and high risk with stem cell transplantation in first remission (hSCT) [25]

12.3 Treatments for AYA-ALL (Table 12.1)

Retrospective comparisons were performed by many cooperative groups throughout the world and examined the outcome of AYA patients treated on pediatric or adult cooperative group trials in ALL. The most of these studies demonstrated a significantly better outcomes when the AYA patient treated by the pediatric cooperative groups [26–29]. As the major explanation for these results, disparities in chemotherapy or in dose-intensity were showed. Higher cumulative dose of asparaginase, vincristine, and corticosteroids and delayed intensifications were employed in pediatric protocols, whereas higher doses of cytarabine and increased rates of hematopoietic stem cell transplantation (HSCT) were taken in adult protocols [29].

Then prospective clinical trials using pediatric regimens for AYA patients with newly diagnosed ALL are designed to address the feasibility and efficacy. These trials have been divided into two types based on their strategy. One strategy was to develop so-called pediatric-inspired regimens for adults up to age 50–60 years. The other was to adopt fully pediatric trials in AYAs up to 40 years of age.

12.3.1 Trials Using Pediatric-Inspired Regimens

The Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL) from France performed trial of a pediatric-inspired therapy in adults with Ph-negative ALL of age 15–60 years (median age, 31 years). At 42 months, event-free survival (EFS) and OS rates were estimated to be 55% and 60%, respectively. For the cohort of age 15–45 in GRAALL, 5-year EFS and OS rate were 58% and 64%, respectively [30]. In North America, Princess Margaret Hospital reported on the treatment of 85 patients of age 18–60 years (median age, 37 years) with Ph negative ALL using a modified Dana-Farber Cancer Institute protocol. For the whole cohort, 3-year disease-free survival (DFS) and OS were 71% and 67%, respectively. For the cohort of age 18–35 years, 3-year DFS and OS were 77% and 83%, respectively [31].

12.3.2 Trial Using Fully Pediatric Regimens

The Japan Adult Leukemia Study Group (JALSG) conducted ALL202-U protocol to examine the efficacy and feasibility of an unmodified pediatric protocol in AYAs of age 15–24 years (median age 19 years) with Ph negative ALL between 2002 and 2009. The outcome of 139 patients included all risk groups were reported. A CR rate was 94%, 5-year DFS and OS rates were 67% and 73%, respectively. Both the DFS and OS rates were significantly better than those of ALL97-U (previous JALSG protocol) patients (44 and 45%, respectively). (Fig. 12.2) There was no observed significant difference in the DFS rate between patients that received HSCT and

Table 12.1 Comparison of pediatric-inspired and fully pediatric trial for AYA-ALL

Trial			Number	Age		Asp		HD-MTX	Corticosteroids		VCR	CR	EFS/DFS		OS		
Type	Group	Duration	Patients	Median	Range		IU/m^2	g/m^2	PSL mg/m^2	DEX mg/m^2	mg/m^2	%	Y	%	Y	%	Ref.
Pediatric inspired trial	PETHEMA ALL-96	1996–2005	81	20	15–30	L-asp	320,000	9	6240	140	19	98	6	EFS, 61	6	69	[48][a]
	PMCC (modified DFCI 91-01)	2000–2006	42	NR	18–35	L-asp	750,000	4	280	1170	60/body	98	3	DFS, 77	3	83	[31]
			85	37	18–60							89	3	DFS, 71	3	67	
	DFCI 01-175	2002–2008	74	28	18–50	L-asp	150,000 (adjust)	4	1120	1620	80/body	86	4	EFS,58	4	67	[49]
	GMALL 07/03	2003–2007	887	NR	15–35		NR	NR	NR	NR	NR	91	5	CRD, 61	5	65	[50]
	PETHEMA HR-ALL11	2003–2010	65	33	16–59	L-asp	140,000	20	1085	0	18	93	2	EFS,46	2	65	[51][b]
	GRAALL-2003/2005	2003–2011	502	24	15–35	L-asp	144,000	9	5460	0	40/body	97	5	EFS, 59	5	65	[52]
	NILG10/07	2007–2010	205	41	18–67		NR	5(T),2.5(B)	NR	NR	NR	83	5	DFS, 48	5	53	[53]
Full pediatric trial	FRALLE 2000-BT	2001–2011	89	NR	15–29		NR	NR	NR	NR	NR	99	5	EFS,61	5	66	[54]
	JALSG ALL202-U	2002–2009	139	19	16–24	L-asp	324,000	9.75	6020		46.5	94	5	DFS, 67	5	73	[32]
	UKALL2003	2003–2011	229	NR	16–24	PEG-asp	4000	ND	0	1198	52.5	NR	5	EFS, 72.3	5	76.4	[55]
	HOVON 70	2005–2007	54	26	17–40	L-asp	126,000	30	7280		42	91	2	EFS, 66	2	72	[56]
	MDACC-A-BFM	2006–2014	106	22	13–39	PEG-asp	17,500	1	1680	860	80/body	93	3	CRD, 70	5	60	[57]
	CALGB 10403	2007–2012	295	24	17–39	PEG-asp	20,000	ND	2520	1040	34.5	89	3	EFS, 59	3	73	[33]
	NOPHO 2008	2008–2014	1509	NR	1–45	PEG-asp	19,000 (IR)	35	1740(IR)	1070(IR)	36/body (IR)		5	EFS 85	5	91	[25]
			221	NR	18–45							NR	5	EFS, 74	5	78	

Asp asparaginase, *HD-MTX* high-dose methotrexate, *VCR* vincristine, *CR* complete remission, *EFS* event free survival, *DFS* disease free survival, *OS* overall survival, *ref.* reference, *Y* years, *NR* not reported, *ND* not done

[a]Only SR risk patients

[b]Only T-ALL(HR) patients

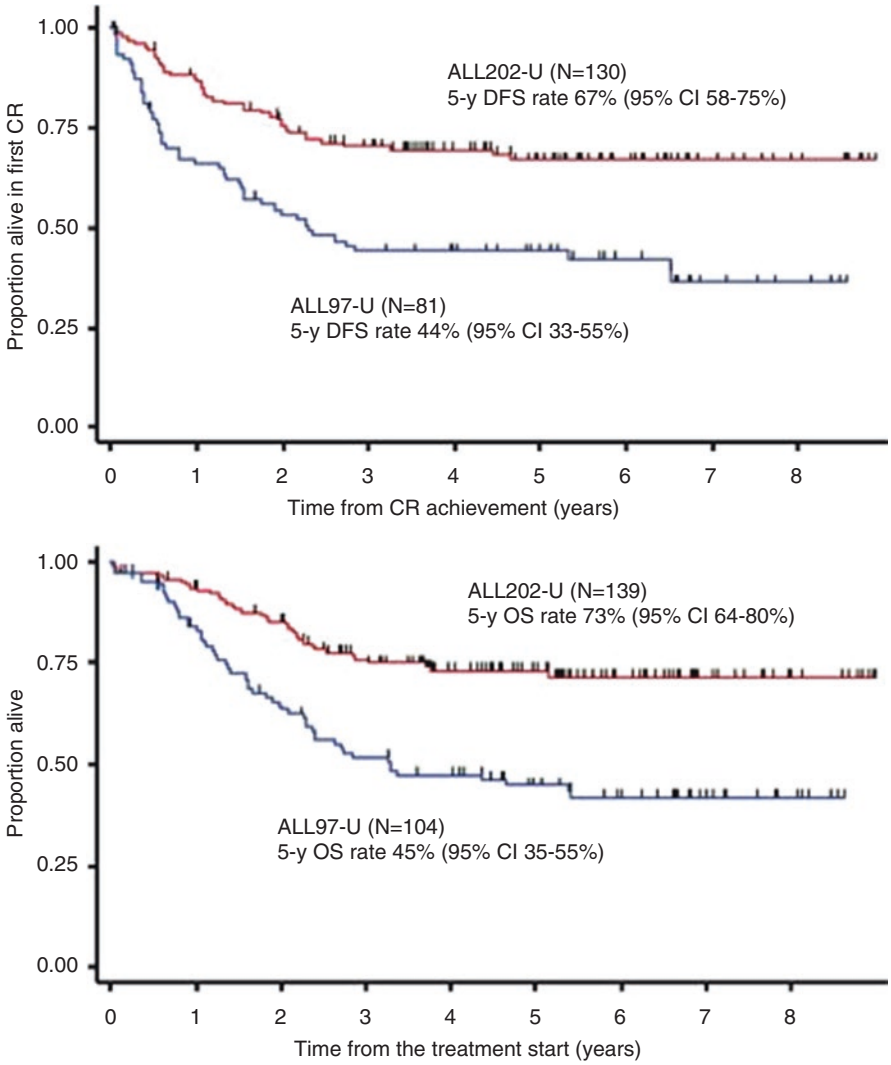


Fig. 12.2 Comparison of disease-free survival (DFS) and overall survival (OS) rates. (Top panel) Comparison of DFS rates between ALL202-U (red line) and ALL97-U (blue line). The median follow-up times were 5.1 and 5.2 years, respectively. (Bottom panel) Comparison of OS rates between ALL202-U (red line) and ALL97-U (blue line). The median follow-up times were 5.1 and 5.8 years, respectively [32]

those who did not, even in the high-risk group. Severe adverse events such as neutropenia, thrombocytopenia, febrile neutropenia, sepsis, hepatic toxicity, pancreatitis, and neuropathy occurred frequently during post-remission therapy. When the toxicities of ALL202-U and ALL97 protocol were compared, sepsis, hepatic toxicity, and neuropathy were more frequent in ALL202-U. However, these severe adverse events, except pancreatitis, occurred more frequently in pediatric patients

using same protocol therapy, and no patients died from the adverse events associated with chemotherapy during post-remission therapy in ALL202-U [32].

The recently published prospective Cancer and Leukemia Group B (CALGB) 10,403 trial included 295 patients age 17–39 years (median 24 years) with Ph negative ALL. The CALGB trial used chemotherapy regimen identical to those in the Children's Oncology Group study AALL0232 protocol. 3-year DFS rate was 66% and OS rate was 73%, both significantly higher than the historical control (3-year DFS, 48%; 3-year OS 58%) for CALGB patients aged 16–29 years. Overall treatment-related mortality was 3% in CALGB 10403 trial. Pretreatment risk factors associated with worse treatment outcomes included obesity and presence of the Ph-like gene expression signature [33].

NOPHO ALL2008 trial was performed for 1509 patients aged 1–45 years with Ph negative ALL. At 5 years, EFS rates were 89%, 80%, 74% for patients age 1–9 years (n = 1022), 10–17 years (n = 266), and 18–45 years (n = 221), respectively. There were significant differences only for non-high risk groups. The incidence of 19 specified toxicities, except for thrombosis, pancreatitis, and osteonecrosis, was not enhanced by age above 10 years [25].

These recent trials revealed the efficacy and feasibility of using a pediatric protocol for AYAs. It is difficult to determine which of these two strategies should be recommended due to the lack of randomized trial and the variability in all these regimens.

12.4 Novel Immunotherapies for AYA-ALL

12.4.1 Inotuzumab Ozogamicin

Inotuzumab ozogamicin (INO) is an anti-CD22 monoclonal antibody conjugated to calicheamicin. In Japan, it was approved for relapse or refractory (R/R) ALL in 2017. INO-VATE ALL trial, a large international phase III study, was performed to compare INO with standard intensive chemotherapy for adults (age 18–79 years, median 47 years) with R/R BCP-ALL. CR rates were 80.7% in INO group and 29.4% in standard therapy group, with higher percentage of MRD-negative cases in INO group (78% versus 28%). Duration of remission was also significantly longer in INO group than standard therapy group (5.0 versus 1.8 months). However, hepatotoxicity is an important non-hematologic toxicity of INO. Sinusoidal obstruction syndrome (SOS) occurred in 11% in INO group and in 1% in standard therapy group [34]. In INO group, CR rates and duration of remission were similar for those aged <55 years and those aged ≥55 years, but OS was longer for younger patients (median, 8.6 versus 5.6 months) [35]. The retrospective data from multiple international pediatric oncology centers that treated children with R/R BCP-ALL using INO were reported recently. A CR rate was 67%, and 71% of responders achieved MRD negativity. SOS occurred 52% of patients who underwent HSCT following INO [36].

12.4.2 Blinatumomab

Blinatumomab is a bispecific anti-CD3/anti-CD19 monoclonal antibody designed to engage and direct endogenous T cells to CD19-positive BCP-ALL leukemic blasts. In Japan, it was approved for R/R ALL in 2018. A large international phase III study was performed to compare blinatumomab with standard intensive chemotherapy for adults (age 18–80 years, mean 41 years) with R/R ALL. CR rates were 34% in the blinatumomab group and 16% in the chemotherapy group, with higher percentage of MRD-negative cases in response patients (76 versus 48%). OS was significantly longer in the blinatumomab group than in the chemotherapy group (7.7 versus 4.0 months). Duration of remission was longer in blinatumomab group than chemotherapy group (7.3 versus 4.6 months). While grade 3 or higher neutropenia or infection was lower with blinatumomab than with chemotherapy, grade 3 or higher cytokine release syndrome and neurologic toxicity was seen in 4.9% and 9.4% of patients with blinatumomab, respectively [37]. A recent study of blinatumomab for patients with B-ALL in CR with high MRD resulted in 91% MRD negativity after 1 cycle in 32 AYAs of age 18–34 years [38].

12.4.3 Chimeric Antigen Receptor T Cells

Cellular immunotherapy with CD19-directed chimeric antigen receptor (CAR) T cells is new promising approach for R/R ALL. In Japan, tisagenlecleucel of anti-CD19 CAR-T was approved in 2019. In international phase I/II study of tisagenlecleucel, 92 children and young adults with R/R CD19 positive BCP-ALL were enrolled, of whom 75 patients (age 3–23 years, median 11 years) received a single infusion. The overall remission rate was 81% with 100% MRD negativity. EFS and OS rates at 12 months were 50 and 76%, respectively. The CAR-T cells persisted as long as 20 months in the blood. The cytokine release syndrome and neurologic toxicity occurred in 77% of and 40% patients, respectively [39]. In recent study of CD19 CAR T-cell (expressing the 19-28z CAR) for adults with relapsed BCP-ALL, 53 patients (age 23–74 years, median 44 years) included 14 patients of age 18–30 years were enrolled. CR rates were 83% in whole cohort and 93% in patients of age 18–30 years. The median EFS and OS were 6.1 and 12.9 months, respectively. Greater incidence of severe cytokine release syndrome and neurotoxic events and shorter survival were observed in patients with a higher burden of disease (≥5% bone marrow blasts or extramedullary disease) [40].

12.5 Long-Term Complications in AYA-ALL Survivors

The improvement of survival in AYA-ALL by pediatric regimens has raised the need for monitoring late effects in this population. AYAs will spend the majority of their lives as cancer survivors, and they have significant risks for long-term complications,

second cancers, and accelerated development of usual age-related comorbid conditions [41]. Long-term toxicities of ALL therapy in AYAs include obesity, insulin resistance, dyslipidemia, venous thrombosis, cardiomyopathy, and cardiometabolic abnormalities [42, 43]. It is well-known fact that these symptoms are risk factors for cardiovascular disease. The Children's Oncology Group and Harmonization Group recommended to obtain an echocardiogram ≤2 years after completion of cardiotoxic therapy, repeat at 5 years after diagnosis, and continue every 5 years thereafter. AYA survivors also have risk of osteonecrosis. The overall incidence of osteonecrosis in patients with ALL is 1–5%, but there is a higher risk in AYAs [44].

Late comorbidities occur not only in physical conditions but also in mental conditions. The higher rates of depression, post-traumatic stress symptoms, fatigue, poor attention, and sexual dysfunction were shown in AYA cancer survivors in several reports [41, 45].

The Adolescent and Young Adult Health Outcome and Patient Experience (AYA HOPE) study included AYA-ALL patients is a population-based study of medical care, physical, and mental health outcomes for AYAs with cancer in the United States. They revealed that AYAs with cancer reported significantly worse physical and mental health than did similarly aged general and healthy population in health-related quality of life [46]. Moreover, the overall mortality rate for AYA survivors (age 20–24 years) was shown almost six-fold higher compared with the general population by British Columbia Cancer Registry [47].

12.6 Conclusion

Progress in the AYA-ALL has been remarkable, which is contributed by the understanding of pathophysiology and the adoption of pediatric protocols. The firstline introduction of immunotherapy in BCP-ALL, kinase inhibitors in Ph-like ALL, and nelarabine in T-ALL will further improve the outcome of AYA-ALL. There remains a critical lack of post-treatment survivorship care specific for AYA-ALL. The NCCN has developed AYA-specific guidelines that include topics relevant to the care of AYA patients diagnosed with cancer from the time of diagnosis through survivorship and/or palliative care. Involvement of multidisciplinary care teams trained in AYAs-specific needs will be important to further improve survival and quality of life.

References

1. Hunger SP, Mullighan CG. Acute Lymphoblastic leukemia in children. N Engl J Med. 2015;373(16):1541–52. https://doi.org/10.1056/NEJMra1400972.
2. Kantarjian BHM, Brien SO, Smith TL, Cortes J, Giles FJ, Beran M, et al. Results of treatment With hyper-CVAD, a dose-intensive regimen, in adult Acute lymphocytic leukemia. J Clin Oncol. 2000;18(3):547–61.

3. Stock W, Johnson JL, Stone RM, Kolitz JE, Powell BL, Wetzler M, et al. Dose intensification of daunorubicin and cytarabine during treatment of adult acute lymphoblastic leukemia: results of cancer and leukemia group B study 19802. Cancer. 2013;119(1):90–8.
4. Pulte D, Gondos A, Brenner H. Improvement in survival in younger patients with acute lymphoblastic leukemia from the 1980s to the early 21st century. Blood. 2009;113(7):1408–11.
5. Pulte D, Gondos A, Brenner H. Trends in 5- and 10-year survival after diagnosis with childhood hematologic malignancies in the United States, 1990–2004. J Natl Cancer Inst. 2008;100(18):1301–9.
6. Trama A, Botta L, Foschi R, Ferrari A, Stiller C, Desandes E, et al. Survival of European adolescents and young adults diagnosed with cancer in 2000–07: population-based data from EUROCARE-5. Lancet Oncol. 2016;17(7):896–906. https://doi.org/10.1016/S1470-2045(16)00162-5.
7. Harrison CJ. Cytogenetics of paediatric and adolescent acute lymphoblastic leukaemia. Br J Haematol. 2009;144(2):147–56.
8. Roberts KG. Genetics and prognosis of ALL in children vs adults. Hematology Am Soc Hematol Educ Program. 2018;2018(1):137–45. http://www.ncbi.nlm.nih.gov/pubmed/30504302%0A, http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC6245970
9. Reismüller B, Steiner M, Pichler H, Dworzak M, Urban C, Meister B, et al. High hyperdiploid acute lymphoblastic leukemia (ALL)—a 25-year population-based survey of the Austrian ALL-BFM (Berlin-Frankfurt-Münster) study group. Pediatr Blood Cancer. 2017;64(6):1–8.
10. Moorman AV, Chilton L, Wilkinson J, Ensor HM, Bown N, Proctor SJ. A population-based cytogenetic study of adults with acute lymphoblastic leukemia. Blood. 2010; 115(2):206–14.
11. Burmeister T, Gökbuget N, Schwartz S, Fischer L, Hubert D, Sindram A, et al. Clinical features and prognostic implications of TCF3-PBX1 and ETV6-RUNX1 in adult acute lymphoblastic leukemia. Haematologica. 2010;95(2):241–6.
12. Bassan R, Bourquin JP, DeAngelo DJ, Chiaretti S. New approaches to the management of adult acute lymphoblastic leukemia. J Clin Oncol. 2018;36(35):3504–19.
13. Mullighan CG, Xiaoping S, Zhang J, Radtke I, Phillips LAA, Miller CB, Ma J, Liu W, Cheng C, Schulman BA, Harvey RC, Chen I-M, Clifford RJ, Carroll WL, Reaman G, et al. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N Engl J Med. 2009;360(5):470–80.
14. Roberts KG, Li Y, Payne-Turner D, Harvey RC, Yang Y-L, Pei D, et al. Targetable kinase-activating lesions in Ph-like Acute Lymphoblastic leukemia. N Engl J Med. 2015;371(11):1005–15.
15. Roberts KG, Gu Z, Payne-Turner D, McCastlain K, Harvey RC, Chen IM, et al. High frequency and poor outcome of Philadelphia chromosome-like Acute Lymphoblastic leukemia in adults. J Clin Oncol. 2017;35(4):394–401.
16. Reshmi SC, Harvey RC, Roberts KG, Stonerock E, Smith A, Jenkins H, et al. Targetable kinase gene fusions in high-risk B-ALL: a study from the Children's oncology group. Blood. 2017;129(25):3352–61.
17. Boer JM, Koenders JE, Van Der Holt B, Exalto C, Sanders MA, Cornelissen JJ, et al. Expression profiling of adult acute lymphoblastic leukemia identifies a BCR-ABL1-like subgroup characterized by high non-response and relapse rates. Haematologica. 2015;100(7):e261–4.
18. Boer JM, Marchante JRM, Evans WE, Horstmann MA, Escherich G, Pieters R, et al. BCR-ABL1-like cases in pediatric acute lymphoblastic leukemia: a comparison between DCOG/ErasmusMC and COG/St.Jude signatures. Haematologica. 2015;100:e354–7.
19. Tran TH, Loh ML. Ph-like acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program. 2016;2016(1):561–6.
20. Suzuki K, Okuno Y, Kawashima N, Muramatsu H, Okuno T, Wang X, et al. MEF2D-BCL9 fusion gene is associated with high-risk acute B-cell precursor lymphoblastic leukemia in adolescents. J Clin Oncol. 2016;34(28):3451–9.

21. Li J-F, Dai Y-T, Lilljebjörn H, Shen S-H, Cui B-W, Bai L, et al. Transcriptional landscape of B cell precursor acute lymphoblastic leukemia based on an international study of 1,223 cases. Proc Natl Acad Sci. 2018;115(50):E11711–20. https://doi.org/10.1073/pnas.1814397115.
22. Hirabayashi S, Ohki K, Nakabayashi K, Ichikawa H, Momozawa Y, Okamura K, et al. ZNF384-related fusion genes define a subgroup of childhood B-cell precursor acute lymphoblastic leukemia with a characteristic immunotype. Haematologica. 2017;102(1):118–29.
23. Iacobucci I, Mullighan CG. Genetic basis of acute lymphoblastic leukemia. J Clin Oncol. 2017;35(9):975–83.
24. Hunger SP, Lu X, Devidas M, Camitta BM, Gaynon PS, Winick NJ, 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. 2012;30(14):1663–9.
25. Toft N, Birgens H, Abrahamsson J, Griškevic Ius L, Hallböök H, Heyman M, et al. Results of NOPHO ALL2008 treatment for patients aged 1–45 years with acute lymphoblastic leukemia. Leukemia. 2018;32(3):606–15.
26. Stock W, La M, Sanford B, Bloomfield CD, Vardiman JW, Gaynon P, et al. What determines the outcomes for adolescents and young adults with acutelymphoblastic leukemia treated on cooperative group protocols? A comparison of Children's Cancer Group and Cancer and Leukemia Group B studies. Blood. 2008;112(5):1646–54.
27. Testi AM, Valsecchi MG, Conter V, Vignetti M, Paoloni F, Giona F, et al. Difference in outcome of Adolescents with acute lymphoblastic leukemia (ALL) enrolled in pediatric (AIEOP) and adult (GIMEMA) protocols. Blood. 2004;104(11):1954 LP. http://www.bloodjournal.org/content/104/11/1954.abstract
28. Ramanujachar R, Richards S, Hann I, Goldstone A, Mitchell C, Vora A, Rowe J, Webb D. Adolescents With Acute Lymphoblastic Leukaemia: Outcome on UK National Paediatric (ALL97) and adult(UKALLXII/E2993) trials. Pediatr Blood Cancer. 2007;48:254–61.
29. Boissel N, Auclerc MF, Lhéritier V, Perel Y, Thomas X, Leblanc T, et al. Should adolescents with acute lymphoblastic leukemia be treated as old children or young adults? Comparison of the French FRALLE-93 and LALA-94 trials. J Clin Oncol. 2003;21(5):774–80.
30. Huguet F, Leguay T, Raffoux E, Thomas X, Beldjord K, Delabesse E, et al. Pediatric-inspired therapy in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia: the GRAALL-2003 study. J Clin Oncol. 2009;27(6):911–8.
31. Storring JM, Minden MD, Kao S, Gupta V, Schuh AC, Schimmer AD, et al. Treatment of adults with BCR-ABL negative acute lymphoblastic leukaemia with a modified paediatric regimen. Br J Haematol. 2009;146(1):76–85.
32. Hayakawa F, Sakura T, Yujiri T, Kondo E, Fujimaki K, Sasaki O, et al. Markedly improved outcomes and acceptable toxicity in adolescents and young adults with acute lymphoblastic leukemia following treatment with a pediatric protocol: a phase II study by the Japan adult leukemia study group. Blood Cancer J. 2014;4(10):e252–9. https://doi.org/10.1038/bcj.2014.72.
33. Stock W, Luger SM, Advani AS, Yin J, Harvey RC, Mullighan CG, et al. A pediatric regimen for older adolescents and young adults with acute lymphoblastic leukemia: results of CALGB 10403. Blood. 2019;133(14):1548–59.
34. Kantarjian HM, DeAngelo DJ, Stelljes M, Martinelli G, Liedtke M, Stock W, et al. Inotuzumab Ozogamicin versus standard therapy for Acute Lymphoblastic leukemia. N Engl J Med. 2016;375(8):740–53. https://doi.org/10.1056/NEJMoa1509277.
35. Jabbour EJ, DeAngelo DJ, Stelljes M, Stock W, Liedtke M, Gökbuget N, et al. Efficacy and safety analysis by age cohort of inotuzumab ozogamicin in patients with relapsed or refractory acute lymphoblastic leukemia enrolled in INO-VATE. Cancer. 2018;124(8):1722–32.
36. Bhojwani D, Sposto R, Shah NN, Rodriguez V, Yuan C, Stetler-Stevenson M, et al. Inotuzumab ozogamicin in pediatric patients with relapsed/refractory acute lymphoblastic leukemia. Leukemia. 2019;33(4):884–92. https://doi.org/10.1038/s41375-018-0265-z.
37. Kantarjian H, Stein A, Gökbuget N, Fielding AK, Schuh AC, Ribera J-M, et al. Blinatumomab versus chemotherapy for advanced Acute Lymphoblastic leukemia. N Engl J Med. 2017;376(9):836–47. https://doi.org/10.1056/NEJMoa1609783.

38. Gökbuget N, Dombret H, Bonifacio M, Reichle A, Graux C, Faul C, et al. Blinatumomab for minimal residual disease in adults with B-cell precursor acute lymphoblastic leukemia. Blood. 2018;131(14):1522–31.
39. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in children and young adults with B-cell Lymphoblastic leukemia. N Engl J Med. 2018;378(5):439–48. http://www.ncbi.nlm.nih.gov/pubmed/29385370%0A, http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5996391
40. Park JH, Rivière I, Gonen M, Wang X, Sénéchal B, Curran KJ, et al. Long-term follow-up of CD19 CAR therapy in Acute Lymphoblastic leukemia. N Engl J Med. 2018;378(5):449–59. https://doi.org/10.1056/NEJMoa1709919.
41. Tai E, Buchanan N, Townsend J, Fairley T, Moore A, Richardson LC. Health status of adolescent and young adult cancer survivors. Cancer. 2012;118(19):4884–91. http://www.ncbi.nlm.nih.gov/pubmed/22688896%0A, http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5292773
42. Salvador C, Meister B, Crazzolara R, Kropshofer G. Management of hypertriglyceridemia in children with acute lymphoblastic leukemia under persistent therapy with glucocorticoids and l-asparaginase during induction chemotherapy. Pediatr Blood Cancer. 2012;59(4):771.
43. Armenian SH, Hudson MM, Mulder RL, Chen MH, Constine LS, Dwyer M, et al. Recommendations for cardiomyopathy surveillance for survivors of childhood cancer: a report from the international late effects of childhood cancer guideline harmonization group. Lancet Oncol. 2015;16(3):e123–36. https://doi.org/10.1016/S1470-2045(14)70409-7.
44. Vora A. Management of osteonecrosis in children and young adults with acute lymphoblastic leukaemia. Br J Haematol. 2011;155(5):549–60.
45. Salsman JM, Garcia SF, Yanez B, Sanford SD, Snyder MA. Victorson D. physical, emotional, and social health differences between post-treatment young adults with cancer and matched healthy controls. Cancer. 2014;120(15):2247–54. http://www.ncbi.nlm.nih.gov/pubmed/24888335%0A, http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4121054
46. Smith AW, Keegan T, Hamilton A, Lynch C, Wu XC, Schwartz SM, et al. Understanding care and outcomes in adolescents and young adult with cancer: a review of the AYA HOPE study. Pediatr Blood Cancer. 2019;66(1):1–7.
47. Zhang Y, Goddard K, Spinelli JJ, Gotay C, McBride ML. Risk of late mortality and second malignant neoplasms among 5-year survivors of young adult cancer: a report of the childhood, adolescent, and young adult cancer survivors research program. J Cancer Epidemiol. 2012;2012:1–11.
48. Ribera JM, Oriol A, Sanz MA, Tormo M, Fernández-Abellán P, Del Potro E, et al. Comparison of the results of the treatment of adolescents and young adults with standard-risk acute lymphoblastic leukemia with the programa Español de tratamiento en hematología pediatric-based protocol ALL-96. J Clin Oncol. 2008;26(11):1843–9.
49. DeAngelo DJ, Stevenson KE, Dahlberg SE, Silverman LB, Couban S, Supko JG, et al. Long-term outcome of a pediatric-inspired regimen used for adults aged 18–50 years with newly diagnosed acute lymphoblastic leukemia. Leukemia. 2015;29(3):526–34.
50. Gokbuget N, Beck J, Brandt K, Bruggemann M, Burmeister T, Diedrich H, et al. Significant improvement of outcome in adolescents and young adults (AYAs) aged 15-35 years with acute lymphoblastic leukemia (ALL) with a pediatric derived adult ALL protokol; results of 1529 AYAs in 2 consecutive trial of the German multicenter study group for adult ALL (GMALL). Blood. 2013;122(21):abst 839.
51. Barba P, Morgades M, Montesinos P, Gil C, Fox ML, Ciudad J, et al. Increased survival due to lower toxicity for high-risk T-cell acute lymphoblastic leukemia patients in two consecutive pediatric-inspired PETHEMA trials. Eur J Haematol. 2019;102(1):79–86.
52. Dombret H, Cluzeau T, Huguet F, Boissel N. Pediatric-like therapy for adults with ALL. Curr Hematol Malig Rep. 2014;9(2):158–64.

53. Bassan R, Masciulli A, Intermesoli T, Spinelli O, Tosi M, Pavoni C, et al. Final results of northern Italy leukemia group (NILG) trial 10/07 combining pediatric-type therapy with minimal residual disease study and risk-oriented hematopoietic cell transplantation in adult acute lymphoblastic leukemia (ALL). Blood. 2016;128(21):abst 176.
54. Cluzeau T, Dhedin N, Huguet F, Raffoux E, Maury S, Mannone L, et al. Dose-intensity impacts on survival of adolescents and young adults with acute lymphoblastic leukemia treated in adult departments by a pediatric protocol (FRALLE 2000BT). Blood. 2012;120(21):abst 3561.
55. Hough R, Rowntree C, Goulden N, Mitchell C, Moorman A, Wade R, et al. Efficacy and toxicity of a paediatric protocol in teenagers and young adults with Philadelphia chromosome negative acute lymphoblastic leukaemia: results from UKALL 2003. Br J Haematol. 2016;172(3):439–51.
56. Rijneveld AW, van der Holt B, Daenen SMGJ, Biemond BJ, de Weerdt O, Muus P, et al. Intensified chemotherapy inspired by a pediatric regimen combined with allogeneic transplantation in adult patients with acute lymphoblastic leukemia up to the age of 40. Leukemia. 2011;25(11):1697–703. https://doi.org/10.1038/leu.2011.141.
57. Rytting ME, Jabbour EJ, Jorgensen JL, Ravandi F, Franklin AR, Kadia TM, et al. Final results of a single institution experience with a pediatric-based regimen, the augmented Berlin–Frankfurt–Münster, in adolescents and young adults with acute lymphoblastic leukemia, and comparison to the hyper-CVAD regimen. Am J Hematol. 2016;91(8):819–23.

Chapter 13
Relapsed Pediatric ALL

Ayumu Arakawa

Abstract Although the survival of children with acute lymphoblastic leukemia has considerably improved in the previous two decades, 15–20% of patients experience subsequent relapse. Immunophenotype, duration of first complete remission, and site of relapse are the most widely accepted risk factors used for patient stratification in pediatric relapsed ALL. Patients with bone marrow (BM) relapse of T-ALL or very early or early BM relapse of BCP-ALL receive multi-drug chemotherapy followed by hematopoietic stem cell transplantation (HSCT), while those with late BM relapse of BCP-ALL and negative minimal residual disease after re-induction undergo about 2 years of chemotherapy and can be treated without HSCT. Patients with late BM relapse of BCP-ALL who have poor minimal residual disease (MRD) response after re-induction are scheduled to receive HSCT at the time of second remission. Many novel agents for pediatric relapsed ALL have been developed in the previous decades.

Keywords Pediatric relapsed acute lymphoblastic leukemia · Risk classification Minimal residual disease · Hematological stem cell transplantation Immunotherapy · Molecular targeted drug · Anti-CD19 chimeric-antigen receptor T-cell

13.1 Introduction

Although survival in children with acute lymphoblastic leukemia has considerably increased in the previous two decades, and event-free survival (EFS) rates of 85%–90% have been achieved in the first complete remission (CR) with multi-drug chemotherapy [1–3], 15–20% of the patients experience from subsequent relapse. At relapse, about 40% of the patients can be treated successfully with intensive

A. Arakawa (✉)
Department of Pediatric Oncology, National Cancer Center Hospital, Chuo-ku, Tokyo, Japan

M. Kato (ed.), *Pediatric Acute Lymphoblastic Leukemia*,
https://doi.org/10.1007/978-981-15-0548-5_13

multi-drug chemotherapy and hematopoietic stem cell transplantation (HSCT) [4–6]. However, about 60% of pediatric ALL patients die because of subsequent relapse or treatment-related complications; the introduction of new agents, including cellular therapies, antibodies, and molecular targeted drugs are required to improve outcomes. In this chapter, we discuss the current treatment status for relapsed pediatric ALL with a focus on novel therapeutic options that have different mechanisms of actions.

13.2 Prognostic Factors and Risk Stratification for Relapsed ALL

13.2.1 Clinical Prognostic Factors

Several clinical, laboratory, and molecular risk factors are helpful and are used in the risk stratification at initial diagnosis; however, few have been applied in relapsed patient. Immunophenotype, duration of first CR, and site of relapse are three clinical characteristics that are most widely accepted as risk factors for patient stratification in recent treatment protocols. Although other clinical factors, such as age (>10 years), National Cancer Institute Criteria high risk, male sex, and central nerve system (CNS) involvement are associated with poorer response in a Children's Oncology Group (COG) study; [7] these characteristics are not used in the stratification [8].

In terms of the definition for time point of relapse, slight differences are observed as per the study groups. Relapses occurring before 18 months from diagnosis were considered very early relapses in the Berlin–Frankfurt–Münster (BFM) stratification; early relapses were those occurring from 18 months from first remission to before 6 months of completion of primary therapy, and late relapses were those that occurred ≥6 months after the completion of primary therapy. In the COG stratification, early bone marrow (BM) relapses were those occurring <36 months from the date of diagnosis; late BM relapse was that occurring ≥36 months after the diagnosis, and isolated EM relapses were divided into early and late relapses using the cut-off point of 18 months after achieving first CR. Early relapses behave aggressively and are associated with poorer outcome (survival <30–40%), while late relapses have a much higher chance of cure (survival >50%) [9]. T-cell phenotype relapsed ALL is associated with a poor prognosis with only 3–5 year survival in 10–30% of the subjects, partly owing to the more aggressive characteristics of T-cell ALL as compared to that of BCP-ALL and higher rate of early relapse in T-cell ALL [10, 11]. In terms of the relapse site, isolated BM relapse historically has the worst prognosis; isolated CNS, testicular, or other extramedullary (EM) relapse has better prognosis, and isolated extramedullary relapse exhibits the best prognosis among these three types (5-year EFS, 24%, 39%, and 59%, retrospectively, in a retrospective COG study) [7, 12–16].

13.2.2 Risk Stratification for Pediatric Relapsed ALL

The COG and BFM study groups have developed formal criteria using time point of relapse and site of relapse for risk stratification to identify patients for whom HSCT might be needed once second remission is achieved after multi-agent re-induction therapy (Table 13.1). The BFM study group classified first-relapse ALL into four risk categories (S classification); S1/S2 is considered the standard-risk group, and S3/S4 is considered the high-risk group. The 5-year overall survival of S1, S2, S3, and S4 were reported to be 60–70%, 60%, 30%, and 25%, respectively [17]. In contrast, the COG study group classified into the following three risk groups: low, intermediate, and high. The BFM study group classified T-cell relapse as being at a higher risk than BCP-ALL and separated these two types in their stratification, while the COG group did not separate T-cell and BCP-ALL in their classification. After several national phase III trials having been performed during the previous three decades all over Europe, an international study for treatment of childhood relapsed ALL (IntReALL) was organized in 2010; more than 20 countries, including Japan have participated in this ongoing study. The IntReALL 2010 study has divided patients into the standard-risk (SR) group and the high-risk (HR) group. Given that the treatment results of very early EM relapse that used to be categorized into S2 were unsatisfactory, this group is now categorized into the HR group in the IntReALL 2010 study.

13.2.3 Molecular Risk Factors

In addition to clinical prognostic factors, molecular markers are related to worse outcomes after relapse. ETV-RUNX1 positive ALL that has excellent outcomes at initial diagnosis mostly involves late relapse, and 80% of the cases of late relapse of ETV-RUNX1 positive ALL are cured successfully, thus, indicating good prognosis [18].

Based on the analyses of the ALL-REZ BFM 2002 study, deletion of IKZF1 and TP53 mutation was identified as a poor prognostic factor after relapse [19, 20]. The United kingdom (UK) study group has reported that TP53 alterations and NR3C1/BTG1 deletions are associated with a higher risk of progression, and NRAS mutations in high hyperdiploidy were associated with a poor outcome in pediatric relapsed BCP-ALL [21]. They propose an integration of the genetic and clinical risk factors for risk stratification instead of the current stratification that is based only on clinical information.

13.2.4 Minimal Residual Disease (MRD) after Re-Induction Therapy

Early response to induction therapy, evaluated as a MRD, with molecular techniques or through flow-cytometry has proven to have high prognostic significance in frontline therapy for pediatric ALL [22–25]. MRD has been shown to be a highly

Table 13.1 Risk classification of first-relapse ALL

		Immunophenotype: non-T			Immunophenotype: T		
		Isolated EM	Combined	Isolated BM	Isolated EM	Combined	Isolated BM
BFM S classification							
Very early	<18 months after primary diagnosis and <6 months after completion of primary therapy	S2	S4	S4	S2	S4	S4
Early	≥18 months after primary diagnosis and <6 months after completion of primary therapy	S2	S2	S3	S2	S4	S4
Late	≥6 months after completion of primary therapy	S1	S2	S2	S1	S4	S4
IntReALL classification							
Very early	<18 months after primary diagnosis and <6 months after completion of primary therapy	High risk	High risk	High risk	High risk	High risk	High risk
Early	≥18 months after primary diagnosis and <6 months after completion of primary therapy	Standard risk	Standard risk	High risk	Standard risk	High risk	High risk
Late	≥6 months after completion of primary therapy	Standard risk	Standard risk	Standard risk	Standard risk	High risk	High risk
COG classification							
Early	<36 months after primary diagnosis for combined/isolated BM <18 months after achieving CR1 for isolated EM	Intermediate	High	High	Intermediate	High	High
Late	≥36 months after primary diagnosis for combined/isolated BM ≥18 months after achieving CR1 for isolated EM	Low	Intermediate	Intermediate	Low	Intermediate	Intermediate

BFM Berlin–Frankfurt–Münster, *BM* bone marrow, *COG* Children's Oncology Group, *EM* extramedullary, *IntReALL* international study for treatment of childhood relapsed ALL, *CR1* first complete remission

predictive risk factor in pediatric relapsed ALL [26–29], and MRD response is now used to determine the indication of hematological stem cell transplantation (HSCT) in patients achieving second CR. MRD before allogenic transplantation is a strong prognostic factor for high risk of disease recurrence after HSCT [30].

13.3 Treatment of Relapsed Pediatric ALL

The algorithm in Fig. 13.1 gives an overview of our treatment strategy for first relapse of ALL.

13.3.1 Treatment of Isolated and Combined BM Relapse

There is slight difference in the time point of relapse defined by major study groups; therefore, we use the definition from the BFM classification in this section. In general, patients with BM relapse of T-ALL or very early or early BM relapse of BCP-ALL receive multi-drug chemotherapy followed by HSCT, while those with late BM relapse of BCP-ALL receive about 2 years of chemotherapy, including intrathecal chemotherapy and oral maintenance therapy [16]. Indication of HSCT in patients with late BM relapse of BCP-ALL is decided based on the MRD response after re-induction chemotherapy [6, 31].

Standard re-induction regimens for pediatric relapsed ALL basically comprise different combinations of the same drugs used in frontline therapies and normally

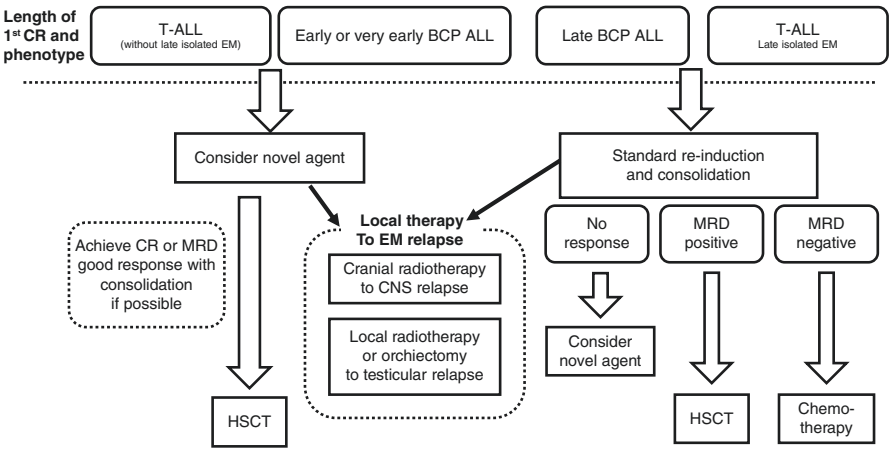


Fig. 13.1 Algorithm for treatment of pediatric relapsed acute lymphoblastic leukemia (ALL)

involve a combination of vincristine, glucocorticoid (prednisolone or dexamethasone), and asparaginase, plus anthracycline, methotrexate, or cytarabine in varying doses and schedules. Multiple studies have shown that remission can be accomplished in >70% of early relapse and >90% of late relapse cases after standard re-induction therapy [9]. After achieving CR, MRD assessment after re-induction therapy is an essential and defining factor that influences the outcome in patients with late BM relapse of BCP-ALL. In the ALL-REZ BFM P95/96 study, 10 year EFS of early/late combined BM relapse or late isolated BM relapse of BCP-ALL with negative MRD ($<10^{-3}$ cells) after re-induction was 76%, while the 10-year EFS rate of patients with positive MRD was 18% [26]. Based on this result, the subsequent ALL-REZ BFM 2002 study allocated these patients with MRD poor response to allogenic HSCT post consolidation, and the survival rate in this subgroup improved to 64% that was not significantly different from the survival rate of 70% in patients with negative MRD [31]. In the UKALL R3 study, the cut-off limit was 10^{-4} cells because duration re-induction therapy is longer, and the intensity of chemotherapy is stronger than that in the REZ-BFM protocol. Among first-relapse patients with late isolated or combined BM relapse, MRD-positive patients at the end of re-induction therapy underwent HSCT, and the 6-year OS was 64%. MRD-negative patients were allocated to receive chemotherapy and achieved good outcomes, with a 6-year OS of 87% [6, 27]. These studies proved that HSCT is not necessary for patients with late BM relapse of BCP-ALL if patients achieve good MRD response after re-induction chemotherapy; further, patients with poor MRD response can be treated with subsequent HSCT after consolidation. In the current IntReALL 2010 SR study, randomizing between the UK and BFM approaches, the MRD levels have been retained as per the protocol that was used to stratify for allo-SCT.

Although several trials have investigated various combinations of intensified chemotherapy, the prognosis in patients with first BM relapse of T-ALL or very early or early BM relapse of BCP-ALL remains poor, and the EFS rate remains 30% [4, 10, 32]. Patients with multiple relapse have a lower chance of cure and remission with every subsequent relapse. In a retrospective review from North America, the 5-year disease-free survival in pediatric ALL patients after the second and third relapse was 27% and 15%, respectively [33]. In contrast, the result of the AIEOP REC 2003 study by an Italian group showed that these patients can be cured if MRD good response is obtained after re-induction therapy before HSCT [28]. Re-induction therapy comprises fludarabine, cytarabine plus liposomal doxorubicin, or cytarabine, and idarubicin. The 3-year EFS rate in patients with negative MRD ($<10^{-4}$ cells) was 73%, although the proportion of patients with negative MRD was small (15%). Similarly, in the REZ2002 study and UKALL R3, around one-third of the patients with early or very early BM relapse who achieved negative MRD after re-induction had an EFS rate of 60% and 63%, respectively, compared to 31% and 21%, respectively, in patients with positive MRD [6, 31]. This result highlights that the selection of effective re-induction therapy to achieve good MRD response is crucial for successful treatment.

This subgroup may have chemotherapy-resistant leukemic blasts; therefore, further intensification of known conventional chemotherapy is not realistic, and the introduction of novel therapies, including immunotherapy and molecular targeted drugs is expected. In the section "13.4. Novel therapies for relapsed pediatric ALL", choices and current development situation of new agents are reviewed briefly.

13.3.2 Treatment of Isolated Extramedullary Relapse

With advances in the method of detecting submicroscopic BM involvement, extramedullary relapse of ALL has been detected as rarely, truly "isolated" [16]. In one study on 64 patients with apparent isolated extramedullary relapse, 47 patients (73%) had detectable BM involvement ($\geq 10^{-4}$), and the 5-year EFS with and without detectable BM involvement was 60% and 30%, respectively [34].

The CNS is the most frequent extramedullary relapse site, and about 0.6–5% of ALL cases exhibit a CNS relapse [35]. Similar to BM relapse, the time to relapse was also a prognostic factor in patients with CNS relapse. In the retrospective analysis from COG data, 5-year survival after early (<18 months), intermediate (18–36 months), late (≥36 months) isolated CNS relapse was 43%, 68%, and 78%, respectively [7].

Radiotherapy together with systemic and intrathecal chemotherapy is a standard therapy for CNS relapse of ALL. For the minimization of long-term neurocognitive sequelae, whether the reduction of irradiation dose is possible without worsening the outcome is an area of concern. In a Pediatric Oncology Group (POG) 9412 study, isolated CNS relapse ALL patients who relapsed 18 months or more after the first CR were treated with a combination of chemotherapy and reduced cranial irradiation (18 Gy, cranial only) compared to that in a previous POG trial (24 Gy cranial or 15 Gy spinal), and this group achieved a 4-year EFS rate of 78% [36]. In contrast, patients who relapsed <18 months from the first CR had a 4-year EFS rate of only 52%. In the ALL-R3 study, early and very early isolated CNS relapse patients were recommended to undergo HSCT. Among the patients with CNS relapse who were eligible but did not undergo HSCT, 13 patients (76.5%) had a subsequent relapse [37]. In sum, late isolated CNS relapse patients can be successfully treated with a combination of systematic chemotherapy and radiation, and methods to further reduce the irradiation dose should be investigated in prospective trials. The outcomes of early and very early isolated CNS relapse should be improved, and these groups may be indicated for HSCT [17]. The earlier COG study did not show any clinical advantage of HSCT over chemotherapy and cranial irradiation only [38]; therefore, further research is necessary to determine a clear indication of HSCT in isolated CNS relapse patients.

Testis is the second most-common extramedullary relapse site in boys with ALL, and early testicular relapse and bilateral involvement are adverse prognostic

factors [16]. In combination with chemotherapy, orchitectomy of the involved testis and/or bilateral testicular irradiation (including clinically normally testes) has been generally used [10, 39, 40]. There are no data supporting one approach over the other, although orchitectomy may provide a greater chance for eradication of testicular involvement [17]. Similar to that of CNS relapse, submicroscopic BM involvement was found in 57% of the patients with isolated testicular relapse [34]; therefore, systemic chemotherapy is an essential component for this subgroup. In order to preserve fertility and Leydig cell function for spontaneous pubertal development, attempts have been made to reduce or avoid testicular irradiation in some patients. As optimum dose of either therapeutic radiotherapy for involved testis or prophylactic radiotherapy for contralateral testis has not been confirmed, further research is required to determine the adequate dose of irradiation.

13.3.3 Role of Transplantation in Relapsed Pediatric ALL

Several studies have shown the benefit of HSCT over chemotherapy in patients with T-ALL, early BM relapse, or very early BM relapse in BCP-ALL [13, 41–43]. In the pair-matched analysis from REZ-BFM, the 5-year EFS rate was significantly better with HSCT from unrelated donor in patients with T-ALL, early BM relapse, or very early BM relapse in BCP-ALL than with chemotherapy (44% vs. 0%), while no difference was observed in patients with late BM relapse of BCP-ALL(39% vs. 49%) [43].

As mentioned above, patients with late BM relapse of BCP-ALL with poor MRD response after re-induction are allocated to receive HSCT in the second remission. Although the indication of transplantation for isolated EM relapse has not been confirmed in a prospective study, early or very early EM relapse of both BCP and T-ALL are considered to be allocated to transplantation [17] based on the poor outcome of these subgroups when treated with chemotherapy only. In addition, it is important to reduce the tumor burden before HSCT to the level of MRD negativity because in the retrospective analysis of the REZ-BFM group, patients with positive MRD ($\geq 10^{-4}$) had a significantly lower probability of EFS (pEFS) of 27% as compared to those with negative MRD (10^{-4}) who had pEFS of 60% ($p = 0.004$) [30]. In patients who do not achieve negative MRD, transplantation can still benefit a small subset of patients with positive MRD [44] and occasionally, patients with re-induction failure [16, 45]. HSCT from an HLA-haploidentical relative offers an immediate transplant option for patients who do not have a matched donor or suitable cord blood and makes it possible to identify a donor for nearly all patients [46–48]. Details on the choices of donor and pre-conditioning treatment for pediatric ALL are described in Chap. 15.

13.4 Novel Therapies for Pediatric Relapsed ALL

The choice of effective drugs against pediatric relapsed ALL has remained almost the same for several years; currently, many novel agents, including molecular targeted drugs, immunotherapy, and nucleoside analogs have been developed in the last decades or are under investigation (Table 13.2). Among them, novel agents that are reported to be effective in pediatric trials or in studies with a relatively large number of pediatric relapsed ALL patients are briefly reviewed in the next section.

Table 13.2 Novel therapies for treatment of pediatric relapsed acute lymphoblastic leukemia

	Drug properties	Study design	Number of patients	Response rate
Immunotherapy				
Blinatumomab	Aniti-CD19 Bi-specific T-cell engagers	Phase I/II [49]	Phase I 49 Phase II 43	39% (within 2 cycles)
Inotuzumab	Anti-CD22 monoclonal Antibody conjugated to Calicheamycin	Compassionate use Program [50]	51	67%
Tisagenlecleucel	Anti-CD19 CAR-T-cell	Phase II [51]	75	81%
Molecular targeted drugs and cytotoxic drugs				
Bortezomib	Proteasome inhibitor	Phase II (TACL) [52]	22	73%
		Phase II (COG) [53]	135	68% (BCP-ALL, $n = 100$) 68% (T-ALL, $n = 22$)
Clofarabine	Second-generation purine nucleoside analog	Phase II (single agent) [54]	61	30%
		Phase II (Clo + VP16 + Cy) [55]	25	44%
Nelarabine	Inihibitor of purine nucleoside phospharylase	Phase II (single agent) [56]	12	33%
		Treatment experience (Nelarabine+VP-16 + Cy) [57]	7 (T-ALL+T-LL)	57% (4/7 Pts)

CAR chimeric antigen receptor, *TACL* Therapeutic Advances in Childhood Leukemia & lymphoma, *COG* Children's Oncology Group, *BCP-ALL* B-cell precursor acute lymphoblastic leukemia, *Clo* clofarabine, *VP16* etoposide, *Cy* cyclophosphamide, *T-ALL* T-cell acute lymphoblastic leukemia, *T-LL* T-cell lymphoblastic lymphoma

13.4.1 Immunotherapy

Figure 13.2 provides an overview of representative novel immunotherapeutic drugs for relapse or refractory ALL.

Blinatumomab is a bispecific T-cell engager antibody construct with dual specificity for CD19 and CD3 [58]. Blinatumomab directs CD3-positive effector memory T-cells to CD19-positive target cells, triggering T-cell-mediated serial lysis of normal and malignant B cells [59, 60]. In a large phase II study on adults with relapsed/refractory BCP-ALL, 180 patients were treated with 28-day continuous infusion of blinatumomab per cycle, and the response rate was 43% [61]. In an adult phase III randomized trial that compared blinatumomab with standard-of-care chemotherapy in relapsed/refractory BCP-ALL, the median overall survival was significantly longer in the blinatumomab group (7.7 months) than in the chemotherapy group (4 months, $p = 0.01$) [62]. The remission rates within 12 weeks after treatment initiation were also significantly higher in the blinatumomab group than in the chemotherapy group (34% vs. 16%, $p < 0.001$). In a pediatric phase I/II study in relapsed/refractory BCP-ALL patients, 39% achieved CR within the first two cycles, 52% achieved complete MRD response [49]. The most common grade 3+ adverse effects were anemia (36%), thrombocytopenia (21%), and hypokalemia (17%); 4% of the patients experienced ≥grade 3 cytokine-release syndrome.

Inotuzumab ozogamicin (InO) is a humanized anti-CD22 monoclonal antibody conjugated to calicheamycin, a cytotoxic agent [63–65]. CD22 is widely expressed on BCP-ALL blasts and after the conjugate binds to cell-surface CD22, the CD22-conjugate-calicheamicin complex is rapidly internalized; thereafter, calicheamicin is released and induces subsequent apoptosis of the leukemic cells [63, 65–67].

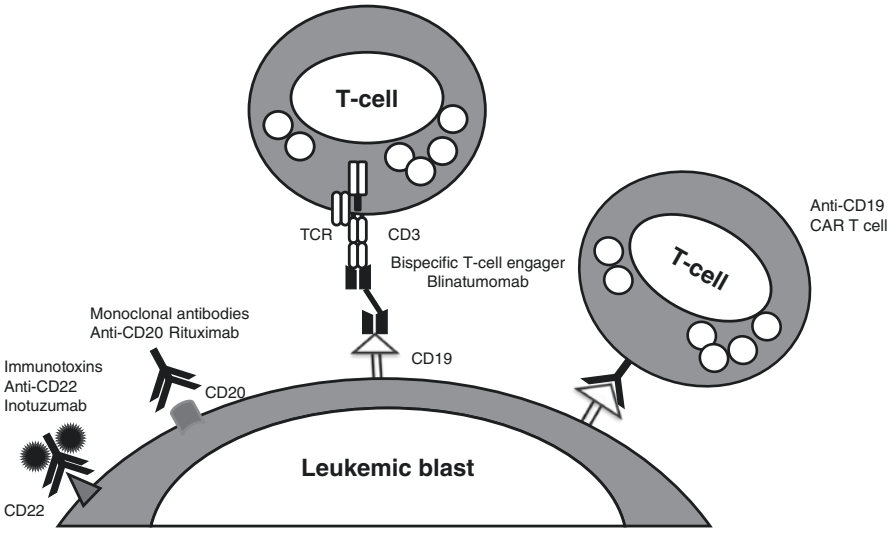


Fig. 13.2 New options of immunotherapy for pediatric relapsed acute lymphoblastic leukemia (ALL)

In a phase 3 study for relapsed/refractory CD22 positive ALL, all the patients were randomized to InO or standard chemotherapy; the CR rate was 80.7% in the InO group and 33.3% in the standard chemotherapy group ($p < 0.001$) [68]. MRD negativity was achieved at a higher percentage in the InO arm (78.4 vs. 28.1%, $p < 0.0001$), and the median duration of remission was longer (4.6 months vs. 3.1 months, $p = 0.03$). InO is relatively well tolerated in adult patients. However, sinusoidal obstructive syndrome (SOS) occurred in 11% of the patients in this study, and the incidence of SOS was associated with HSCT after InO monotherapy. In 51 pediatric relapsed/refractory ALL patients who were treated with InO in the compassionate program, CR was achieved in 67% of patients, of whom, 71% were MRD-negative [50]. With respect to safety, no patient developed sinusoidal obstruction syndrome during InO therapy; however, 52% of the patients who underwent HSCT following InO developed SOS.

Recent advances in adoptive immunotherapy using autologous T-cells transduced with a chimeric-antigen receptor (CAR) targeting CD19 resulted in high rates of clinical remission of relapsed and refractory ALL in multiple studies [51, 69–71]. Tisagenlecleucel, one of an anti-CD19 CAR-T-cell, showed durable remission with long-term persistence in pediatric and young adult patients with relapsed or refractory B-cell ALL in a phase 2 study [51]. The overall remission rate within 3 months was 81%, and the 1-year EFS and 1-year OS was 50% and 76%, respectively, in 75 relapsed/refractory patients. Grade-3 or -4 adverse effects related to tisagenlecleucel occurred in 88% of the patients, and cytokine-release syndrome (CRS) occurred in 77% of the patients; 47% of the patients were admitted to the intensive care unit, and 13% received mechanical ventilation because of CRS.

13.4.2 Molecular Targeted Drugs and Cytotoxic Drugs

Bortezomib is a selective inhibitor of the ubiquitin-proteasome pathway and inhibits NF-kb that is postulated to be involved in its anti-cancer effects. In preclinical studies, bortezomib has shown synergy with dexamethasone, asparaginase, vincristine, and doxorubicin. In a pediatric phase II expansion study of bortezomib on the combination of these four drugs for relapsed/refractory ALL, the overall response rate was 73%, and 16/20 patients with BCP-ALL achieved CR [52]. Grade 3 peripheral neuropathy developed in 9% of the patients, and infection of grade 3 or higher was seen in 45% of the patients.

Clofarabine is a second-generation purine nucleoside analog capable of inhibiting DNA synthesis/repair and inducing cell death [72]. Clofarabine has shown a synergistic effect with cyclophosphamide by inhibiting DNA repair, and the combination of clofarabine with cyclophosphamide and etoposide has been evaluated in several studies [55, 73, 74]. In a pediatric phase 2 trial reported by Hijiya, among the 25 patients with R/R pediatric ALL, the overall response rate was 44% (7CR, 4CRp), and the median duration of remission was 67.3 weeks. Moreover, six patients (24%) died because of treatment-related AEs associated with infection, hepatotoxicity, and/or multiple organ failure. Four of the eight patients who received clofarabine after HSCT developed veno-occlusive disease.

Nelarabine is an inhibitor of purine nucleoside phosphorylase. A single agent phase II pediatric trial in children with refractory T-ALL or T-cell lymphoblastic lymphoma showed a 48% CR2 rate and 23% CR3 rate for R/R T-ALL [56]. In patients with R/R T-ALL, nelarabine in combination with etoposide and cyclophosphamide was used as a salvage therapy [57] and in patients with newly diagnosed HR T-ALL, nelarabine, in combination with BFM 86 chemotherapy, was evaluated in a pilot study conducted by COG [75]. In addition to hematological AEs, neurological toxicities are a major concern; grade ≥3 neurological toxicities have been reported in 18% of children and young adults. Severe neurological-associated AEs include altered mental states, CNS effects, and peripheral neuropathies [56].

There have been several case reports that showed tyrosine kinase inhibitors successfully induced remission in refractory Philadelphia chromosome-negative ALL patients including T-ALL [76–78]. Currently, an individual approach to seek the best matched novel agent as per molecular profiling and drug-response profiling is under development, especially for pediatric R/R T-ALL patients [79].

13.4.3 Integration of Novel Therapies in the Treatment Strategy of Relapsed ALL

The combination of conventional cytotoxic drugs is insufficient as re-induction therapy for T-ALL, early relapse, or very early relapse of BCP-ALL; therefore, novel therapies, such as blinatumomab, bortezomib combination, InO, and anti-CD19 CAR-T cell, are new candidates for re-induction therapy in these subgroups. The IntReALL HR study is now investigating the efficacy as a re-induction of bortezomib in addition to UK R3 backbone in a randomized controlled trial. The best strategy for achieving second remission in these HR subgroups is yet to be determined and should be investigated in future trials.

After achieving second or higher CR, HSCT is essential to treat T-ALL, early BM relapse, or very early BM relapse of BCP-ALL, as mentioned in this chapter. Although CD19 CAR-T cell has shown promising outcome in relapsed pediatric BCP- ALL, the duration of response is insufficient; thus, HSCT may still not be omitted after the achievement of CR with CD19 CAR-T cell. In the future, novel strategies that can replace HSCT as a cure option in pediatric relapsed ALL are expected to be established.

References

1. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. N Engl J Med. 2006;354(2):166–78.
2. Tsuchida M, Ohara A, Manabe A, Kumagai M, Shimada H, Kikuchi A, et al. Long-term results of Tokyo Children's cancer study group trials for childhood acute lymphoblastic leukemia, 1984–1999. Leukemia. 2010;24(2):383.

3. Schrappe M, Bleckmann K, Zimmermann M, Biondi A, Möricke A, Locatelli F, et al. Reduced-intensity delayed intensification in standard-risk pediatric acute lymphoblastic leukemia defined by undetectable minimal residual disease: results of an international randomized trial (AIEOP-BFM ALL 2000). J Clin Oncol. 2018;36(3):244–53.
4. Tallen G, Ratei R, Mann G, Kaspers G, Niggli F, Karachunsky A, et al. Long-term outcome in children with relapsed acute lymphoblastic leukemia after time-point and site-of-relapse stratification and intensified short-course multidrug chemotherapy: results of trial ALL-REZ BFM 90. J Clin Oncol. 2010;28(14):2339–47.
5. Gaynon PS, Harris RE, Altman AJ, Bostrom BC, Breneman JC, Hawks R, et al. Bone marrow transplantation versus prolonged intensive chemotherapy for children with acute lymphoblastic leukemia and an initial bone marrow relapse within 12 months of the completion of primary therapy: children's oncology group study CCG-1941. J Clin Oncol. 2006;24(19):3150–6.
6. Parker C, Waters R, Leighton C, Hancock J, Sutton R, Moorman AV, et al. Effect of mitoxantrone on outcome of children with first relapse of acute lymphoblastic leukaemia (ALL R3): an open-label randomised trial. Lancet. 2010;376(9757):2009–17.
7. Nguyen K, Devidas M, Cheng SC, La M, Raetz EA, Carroll WL, et al. Factors influencing survival after relapse from acute lymphoblastic leukemia: a Children's oncology group study. Leukemia. 2008;22(12):2142–50.
8. Weston BW, Hayden MA, Roberts KG, Bowyer S, Hsu J, Fedoriw G, et al. Tyrosine kinase inhibitor therapy induces remission in a patient with refractory EBF1-PDGFRB-positive acute lymphoblastic leukemia. J Clin Oncol. 2013;31(25):e413–6.
9. Bailey CL, Lange BJ, Rheingold SR, Bunin NJ. Bone-marrow relapse in paediatric acute lymphoblastic leukaemia. Lancet Oncol. 2008;9(9):873–83.
10. Gaynon PS, Qu RP, Chappell RJ, Willoughby ML, Tubergen DG, Steinherz PG, et al. Survival after relapse in childhood acute lymphoblastic leukemia: impact of site and time to first relapse- 331(the Children's cancer group experience). Cancer. 1998;82(7):1387–95.
11. Barrett AJ, Horowitz MM, Pollock BH, Zang MJ, Bortin MM, Buchanan GR, Camitta BM, et al. Bone marrow transplants from HLA-identical siblings as compared with chemotherapy for children with acute lymphoblastic leukemia in a second remission. N Engl J Med. 1994;331(19):1253–8.
12. Lawson SE, Harrison G, Richards S, Oakhill A, Stevens R, Eden OG, et al. The UK experience in treating relapsed childhood acute lymphoblastic leukaemia: a report on the medical research council UKALLR1 study. Br J Haematol. 2000;108(3):531–43.
13. Einsiedel H, von Stackelberg A, Hartmann R, Fengler R, Schrappe M, Janka-Schaub G, et al. Long-term outcome in children with relapsed ALL by risk-stratified salvage therapy: results of trial acute lymphoblastic leukemia-relapse study of the Berlin-Frankfurt-Münster group 87. J Clin Oncol. 2005;23(31):7942–50.
14. von Stackelberg A, Hartmann R, Bührer C, Fengler R, Janka-Schaub G, Reiter A, et al. High-dose compared with intermediate-dose methotrexate in children with a first relapse of acute lymphoblastic leukemia. Blood. 2008;111(5):2573–80.
15. Gaynon PS. Childhood acute lymphoblastic leukaemia and relapse. Br J Haematol. 2005;131(5):579–87.
16. Bhojwani D, Pui C-H. Relapsed childhood acute lymphoblastic leukaemia. Lancet Oncol. 2013;14(6):e205–17.
17. Locatelli F, Schrappe M, Bernardo M, Rutella S. How I treat relapsed childhood acute lymphoblastic leukemia. Blood. 2012;120(14):2807–16.
18. Gandemer V, Chevret S, Petit A, Vermylen C, Leblanc T, Michel G, et al. Excellent prognosis of late relapses of ETV6/RUNX1-positive childhood acute lymphoblastic leukemia: lessons from the FRALLE 93 protocol. Haematologica. 2012;97(11):1743–50.
19. Krentz S, Hof J, Mendioroz A, Vaggopoulou R, Dörge P, Lottaz C, et al. Prognostic value of genetic alterations in children with first bone marrow relapse of childhood B-cell precursor acute lymphoblastic leukemia. Leukemia. 2013;27(2):295.

20. Hof J, Krentz S, van Schewick C, Körner G, Shalapour S, Rhein P, et al. Mutations and deletions of the TP53 gene predict nonresponse to treatment and poor outcome in first relapse of childhood acute lymphoblastic leukemia. J Clin Oncol. 2011;29(23):3185–93.
21. Irving JAE, Enshaei A, Parker CA, Sutton R, Kuiper RP, Erhorn A, et al. Integration of genetic and clinical risk factors improves prognostication in relapsed childhood B-cell precursor acute lymphoblastic leukemia. Blood. 2016;128(7):911–22.
22. Dworzak MN, Fröschl G, Printz D, Mann G, Pötschger U, Mühlegger N, et al. Prognostic significance and modalities of flow cytometric minimal residual disease detection in childhood acute lymphoblastic leukemia. Blood. 2002;99(6):1952–8.
23. Coustan-Smith E, Sancho J, Hancock ML, Boyett JM, Behm FG, Raimondi SC, et al. Clinical importance of minimal residual disease in childhood acute lymphoblastic leukemia. Blood. 2000;96(8):2961–6.
24. Borowitz MJ, Devidas M, Hunger SP, Bowman WP, Carroll AJ, Carroll WL. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's oncology group study. Blood. 2008;111(12):5457–85.
25. Conter V, Bartram CR, Valsecchi MG, Schrauder A, Panzer-Grümayer MA, 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. 2010;115(16):3206–14.
26. Eckert C, von Stackelberg A, Seeger K, Groeneveld T, Peters C, Klingebiel T, et al. Minimal residual disease after induction is the strongest predictor of prognosis in intermediate risk relapsed acute lymphoblastic leukaemia – long-term results of trial ALL-REZ BFM P95/96. Eur J Cancer. 2013;49(6):1346–55.
27. Parker C, Krishnan S, Hamadeh L, Irving JAE, Kuiper RP, Révész T, et al. Outcomes of patients with childhood B-cell precursor acute lymphoblastic leukaemia with late bone marrow relapses: long-term follow-up of the ALLR3 open-label randomised trial. Lancet Haematol. 2019;6(4):e204–16.
28. Paganin M, Zecca M, Fabbri G, Polato K, Biondi A, Rizzari C, et al. Minimal residual disease is an important predictive factor of outcome in children with relapsed 'high-risk' acute lymphoblastic leukemia. Leukemia. 2008;22(12):2193.
29. Karawajew L, Dworzak M, Ratei R, Rhein P, Gaipa G, Buldini B, et al. Minimal residual disease analysis by eight-color flow cytometry in relapsed childhood acute lymphoblastic leukemia. Haematologica. 2015;100(7):935–44.
30. Bader P, Kreyenberg H, Henze GHR, Eckert C, Reising M, Willasch A, et al. Prognostic value of minimal residual disease quantification before allogeneic stem-cell transplantation in relapsed childhood acute lymphoblastic leukemia: the ALL-REZ BFM study group. J Clin Oncol. 2008;27(3):377–84.
31. Eckert C, Henze G, Seeger K, Hagedorn N, Mann G, Panzer-Grümayer R, et al. Use of allogeneic hematopoietic stem-cell transplantation based on minimal residual disease response improves outcomes for children with relapsed acute lymphoblastic leukemia in the intermediate-risk group. J Clin Oncol. 2013;31(21):2736–42.
32. Reismüller B, Attarbaschi A, Peters C, Dworzak MN, Pötschger U, Urban C, et al. Long-term outcome of initially homogenously treated and relapsed childhood acute lymphoblastic leukaemia in Austria stem-cell trans-based report of the Austrian Berlin-Frankfurt-Münster (BFM) study group. Br J Haematol. 2009;144(4):559–70.
33. Ko RH, Ji L, Barnette P, Bostrom B, Hutchinson R, Raetz E, et al. Outcome of patients treated for relapsed or refractory acute lymphoblastic leukemia: a therapeutic advances in childhood leukemia consortium study. J Clin Oncol. 2009;28(4):648–54.
34. Hagedorn N, Acquaviva C, Fronkova E, von Stackelberg A, Barth A, zur Stadt U, et al. Submicroscopic bone marrow involvement in isolated extramedullary relapses in childhood acute lymphoblastic leukemia: a more precise definition of "isolated" and its possible clinical

implications, a collaborative study of the resistant disease Committee of the International BFM study group. Blood. 2007;110(12):4022–9.
35. Pui C-H, Howard SC. Current management and challenges of malignant disease in the CNS in paediatric leukaemia. Lancet Oncol. 2008;9(3):257–68.
36. Barredo JC, Devidas M, Lauer SJ, Billett A, Marymont M, Pullen J, et al. Isolated CNS relapse of acute lymphoblastic leukemia treated with intensive systemic chemotherapy and delayed CNS radiation: a pediatric oncology group study. J Clin Oncol. 2006;24(19):3142–9.
37. Masurekar AN, Parker CA, Shanyinde M, Moorman AV, Hancock JP, Sutton R, et al. Outcome of central nervous system relapses in childhood acute lymphoblastic leukaemia--prospective open cohort analyses of the ALLR3 trial. PLoS One. 2014;9(10):e108107.
38. Eapen M, Zhang MJJ, Devidas M, Raetz E, Barredo JC, Ritchey AK, et al. Outcomes after HLA-matched sibling transplantation or chemotherapy in children with acute lymphoblastic leukemia in a second remission after an isolated central nervous system relapse: a collaborative study of the Children's oncology group and the Center for International Blood and Marrow Transplant Research. Leukemia. 2008;22(2):281–6.
39. van den Berg H, Langeveld NE, Veenhof CHN, Behrendt H. Treatment of isolated testicular recurrence of acute lymphoblastic leukemia without radiotherapy. Cancer. 1997;79(11):2257–62.
40. Berg H, Langeveld NE, Veenhof CHN, Behrendt H. Treatment of isolated testicular recurrence of acute lymphoblastic leukemia without radiotherapy: report from the Dutch late effects study group. Cancer. 1997;79(11):2257–62.
41. Eapen M, Raetz E, Zhang M-J, Muehlenbein C, Devidas M, Abshire T, et al. Outcomes after HLA-matched sibling transplantation or chemotherapy in children with B-precursor acute lymphoblastic leukemia in a second remission: a collaborative study of the Children's oncology group and the Center for International Blood and Marrow Transplant Research. Blood. 2006;107(12):4961–7.
42. Uderzo C, Valsecchi MG, Bacigalupo A, Meloni G, Messina C, Polchi P, et al. Treatment of childhood acute lymphoblastic leukemia in second remission with allogeneic bone marrow transplantation and chemotherapy: ten-year experience of the Italian bone marrow transplantation group and the Italian pediatric hematology oncology association. J Clin Oncol. 1995;13(2):352–8.
43. Borgmann A, von Stackelberg A, Hartmann R, Ebell W, Klingebiel T, Peters C, et al. Unrelated donor stem cell transplantation compared with chemotherapy for children with acute lymphoblastic leukemia in a second remission: a matched-pair analysis. Blood. 2003;101(10):3835–9.
44. Leung W, Pui C-H, Coustan-Smith E, Yang J, Pei D, Gan K, et al. Detectable minimal residual disease before hematopoietic cell transplantation is prognostic but does not preclude cure for children with very-high-risk leukemia. Blood. 2012;120(2):468–72.
45. Duval M, Klein JP, He W, Cahn JY, Cairo M, Camitta BM, et al. Hematopoietic stem-cell transplantation for acute leukemia in relapse or primary induction failure. J Clin Oncol. 2010;28(23):3730–8.
46. Bertaina A, Zecca M, Buldini B, Sacchi N, Algeri M, Saglio F, et al. Unrelated donor vs HLA-haploidentical α/β T-cell- and B-cell-depleted HSCT in children with acute leukemia. Blood. 2018;132(24):2594–607.
47. Sano H, Mochizuki K, Kobayashi S, Ohara Y, Ito M, Waragai T, et al. T-cell-replete haploidentical stem cell transplantation using low-dose antithymocyte globulin in children with relapsed or refractory acute leukemia. Int J Hematol. 2018;108(1):76–84.
48. Kobayashi S, Ito M, Sano H, Mochizuki K, Akaihata M, Waragai T, et al. T-cell-replete haploidentical stem cell transplantation is highly efficacious for relapsed and refractory childhood acute leukaemia. Transfus Med. 2014;24(5):305–10.
49. von Stackelberg A, Locatelli F, Zugmaier G, Handgretinger R, Trippett TM, Rizzari C, et al. Phase I/phase II study of blinatumomab in pediatric patients with relapsed/refractory acute lymphoblastic leukemia. J Clin Oncol. 2016;34(36):4381–9.

50. Bhojwani D, Sposto R, Shah NN, Rodriguez V, Yuan C, Stetler-Stevenson M, et al. Inotuzumab ozogamicin in pediatric patients with relapsed/refractory acute lymphoblastic leukemia. Leukemia. 2019;33(4):884–92.
51. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med. 2018;378(5):439–48.
52. Messinger YH, Gaynon PS, Sposto R, van der Giessen J, Eckroth E, Malvar J, et al. Bortezomib with chemotherapy is highly active in advanced B-precursor acute lymphoblastic leukemia: therapeutic advances in Childhood Leukemia & Lymphoma (TACL) study. Blood. 2012;120(2):285–90.
53. Horton TM, Whitlock JA, Lu X, O'Brien MM, Borowitz MJ, Devidas M, et al. Bortezomib reinduction chemotherapy in high-risk ALL in first relapse: a report from the Children's oncology group. Br J Haematol. 2019;186(2):274–85.. [Epub ahead of print]
54. Jeha S, Gaynon PS, Razzouk BI, Franklin J, Kadota R, Shen V, et al. Phase II study of clofarabine in pediatric patients with refractory or relapsed acute lymphoblastic leukemia. J Clin Oncol. 2006;24(12):1917–23.
55. Hijiya N, Thomson B, Isakoff MS, Silverman LB, Steinherz PG, Borowitz MJ, et al. Phase 2 trial of clofarabine in combination with etoposide and cyclophosphamide in pediatric patients with refractory or relapsed acute lymphoblastic leukemia. Blood. 2011;118(23):6043–9.
56. Berg SL, Blaney SM, Devidas M, Lampkin TA, Murgo A, Bernstein M, et al. Phase II study of nelarabine (compound 506U78) in children and young adults with refractory T-cell malignancies: a report from the children's oncology group. J Clin Oncol. 2005;23(15):3376–82.
57. Commander LA, Seif AE, Insogna IG, Rheingold SR. Salvage therapy with nelarabine, etoposide, and cyclophosphamide in relapsed/refractory paediatric T-cell lymphoblastic leukaemia and lymphoma. Br J Haematol. 2010;150(3):345–51.
58. Löffler A, Gruen M, Wuchter C, Schriever F, Kufer P, Dreier T, et al. Efficient elimination of chronic lymphocytic leukaemia B cells by autologous T cells with a bispecific anti-CD19/anti-CD3 single-chain antibody construct. Leukemia. 2003;17(5):900–9.
59. Dreier T, Lorenczewski G, Brandl C, Hoffmann P, Syring U, Hanakam F, et al. Extremely potent, rapid and co-stimulation-independent cytotoxic T-cell response against lymphoma cells catalyzed by a single-chain bispecific antibody. Int J Cancer. 2002;100(6):690–7.
60. Hoffmann P, Hofmeister R, Brischwein K, Brandl C, Crommer S, Bargou R, et al. Serial killing of tumor cells by cytotoxic T cells redirected with a CD19-/CD3-bispecific single-chain antibody construct. Int J Cancer. 2005;115(1):98–104.
61. Topp MS, Gökbuget N, Stein AS, Zugmaier G, O'Brien S, Bargou RC, et al. Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multicentre, single-arm, phase 2 study. Lancet Oncol. 2015;16(1):57–66.
62. Kantarjian H, Stein A, Gökbuget N, Fielding AK, Schuh AC, Ribera J-M, et al. Blinatumomab versus chemotherapy for advanced acute lymphoblastic leukemia. N Engl J Med. 2017;376(9):836–47.
63. DiJoseph JF, Armellino DC, Boghaert ER, Khandke K, Dougher MM, Sridharan L, et al. Antibody-targeted chemotherapy with CMC-544: a CD22-targeted immunoconjugate of calicheamicin for the treatment of B-lymphoid malignancies. Blood. 2004;103(5):1807–14.
64. Hinman LM, Hamann PR, Wallace R, Menendez AT, Durr FE, Upeslacis J. Preparation and characterization of monoclonal antibody conjugates of the calicheamicins: a novel and potent family of antitumor antibiotics. Cancer Res. 1993;53(14):3336–42.
65. Shor B, Gerber H-P, Sapra P. Preclinical and clinical development of inotuzumab-ozogamicin in hematological malignancies. Mol Immunol. 2015;67(2):107–16.
66. Hanna R, Ong GL, Mattes MJ. Processing of antibodies bound to B-cell lymphomas and other hematological malignancies. Cancer Res. 1996;56(13):3062–8.
67. Bouchard H, Viskov C, Garcia-Echeverria C. Antibody–drug conjugates—a new wave of cancer drugs. Bioorg Med Chem Lett. 2014;24(23):5357–63.

68. Kantarjian HM, DeAngelo DJ, Stelljes M, Martinelli G, Liedtke M, Stock W, et al. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. N Engl J Med. 2016;375(8):740–53.
69. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med. 2014;371(16):1507–17.
70. Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet. 2015;385(9967):517–28.
71. Gardner RA, Finney O, Annesley C, Brakke H, Summers C, Leger K, et al. Intent to treat leukemia remission by CD19CAR T cells of defined formulation and dose in children and young adults. Blood. 2017;129(25):3322–31.
72. Jeha S, Gandhi V, Chan K, McDonald L, Ramirez I, Madden R, et al. Clofarabine, a novel nucleoside analog, is active in pediatric patients with advanced leukemia. Blood. 2004;103(3):784–9.
73. O'Connor D, Sibson K, Caswell M, Connor P, Cummins M, Mitchell C, et al. Early UK experience in the use of clofarabine in the treatment of relapsed and refractory paediatric acute lymphoblastic leukaemia. Br J Haematol. 2011;154(4):482–5.
74. Locatelli F, Testi AM, Bernardo M, Rizzari C, Bertaina A, Merli P, et al. Clofarabine, cyclophosphamide and etoposide as single-course re-induction therapy for children with refractory/multiple relapsed acute lymphoblastic leukaemia. Br J Haematol. 2009;147(3):371–8.
75. Dunsmore KP, Devidas M, Linda SB, Borowitz MJ, Winick N, Hunger SP, et al. Pilot study of nelarabine in combination with intensive chemotherapy in high-risk T-cell acute lymphoblastic leukemia: a report from the children's oncology group. J Clin Oncol. 2012;30(22):2753–9.
76. Crombet O, Lastrapes K, Zieske A, Morales-Arias J. Complete morphologic and molecular remission after introduction of dasatinib in the treatment of a pediatric patient with t-cell acute lymphoblastic leukemia and ABL1 amplification. Pediatr Blood Cancer. 2012;59(2):333–4.
77. Glover JM, Loriaux M, Tyner JW, Druker BJ, Chang BH. In vitro sensitivity to dasatinib in lymphoblasts from a patient with t(17;19) (q22;p13) gene rearrangement pre-B acute lymphoblastic leukemia. Pediatr Blood Cancer. 2012;59(3):576–9.
78. Goto H. Childhood relapsed acute lymphoblastic leukemia: biology and recent treatment progress. Pediatr Int. 2015;57(6):1059–66.
79. Bassan R, Bourquin J-P, DeAngelo DJ, Chiaretti S. New approaches to the management of adult acute lymphoblastic leukemia. J Clin Oncol. 2018;36:3504–19.

Chapter 14
Acute Leukemia of Ambiguous Lineage (ALAL)

Shunsuke Nakagawa

Abstract Ambiguous lineage acute leukemia (ALAL) is a rare subtype of acute leukemia and is defined immunologically. ALAL consists of mixed phenotype acute leukemia (MPAL) and acute undifferentiated leukemia (AUL). MPAL is further divided into subtypes such as B/M MPAL, T/M MPAL, and B/T MPAL. Recently, the genetic basis of MPAL has been revealed as an acquisition of mutations in immature hematopoietic progenitors. There are shared genetic features between B/M MPAL with ZNF384 and B-cell acute lymphoblastic leukemia (ALL) with ZNF384 as well as T/M MPAL and early T-cell precursor ALL. Treatment for ALAL has not been established. However, treatment of ALL-type is significantly more effective than treatment of acute myelogenous leukemia. Generally, the prognosis of pediatric ALAL is worse than that of pediatric ALL. Treatment selection based on the genetic background is recommended. ALAL with genetic features of ALL, such as BCR-ABL or KMT2A alterations, and CD19 positive ALAL should be treated using ALL treatment. Treatment switching, either ALL-type to AML-type or vice versa, is beneficial for few ALAL cases. Hematopoietic stem cell transplantation is indicated for patients with poor efficacy of induction treatment.

Keywords Acute leukemia of ambiguous lineage · Mixed phenotype acute leukemia · ALL-type treatment · AML-type treatment · Switching treatments

S. Nakagawa (✉)
Department of Pediatrics, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Kagoshima, Japan
e-mail: ngawa@m2.kufm.kagoshima-u.ac.jp

M. Kato (ed.), *Pediatric Acute Lymphoblastic Leukemia*,
https://doi.org/10.1007/978-981-15-0548-5_14

14.1 Introduction

Acute lymphoblastic leukemia (ALL) is the most common blood cancer in children. It is divided into two subtypes (B-ALL or T-ALL) by immunological method. However, a rare subtype of ALL exists, showing features of both B-ALL and T-ALL. Many researchers have been trying to define and establish the optimal treatment for this rare subtype. The European Group for Immunological Characterization of Leukemias (EGIL) proposed the classification of "biphenotypic acute leukemia" in 1995 [1]. The World Health Organization (WHO) further classified "biphenotypic acute leukemia" by adding cytochemical, karyotypic, and clinical information to the EGIL criteria. The WHO used a simplified method of classification and renamed this subtype "Mixed Phenotype Acute Leukemia" in 2008, with revisions made in 2016 [2]. ALAL has recently become widely recognized and highlighted in the studies. Significant findings around the genetic background and treatment of ALAL have improved our understanding of the disease in recent years, especially in 2018.

14.2 Definition and Diagnosis

The current WHO criteria of ALAL consist of MPAL and acute undifferentiated leukemia (AUL). WHO defines ALAL with five subtypes: MPAL with BCR-ABL1, MPAL with MLL rearrangement, B/M MPAL, T/M MPAL, and AUL. AUL is defined as lacking lineage defining features [2].

Outside of the WHO criteria, there are two patterns of lineage ambiguity: "Biphenotypic" MPAL, which shares immunophenotypic features of lymphoblastic and myeloid acute leukemia in a single population, and "Bilineal" MPAL, in which two separate clones of different lineages coexist.

Although rare, acute leukemia whose phenotype changes during induction therapy is also included in ALAL. Most of these cases have mutations associated with KMT2A and have a poor prognosis [3].

EGIL and WHO criteria are used for diagnosis of MPAL, and both principally rely on flow cytometry (FCM). The differences in definition are shown in Tables 14.1 and 14.2.

Table 14.1 EGIL definition. Score of >2.0 for B or T-cell lineage and for M lineage

Scoring points	B lineage	T lineage	M lineage
2	CD79a, intra IgM, CD22	CD3, TCRαβ, TCRγδ	Intra MPO
1	CD19, CD10, CD20	CD2, CD5, CD8, CD10	CD13, CD33, CD65
0.5	Intra TdT, CD24	Intra TdT, CD7, CD1a	CD14, CD15, CD64, CD117

Table 14.2 WHO definition fulfills criteria for at least two lineages

	Criteria definition
M lineage	Intra MPO or Monocytic differentiation (at least 2 of the following: Nonspecific esterase cytochemistry, CD11c, CD14, CD64, lysozyme)
T lineage	Strong[a] intra CD3 or Surface CD3
B lineage	Strong[a] CD19 with at least 1 of the following strongly expressed: CD79a, intraCD22, or CD10 or Weak CD19 with at least 2 of the following strongly expressed: CD79a, intraCD22, or CD10

[a]Strong defined as equal or brighter than the normal B- or T-cells in the sample

14.3 Case Presentation

14.3.1 Case 1: B/M MPAL

A 5-year-old male patient presented with fever, lymphadenopathy, hepatosplenomegaly, and purpura. Blood examination indicated leukocytosis with a blast count of 251,000/μL, anemia, and thrombocytopenia. The blasts were lymphoblasts and myeloid blasts. FCM indicated that 70% of the blasts were of B-cell lineage (positive markers were CD19, CD22, and cyCD79a) and 30% were of myeloid lineage (positive markers were MPO, CD13, and CD33) (Fig. 14.1). Karyotypic studies showed 47,XY,+X,t(11;19)(q23;p13.3) [3]/46,XY[13]. Fluorescence in situ hybridization (FISH) analysis confirmed the presence of an MLL-ENL fusion.

Response to prednisolone was good and ALL-type induction treatment resulted in complete remission (CR), but minimal residual disease (MRD) was positive. The patient relapsed during maintenance therapy and switched to AML-type treatment. However, complete remission was not achieved, and the patient died.

14.3.2 Case 2: T/M MPAL

An 11-year-old male patient presented with lymphadenopathy. Blood examination indicated mild leukocytosis with a blast count of 4000/μL. Computed tomography showed cervical and mediastinal lymphadenopathy. Bone marrow (BM) pathology revealed 81.0% blasts, with a minority of lymphoid origin and a majority of myeloid origin. FCM indicated that 10% of blasts were early T-cell precursors (positive markers were CD2, CD3, CD7, CD10, and CD34; negative markers were CD1a, CD5, and CD8), and the remaining 90% of blasts were of myeloid lineage (positive markers were MPO, CD13, CD33, CD15, and CD11b) (Fig. 14.2). MLL rearrangement, BCR-ABL, and PML-RARA were all negative.

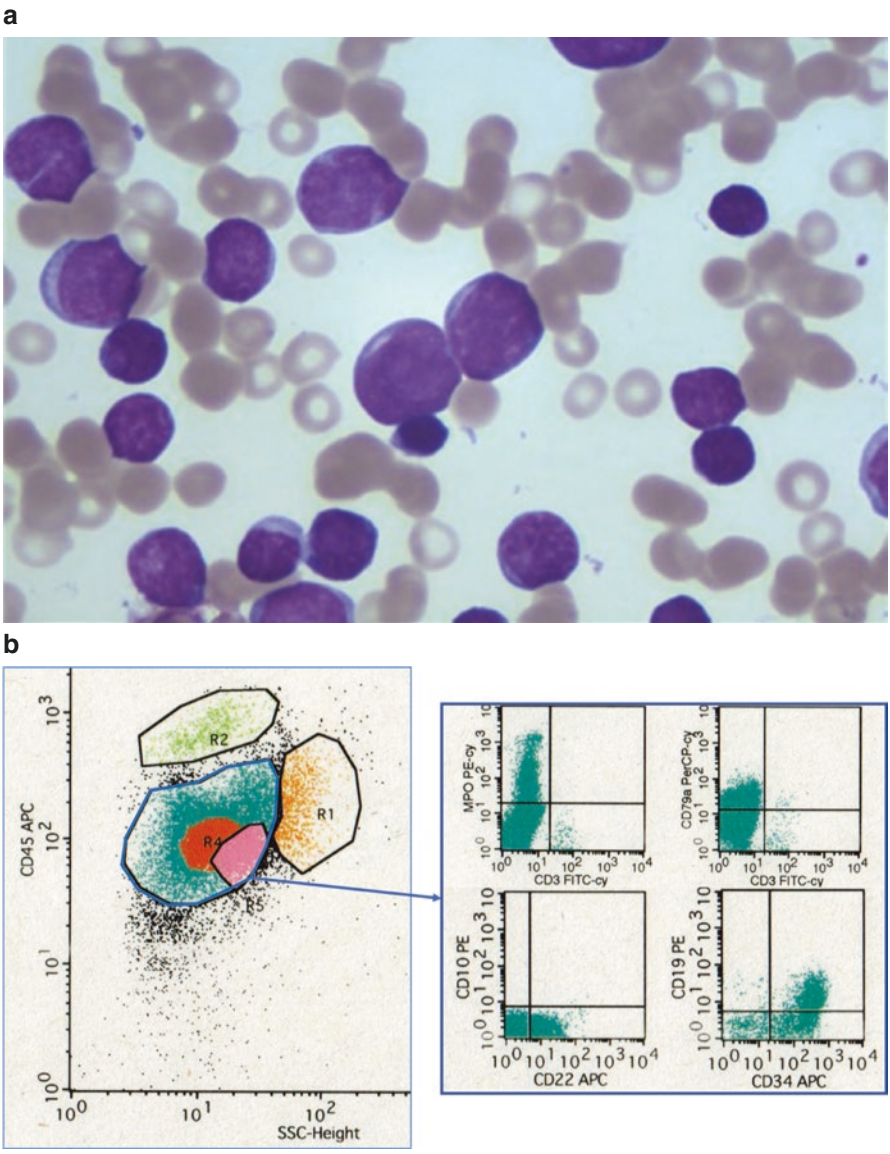


Fig. 14.1 Two distinguishable blasts are observed in the smear (**a**). FCM showed B-cell lineage and myeloid lineage (**b**)

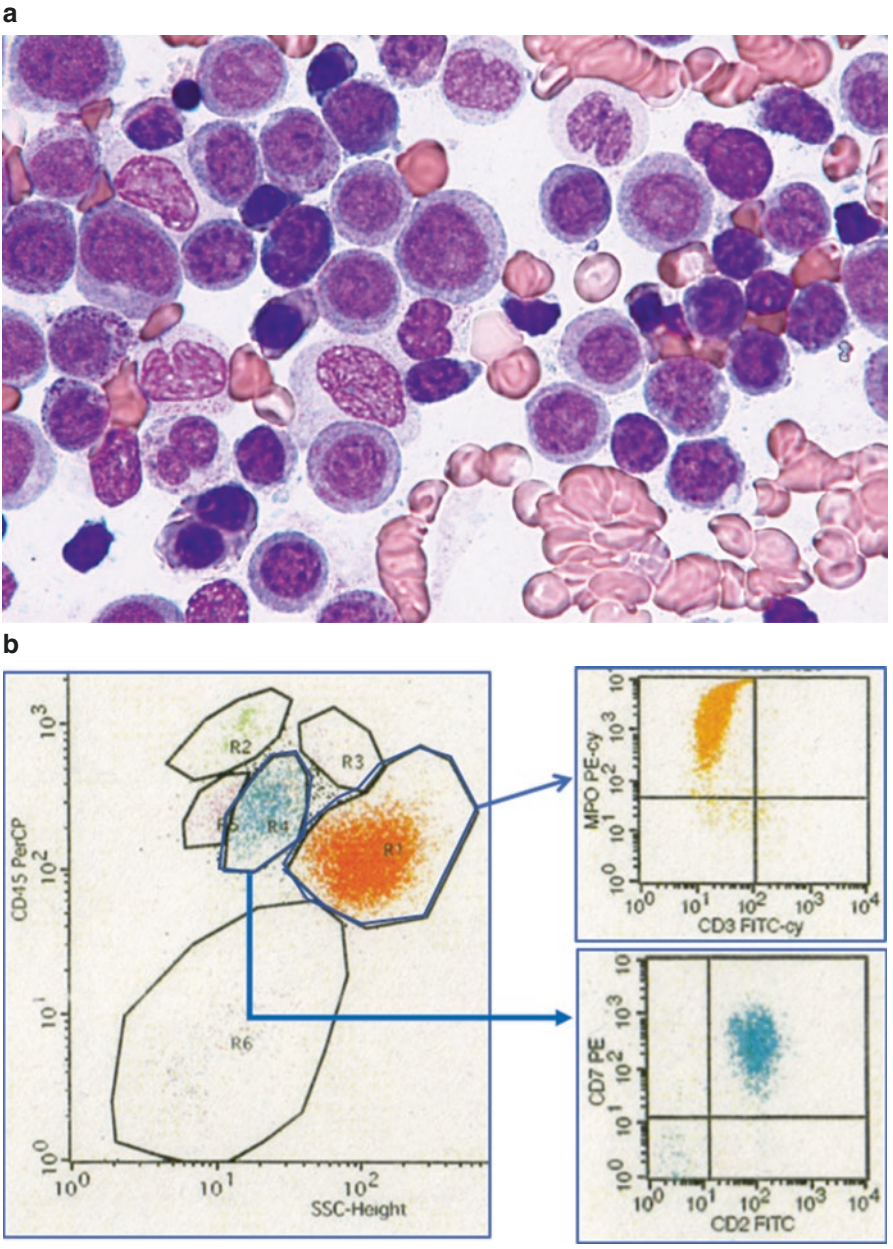


Fig. 14.2 Most blasts are myeloid, but a few lymphoblasts are observed (**a**). Lymphoid blasts showed T-cell lineage markers in the FCM (**b**)

Response to prednisolone was very poor, but the patient achieved CR at the end of induction therapy, with residual MRD. After the consolidation therapy, peripheral blood stem cell transplantation was performed, and the patient maintained CR.

14.4 Etiology

ALAL accounts for 2–5% [4, 5] of acute leukemia cases, MPAL represents 2–3% of pediatric acute leukemia, and AUL is rare [6]. B/M MPAL is most common (45–70% of MPAL), followed by T/M MPAL (30–40% of MPAL). B/T and B/T/M MPAL are rare, 0–14% and 0–2% of MPAL, respectively [5]. Rearrangement of BCR-ABL and KMT2A (also known as MLL) is detected in 1–20% and 8–11% of MPAL, respectively [4–6]. Biphenotypic MPAL is more frequent than bilineage MPAL (75–90% and less than 30%, respectively) [5].

There are no specific clinical characteristics of MPAL. Hyperleukocytosis is not common to MPAL, whose median WBC at diagnosis is 12,000–28,000/μL. CNS disease at diagnosis is also uncommon, presenting in only 6–17% of affected patients [5].

14.5 Genetic Basis

It has been speculated that ALAL has genetic diversity because of its immunological phenotype. Alexander et al. reported in 2018 that research was near to uncovering the pathogenesis of pediatric MPAL, especially in B/M MPAL and T/M MPAL [6]. As a result of an exhaustive examination of genetic abnormalities of MPAL in 159 children from all over the world, important discoveries were made showing that pediatric B/M MPAL and T/M MPAL are genetically distinct.

Pediatric B/M MPAL has features of frequent ZNF384 fusions; Ras pathway mutations; and PAX5, ETV6, CDKN2A/B, and VPREB1 mutations. These features support that patients with B/M MPAL should be treated with ALL-type treatment. Genomic features of B/M MPAL with ZNF384 rearrangement are like those of B-ALL with ZNF384 rearrangement, whose gene expression profiles are indistinguishable. B/M MPAL with ZNF384 rearrangement exhibits higher FLT3 expression compared with other types of MPAL.

Genomic features of T/M MPAL are similar to those of ETP-ALL, but distinguishable from those of T-ALL. The WT1 mutation is common in T/M MPAL and ETP-ALL, but not in T-ALL. Ras and JAK–STAT pathway mutations are common in T/M MPAL and ETP-ALL, and phosphotidylinositol 3-kinase (PI3K) signaling pathway mutations are common in T-ALL. These features support ALL-type treatment for T/M MPAL, which is considered as high risk ALL.

Mi et al. reported nine cases of B/T MPAL, a rare subtype of MPAL, in 2018. B/T MPAL frequently has mutations in PHF6, JAK-STAT, and the Ras signaling

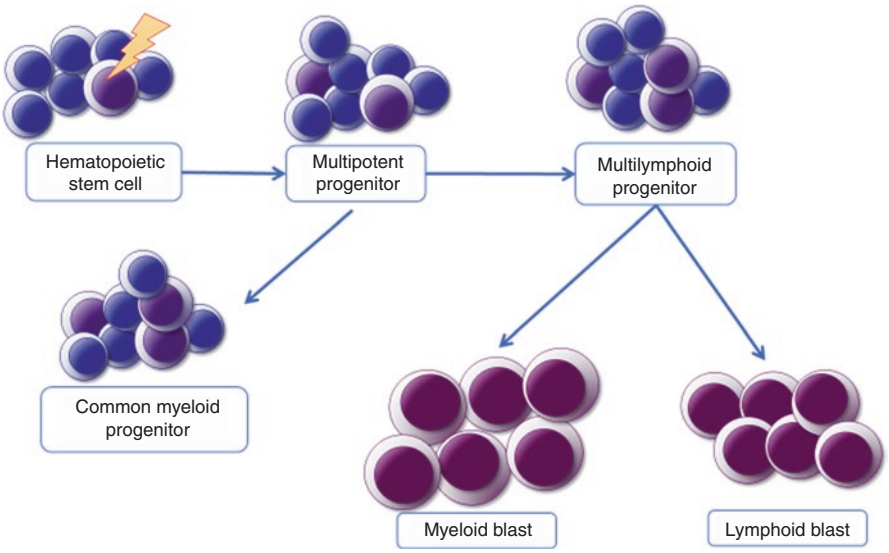


Fig. 14.3 A model of leukemogenesis for MPAL. Mutations are acquired in an early hematopoietic progenitor

pathway [7]. The genomic landscape shares many features with ETP-ALL, which is distinguishable from T-ALL.

The common notion is that ALL requires clonal evolution derived from subclonal genomic variation during disease progression. In contrast, the acquisition of mutations in immature hematopoietic progenitors is required for the ambiguous phenotype of MPAL (Fig. 14.3). These notable findings are revealed from the common genomic features of B-ALL and MPAL with ZNF384 rearrangement, reconstitution of MPAL from subclones, shared genetic features of the subpopulations, and identification of leukemia initiating mutations in early hematopoietic progenitors. Since it is derived from mutations in immature hematopoietic progenitors, ALAL should be considered high-risk leukemia and patients with poor therapeutic efficacy should be treated with hematopoietic stem cell transplantation (HSCT).

14.6 Treatment and Prognosis

Previously, several small cohort studies have suggested that the ALL-type treatment was better than the AML-type treatment [8, 9]. However, there was no prospective clinical trial for ALAL and a lack of consensus regarding appropriate therapy for ALAL.

In 2018, meta-analysis for children and adult MPAL revealed that ALL-type treatment has a significantly higher complete remission rate and overall survival (OS) compared to AML type treatment [5]. The combined type showed a CR rate

equal to that of the ALL type, but its OS was worse than those of ALL type and AML type. In the study, children had a better prognosis than adults when MPAL was evaluated by age. A difference in ALL treatment regimens between children and adults may affect the prognosis. There is a difference in the CR rate among subtypes of MPAL. T/M and B/T/M MPAL have lower CR rates than B/M and B/T MPAL [5]. The advantages of each classification, EGIL or WHO, are controversial [5]. It is likely that both criteria are useful for diagnosing MPAL. Patients with bilineal ALAL were worse off than patients with biphenotypic ALAL. However, regardless of ambiguity types, ALL-type treatment is superior to AML-type or combined-type treatment. The effectiveness of ALL-type treatment is even more pronounced in CD19 positive MPAL [4].

There are limited reports on the dominance of the lineage and treatment. Although AML-type therapy may be selected if the AML population dominates, the prognosis is also poor [4]. Even if the myeloid population dominates over the lymphoid subpopulation, ALL-type induction therapy can achieve CR [10]. The lineage domination does not affect choice of treatment type.

14.7 Current Recommendations for Treatment

Based on the above findings, treatment strategy for ALAL is recommended. Induction therapy of ALAL should be started with a high-risk group regimen. ALL- or AML-specific fusions should be used with each treatment. Patients with BCR-ABL or PML-RARA fusion should have their respective specific treatments added, such as a tyrosine kinase inhibitor or all-trans retinoic acid/arsenic trioxide. CD19 positive patients should avoid starting with AML-type treatment. Because ALL-type induction treatment is superior to AML-type, patients with CD19 negativity and presence of lymphoid markers should be treated with ALL-type. If it is difficult to choose whether to treat with ALL-type or AML-type treatment, the ALL-type treatment should be chosen. It is important to note that the dominance of the lineage should not be the basis for selecting treatment type.

14.8 Switching Treatments

If the treatment response is poor, switching treatments, such as changing ALL-type treatment to AML-type treatment or vice versa, has been tried to improve the treatment efficacy. This treatment switching is a characteristic problem for MPAL. PSL response is one of the most important prognostic factors for B-ALL or T-ALL, and the treatment of patients with prednisolone poor response (PPR) should step up to high-risk treatment. However, in MPAL, treatment switching for patients with PPR is not beneficial. ALL-type treatment should start with a high-risk protocol, and induction therapy should be completed even with PPR or poor early response in the BM [10]. Case 2 was PPR and peripheral blast was increasing during the 2 weeks of

induction therapy, but he achieved complete remission at the end of induction therapy. ALL-type treatment patients with very poor treatment response, such as 5% or more MRD at the end of induction therapy, should be considered for a treatment switch to AML-type therapy.

14.9 Role of HSCT in ALAL

Chemotherapy only is inferior to treatment with HSCT [11]. However, HSCT is not necessary for every patient with MPAL [4]. Patients with a poor response at the end of induction therapy may be rescued using HSCT. Because the MRD of Case 1 was positive even after the consolidation therapy, he might have had a better prognosis if he had been treated with HSCT in complete remission. Similarly, Case 2 sustained positive MRD, so he was indicated for treatment with HSCT and could maintain complete remission. It is recommended that ALL-type treatment be switched to AML-type treatment followed by HSCT in the case of MRD > 5% at the end of induction therapy, or patients with MRD > 0.01% at the end of consolidation should be considered for indication to HSCT.

References

1. Bene MC, Castoldi G, Knapp W, et al. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia. 1995;9(10):1783–6.
2. Arber DA, Orazi A, Hasserijian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
3. Rossi JG, Bernasconi AR, Alonso CN, et al. Lineage switch in childhood acute leukemia: an unusual event with poor outcome. Am J Hematol. 2012;87(9):890–7.
4. Hrusak O, de Haas V, Stancikova J, et al. International cooperative study identifies treatment strategy in childhood ambiguous lineage leukemia. Blood. 2018;132(3):264–76.
5. Maruffi M, Sposto R, Oberley MJ, et al. Therapy for children and adults with mixed phenotype acute leukemia: a systematic review and meta-analysis. Leukemia. 2018;32(7):1515–28.
6. Alexander TB, Gu Z, Iacobucci I, et al. The genetic basis and cell of origin of mixed phenotype acute leukaemia. Nature. 2018;562(7727):373–9.
7. Mi X, Griffin G, Lee W, et al. Genomic and clinical characterization of B/T mixed phenotype acute leukemia reveals recurrent features and T-ALL like mutations. Am J Hematol. 2018;93(11):1358–67.
8. Gerr H, Zimmermann M, Schrappe M, et al. Acute leukaemias of ambiguous lineage in children: characterization, prognosis and therapy recommendations. Br J Haematol. 2010;149(1):84–92.
9. Matutes E, Pickl WF, Van't Veer M, et al. Mixed-phenotype acute leukemia: clinical and laboratory features and outcome in 100 patients defined according to the WHO 2008 classification. Blood. 2011;117(11):3163–71.
10. Nakagawa S, Okamoto Y, Kodama Y, et al. Importance of acute lymphoblastic leukemia-type therapy for bilineal acute leukemia. J Pediatr Hematol Oncol. 2018;41(6):504–6. [Epub ahead of print]
11. Tian H, Xu Y, Liu L, et al. Comparison of outcomes in mixed phenotype acute leukemia patients treated with chemotherapy and stem cell transplantation versus chemotherapy alone. Leuk Res. 2016;45:40–6.

Chapter 15
Stem Cell Transplantation for Pediatric ALL

Motohiro Kato

Abstract For pediatric ALL with the highest risk of relapse, allogeneic stem cell transplantation (allo-SCT) is performed as the most potent form of consolidation therapy. Considering acute and late complication, allo-SCT should be limitedly indicated for cases with extremely high risk cytogenetic/genomic alterations or very poor response to treatment (including early relapsed cases). If available, human leukocyte antigen (HLA) matched siblings are the best donor, but current studies showed comparable outcomes of allo-SCT from HLA matched unrelated donors or cord blood for pediatric ALL. In terms of conditioning regimen, total body irradiation (TBI)-based myeloablative combination is still the most potent and widely used as a standard therapy for ALL in children aged 1 years or older, while busulfan-based conditioning is the standard for infant ALL. Considering acute and late complication caused by TBI, reduction or avoidance of TBI should be continuously challenged.

Keywords Transplantation · Indication · Total body irradiation · GVHD · Busulfan

15.1 Consideration of Indication for Allogeneic Transplantation

For children with the highest risk of relapse, allogeneic stem cell transplantation (allo-SCT) is performed as the most potent form of consolidation therapy (Fig. 15.1). Myeloablative therapy such as total body irradiation (TBI) or busulfan can be used as a preconditioning therapy for allo-SCT with stem cell rescue to eradicate leukemic cells resistant to standard chemotherapy and support engraftment of stem cells from allogeneic donors. Additionally, allo-immune effects

M. Kato (✉)
Department of Transplantation and Cell Therapy, Children's Cancer Center, National Center for Child Health and Development, Setagaya-ku, Tokyo, Japan
e-mail: kato-mt@ncchd.go.jp

M. Kato (ed.), *Pediatric Acute Lymphoblastic Leukemia*,
https://doi.org/10.1007/978-981-15-0548-5_15

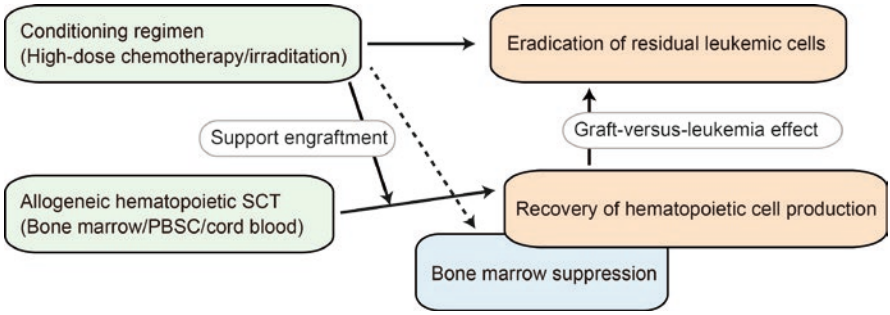


Fig. 15.1 Hypothetical model of allogeneic hematopoietic stem cell transplantation for hematologic malignancy. Conditioning regimen with high-dose chemotherapy and/or total body irradiation eradicates residual leukemic cells, and immunosuppressive effect assists engraftment of transplanted allogeneic hematopoietic stem cells. Allo-reaction of donor cells has anti-leukemic (graft-versus-leukemia) effect

Table 15.1 Indication of transplantation in pediatric ALL

Characteristics	Estimated Percentage in Pediatric ALL
High-risk cytogenetics/genomic alterations	
BCR-ABL1 (with poor response or IKZF1 deletion)	1–5%
Hypodiploid	1–3%
TCF3-HLF	<1%
Poor response to treatment	
High level of MRD	3–5%
Induction failure	1–2%
Relapse (early, except isolated extramedullary)	3–5%

In total, 5–10% of BCP-ALL is indicated for transplantation in the first remission
MRD minimal residual disease

caused by donor cells will eliminate residual leukemic cells derived from the recipient. Based on this graft-versus-leukemia (GVL) concept, graft-versus-host disease (GVHD) is expected to serve as a surrogate marker in decreasing the risk of relapses [1, 2].

Recent advances in transplantation procedure have decreased transplant-related morbidity and mortality, but incidence of treatment-related morbidity and mortality cannot be ignored, and late complications including infertility, growth retardation, metabolic disease, and secondary malignant neoplasms, are unavoidable problem. Thus, allo-SCT should be indicated only for cases that are definitely at high-risk for relapse, such as extremely poor prognostic biological features, poor early response, and relapse (Table 15.1).

On the other hand, we should know that current knowledge is established based on experience with conventional chemotherapy-based approach. An impact of existing prognostic factors and indication for all-SCT may be converted by adaptation immunotherapeutic agents in the near future.

15.1.1 BCR-ABL1

ALL with *BCR-ABL1*, a kinase fusion derived from t(9;22)(q34;q11), is one of the most classical indication for allo-SCT in CR1. ALL with *BCR-ABL1* has a very poor prognosis as low as 20–25% of event-free survival when treated only with conventional chemotherapy. Arico et al. reported an international collaboration study of retrospective review focusing on pediatric ALL with *BCR-ABL1*, including 326 cases diagnosed from 1986 to 1996 [3]. They demonstrated that allo-SCT could improve survival probability for ALL with *BCR-ABL1*, up to 65% of event-free survival at 5 years after transplantation. They also showed transplantation from matched related donors was superior to other types of transplantation. Subsequent analysis is published in 2010, including 610 cases diagnosed as *BCR-ABL1* positive ALL from 1995 to 2005 [4]. Improved outcomes are demonstrated, and 7-year event-free survival of all-SCT from matched related donors or unrelated donors in first remission had better outcome than that of chemotherapy alone, suggesting greater protection against late relapses.

Based on these evidences, *BCR-ABL1*-ALL had been considered as an indication for allo-SCT in CR1. Introduction of tyrosine kinase inhibitor (TKI), directly targeting BCR-ABL1 protein, significantly improved therapeutic outcome. The Children's Oncology Group (COG) AALL0031 trial showed excellent event-free survival with intensive chemotherapy and imatinib as high as 70% of 3-year event-free survival and also suggesting comparable outcome even without all-SCT in CR1 [5]. The similarly improved outcome was reproduced by the EsPhALL trial conducted by the European international group [6]. We should know that, in the era of TKI, a proportion of ALL with *BCR-ABL1* can achieve long-term remission without all-SCT in CR1. On the other hand, even treated with more potent TKI such as dasatinib, an improvement of event-free survival hit the ceiling at around 60–70% [7]. Allo-SCT is still required for *BCR-ABL1* positive ALL with poor early responder, and indication should be considered for cases with *IKZF1* deletion, an independent poor prognostic factor for ALL [7]. Detailed therapeutic strategy is also shown in Chap. 10.

15.1.2 Hypodiploid

Hypodiploidy is poor prognostic cytogenetic feature in pediatric ALL. Especially, ALL with a modal chromosome number of 43 or less had extremely poor prognosis, at ~50% of event-free survival at 8-year after diagnosis [8]. Despite recent improvements of treatment for pediatric ALL, outcome of hypodiploid ALL continued to be poor, and survival probability of recent trials is still around 50% [9]. These poor outcomes had driven most of clinicians to perform allo-SCT for hypodiploid ALL [10]. However, two large retrospective studies simultaneously demonstrated that allo-SCT produced no significant impact on survival probability compared with

chemotherapy alone, irrespective of minimal residual disease (MRD) status [11, 12]. Allo-SCT in CR1 is still a standard option for hypodiploid ALL, but further alternative consolidation therapy to achieve deeper remission is required to improve prognosis for this unfavorable subgroup.

15.1.3 Infant ALL with MLL *Rearrangement*

ALL with *KMT2A* (known as *MLL*) rearrangement is associated with poor outcome [13], and infant ALL, which occurred during the first year of life, had high frequency (>70%) of *MLL* rearrangement. Given the poor prognosis of infant ALL with *MLL* rearrangement, allo-SCT has been adopted, and improved survival outcome has been reported. Kosaka et al. reported that the 3-year event-free survival for 29 cases who received all-SCT in CR1 was 64.4% [14]. Improved outcome by allo-SCT is reproduced by other studies [15]. However, results of recent clinical trials suggested that allo-SCT in infants failed to provide significant difference of event-free survival from intensive chemotherapy [16]. Considering severe late complication, indication of allo-SCT may be limited to high risk group in infants, such as very early onset (e.g., younger than 3 months), high leukocyte counts at diagnosis, or poor treatment response [17]. Detailed therapeutic strategy for infant ALL are also shown in Chap. 9.

15.1.4 TCF3-HLF

TCF3-HLF is a chimeric fusion generated by t(17;19)(q22;p13), which consists of <1% of pediatric ALL. The prognosis of ALL with this fusion is dismal, and almost all cases suffered relapse when treated with chemotherapy only. Although successful experiences by allo-SCT are limited due to its rarity [18, 19], allo-SCT is generally adopted for *TCF3-PBX1* positive ALL. A study suggested *TCF3-HLF* sensitized ALL cells to the GVL effect by upregulating death receptor via expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors [19]. Immunotherapeutic approach may be beneficial to ALL with *TCF3-HLF*.

15.1.5 Poor Response to Treatment

Treatment response is widely recognized as an independent prognostic factor, and poor early response to treatment can predict inferior outcome. More than 95% of children with ALL achieved complete remission after the first course of therapy, while induction failure cases had event-free survival of 32% [20]. Cases with

positive minimal residual disease (MRD) were also associated with high relapse rate [21]. Allo-SCT is generally recommended for these poor early response cases.

Even current chemotherapy, up to 15% of ALL cases suffered relapse. Allo-SCT is routinely performed for cases with early relapse [22], while late relapse (>6 months after completion of therapy) can be salvaged by intensive chemotherapy only. Detailed strategy for relapsed ALL is shown in Chap. 13.

15.2 Selection for Stem Cell Sources

Hematopoietic stem cell source is indispensable to perform stem cell transplantation. Previously, autologous transplantation could provide comparative outcome to chemotherapy [23], but in addition to recognition of GVL effect of allogeneic donors, recent advance of supporting therapy enabled us to perform intensive consolidation therapy without hematopoietic stem cell rescue, reducing necessity of autologous transplantation for pediatric ALL. Currently, autologous SCT is not recommended as standard practice [24]. Thus, currently, most of transplantation uses allogeneic stem cell sources, including related or unrelated donors.

Of note, a retrospective study focusing on GVL effect for hematologic malignancy in children showed that the positive effect of acute and chronic GVHD (Fig. 15.2), which can serve as a surrogate marker for the GVL effect in pediatric leukemia [2]. Considering the limited survival advantage conferred and the longer post-transplantation life of children, excess GVHD should be avoided.

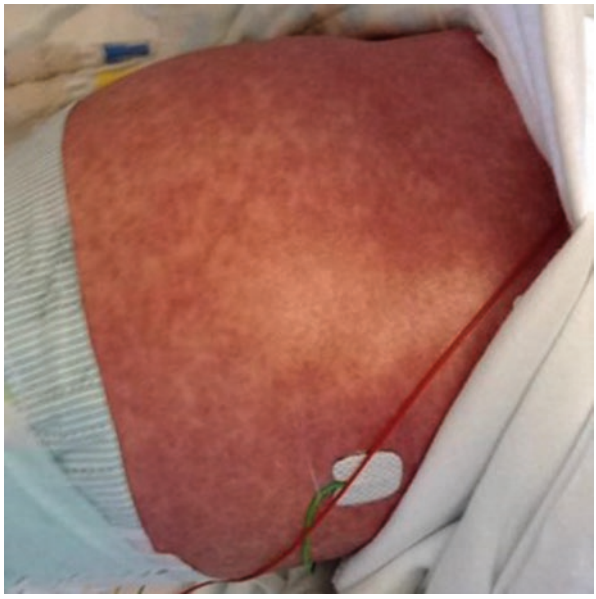


Fig. 15.2 Typical presentation of skin GVHD

15.2.1 Priority of Stem Cell Sources

General considerations for priority of stem cell sources are shown in Fig. 15.3. Although several clinical conditions of patients have to be considered to determine the optimal donor, human leukocyte antigen (HLA) matched siblings are the best donor source. Acquisition of bone marrow (BM) volume equivalent to approximately 15 ml/kg of patients' body weight or collection of peripheral blood stem cells (PBSC) induced by administration of granulocyte colony stimulating factor (G-CSF) are two major methods for hematopoietic stem cell harvest. Randomized controlled trials in adult cases had been repeatedly conducted to compare BM and PBSC [25], and several meta-analyses demonstrated that PBSC had shorter median time to engraftment but higher rate of GVHD than that of BM in transplantation from matched sibling donors. For children receiving SCT from related donors, retrospective studies from the Committee of the International Bone Marrow Transplant Registry (CIBMTR) [26] and the Japan Society for Hematopoietic Cell Transplantation (JSHCT) [27] showed inferior survival probability due to treatment-related mortality and higher incidence of chronic GVHD. Similar results were observed in SCT from unrelated donors for pediatric acute leukemia [28]. Thus, careful prevention and management are required in case of allo-SCT using PBSC for pediatric ALL.

However, HLA-matched sibling donors are not always available, and ethical consideration should be carefully discussed especially when potential donors are young children [29]. Fortunately, comparable survival probability is achieved in SCT from alternative donors (including unrelated donors and cord blood) thanks to recent advances in transplantation [30]. A prospective trial conducted by the Berlin–Frankfurt–Munster (BFM) group revealed that 4-year event-free survival did not differ between patients with SCT from unrelated donors and sibling donors (67% and 71%, respectively) and concluded that outcome among high-risk leukemia in children was not affected by donor type under standardized myeloablative conditioning [31].

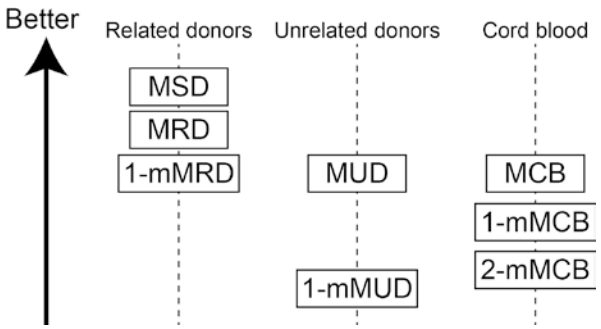


Fig. 15.3 General consideration of priority of stem cell sources. *MSD* matched siblings, *MRD* matched related donors (except MSD), *1-mMRD* one-antigen mismatched related donors, *MUD* matched unrelated donors, *1-mMUD* one-antigen mismatched unrelated donors, *MCB* matched cord blood, *1-mMCB* one-antigen mismatched cord blood, *2-mMCB* two-antigens mismatched cord blood

Furthermore, cord blood transplantation could provide similar outcomes to matched unrelated donor as stem cell source for pediatric ALL. Although a recent retrospective analysis focusing on cord blood transplantation for non-malignant diseases suggested an importance of allele-level HLA matching at HLA-A, HLA-B, HLA-C, and HLA-DRB1, previous studies for acute leukemia in children supported the use of one- or two- antigen HLA-mismatched cord blood [32]. In spite of delayed hematopoietic recovery, overall survival is similar between cord blood and matched unrelated donors, and chronic GVHD was decreased [33]. Cord blood has an advantage of rapid availability, which is more important for advanced stage of hematologic malignancy. The limited number of hematopoietic cells in frozen cord blood units is less problematic for children with small body size. For adults with large body, double-unit cord blood transplantation was challenged to achieving sufficient cell dose and suggested that engraftment and survival were better. On the contrary, a randomized study for children with hematologic malignancy showed that survival rates were similar after single-unit and double-unit cord blood transplantation, but double-unit was associated with higher risk of GVHD [34].

15.2.2 Transplantation from Haploidentical Donors

Historically, SCT from haploidentical donors (mostly 2- or 3- antigens mismatched parents) has been performed to anticipate potent GVL effect. However, SCT from haploidentical donor has a high incidence of severe GVHD, which is eventually associated with morbidity and mortality. In 2000s, Luznik et al. demonstrated that post-transplantation cyclophosphamide (PTCY) has a potent preventive effect for GVHD via selective depletion of allo-reactive T-cell [35, 36]. Numerous studies confirm low incidence of GVHD after haplo-SCT with PTCY [37], including a retrospective study for children [38]. However, of note, GVL effect of haploidentical SCT is similarly attenuated by PTCY, and relapse incidence after haplo-PTCY SCT is eventually similar to that of SCT from HLA-matched donors [39]. Thus, the main role of haplo-SCT with PTCY should be to expand the donor pool. For children without HLA matched donors or suitable cord blood units, SCT from haploidentical donors with PTCY is a reasonable option. Furthermore, recent studies showed that depletion of alpha/beta T-cell and B-cell could prevent GVHD in SCT from haploidentical donor, and the results were similar to that of haplo-SCT with PTCY, with low incidence of overall and extensive chronic GVHD [40]. The priority of donor selection and optimal stem cell source should change according to each era.

15.3 Conditioning Regimen

Conditioning regimen has two major roles in allo-SCT. One is eradiation of residual leukemic cells which was resistant to standard consolidation therapy. The other is immunosuppression to achieve engraftment of allogeneic hematopoietic stem cells

(Fig. 15.1). Myeloablative (12 Gy) TBI is the most traditional conditioning regimen and is still considered as a standard conditioning regimen for ALL in children aged 1 year or older, even though myeloablative TBI causes not only acute toxicity but late complication which is more problematic for children. Advantage of TBI is the most potent effect for lymphoid malignancy. Busulfan (BU) is widely used as an alternative for TBI, but BU had an inferior survival outcome compared with TBI in allo-SCT for pediatric ALL in retrospective [41] and prospective studies. Recent studies in adults showed intravenous BU-containing conditioning led to similar survival following SCT for ALL [42]. In children (except infant), intravenous BU failed to improve outcome compared to oral BU [43], but based on the fact that TBI causes severe late complication for children, reduction or avoidance of TBI should be continuously challenged.

For infant ALL, BU-based conditioning exceptionally provided equal or even better outcome with TBI-based conditioning [44]. Considering potential risks for late complication which could be more severe for those who had received allo-SCT during infancy, BU-based conditioning regimen is assumed to be standard conditioning for infant ALL.

To enhance the cytotoxic and immunosuppressive effects of TBI, chemotherapeutic drugs are concomitantly used, and TBI and cyclophosphamide (CY) are originally used in allo-SCT [45]. However, the outcome with TBI and CY for pediatric ALL had been unsatisfactory mainly due to relapse, even when performed at complete remission. Consequently, several clinicians have attempted to add other agents to TBI-CY or exchange CY for other cytotoxic agents. Gassas et al. reported that etoposide could be replaced by CY with comparable outcomes [46]. A retrospective study showed that additional etoposide to TBI-CY had an advantage on reduction of relapse and better survival [47], but there are some reports with inconsistent results, mainly because of increased transplantation-related toxicity [48]. Other studies adopted cytarabine (AraC), melphalan (LPAM), or thiotepa, but the number of patients included to these studies were limited, with varying disease status and stem cell source. Thus, the optimal partner of TBI for pediatric ALL is still to be established, and the recommended conditioning regimens in recent clinical trials were not uniform, namely, TBI-CY, TBI-etoposide, TBI-etoposide-CY, TBI-AraC-CY, and TBI-LPAM.

SCT using reduced intensity conditioning without myeloablative TBI nor BU was introduced for children with organ damage or infection due to negative reason that they were assumed to be intolerant to myeloablative conditioning. However, recently, reduced intensity of conditioning is selected for children without any organ dysfunction due to positive reason that reduction of conditioning can suppress acute and late complication [49] without compromising disease control [50]. Efficacy of reduced intensity conditioning SCT for pediatric ALL is worth being assessed in clinical trial setting.

References

1. Stern M, de Wreede LC, Brand R, et al. Sensitivity of hematological malignancies to graft-versus-host effects: an EBMT megafile analysis. Leukemia. 2014;28:2235–40.
2. Kato M, Kurata M, Kanda J, et al. Impact of graft-versus-host disease on relapse and survival after allogeneic stem cell transplantation for pediatric leukemia. Bone Marrow Transplant. 2019;54:68–75.
3. Arico M, Valsecchi MG, Camitta B, et al. Outcome of treatment in children with Philadelphia chromosome-positive acute lymphoblastic leukemia. N Engl J Med. 2000;342:998–1006.
4. Arico 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. 2010;28:4755–61.
5. Schultz KR, Bowman WP, Aledo A, et al. Improved early event-free survival with imatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia: a children's oncology group study. J Clin Oncol. 2009;27:5175–81.
6. Biondi A, Schrappe M, De Lorenzo P, et al. Imatinib after induction for treatment of children and adolescents with Philadelphia-chromosome-positive acute lymphoblastic leukaemia (EsPhALL): a randomised, open-label, intergroup study. Lancet Oncol. 2012;13:936–45.
7. Slayton WB, Schultz KR, Kairalla JA, et al. Dasatinib plus intensive chemotherapy in children, adolescents, and young adults with Philadelphia chromosome-positive acute lymphoblastic leukemia: results of children's oncology group trial AALL0622. J Clin Oncol. 2018;36:2306–14.
8. Nachman JB, Heerema NA, Sather H, et al. Outcome of treatment in children with hypodiploid acute lymphoblastic leukemia. Blood. 2007;110:1112–5.
9. Schultz KR, Devidas M, Bowman WP, et al. Philadelphia chromosome-negative very high-risk acute lymphoblastic leukemia in children and adolescents: results from children's oncology group study AALL0031. Leukemia. 2014;28:964–7.
10. Oliansky DM, Camitta B, Gaynon P, et al. Role of cytotoxic therapy with hematopoietic stem cell transplantation in the treatment of pediatric acute lymphoblastic leukemia: update of the 2005 evidence-based review. Biol Blood Marrow Transplant. 2012;18:505–22.
11. McNeer JL, Devidas M, Dai Y, et al. Hematopoietic stem-cell transplantation does not improve the poor outcome of children with hypodiploid acute lymphoblastic leukemia: a report from children's oncology group. J Clin Oncol. 2019;37:780–9.
12. Pui CH, Rebora P, Schrappe M, et al. Outcome of children with hypodiploid acute lymphoblastic leukemia: a retrospective multinational study. J Clin Oncol. 2019;37:770–9.
13. Pui CH, Gaynon PS, Boyett JM, et al. Outcome of treatment in childhood acute lymphoblastic leukaemia with rearrangements of the 11q23 chromosomal region. Lancet. 2002;359:1909–15.
14. Kosaka Y, Koh K, Kinukawa N, et al. Infant acute lymphoblastic leukemia with MLL gene rearrangements: outcome following intensive chemotherapy and hematopoietic stem cell transplantation. Blood. 2004;104:3527–34.
15. Jacobsohn DA, Hewlett B, Morgan E, Tse W, Duerst RE, Kletzel M. Favorable outcome for infant acute lymphoblastic leukemia after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2005;11:999–1005.
16. Dreyer ZE, Dinndorf PA, Camitta B, et al. Analysis of the role of hematopoietic stem-cell transplantation in infants with acute lymphoblastic leukemia in first remission and MLL gene rearrangements: a report from the Children's oncology group. J Clin Oncol. 2011;29:214–22.
17. Mann G, Attarbaschi A, Schrappe M, et al. Improved outcome with hematopoietic stem cell transplantation in a poor prognostic subgroup of infants with mixed-lineage-leukemia

(MLL)-rearranged acute lymphoblastic leukemia: results from the Interfant-99 study. Blood. 2010;116:2644–50.
18. Mouttet B, Vinti L, Ancliff P, et al. Durable remissions in TCF3-HLF positive acute lymphoblastic leukemia with blinatumomab and stem cell transplantation. Haematologica. 2019;104:e244–7.
19. Zhang X, Inukai T, Hirose K, et al. Oncogenic fusion E2A-HLF sensitizes t(17;19)-positive acute lymphoblastic leukemia to TRAIL-mediated apoptosis by upregulating the expression of death receptors. Leukemia. 2012;26:2483–93.
20. Schrappe M, Hunger SP, Pui CH, et al. Outcomes after induction failure in childhood acute lymphoblastic leukemia. N Engl J Med. 2012;366:1371–81.
21. 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. 2010;115:3206–14.
22. Eckert C, Henze G, Seeger K, et al. Use of allogeneic hematopoietic stem-cell transplantation based on minimal residual disease response improves outcomes for children with relapsed acute lymphoblastic leukemia in the intermediate-risk group. J Clin Oncol. 2013;31:2736–42.
23. Messina C, Cesaro S, Rondelli R, et al. Autologous bone marrow transplantation for childhood acute lymphoblastic leukaemia in Italy. AIEOP/FONOP-TMO group. Italian association of paediatric haemato-oncology. Bone Marrow Transplant. 1998;21:1015–21.
24. Majhail NS, Farnia SH, Carpenter PA, et al. Indications for autologous and allogeneic hematopoietic cell transplantation: guidelines from the American society for blood and marrow transplantation. Biol Blood Marrow Transplant. 2015;21:1863–9.
25. Bensinger WI, Martin PJ, Storer B, et al. Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. N Engl J Med. 2001;344:175–81.
26. Eapen M, Horowitz MM, Klein JP, et al. Higher mortality after allogeneic peripheral-blood transplantation compared with bone marrow in children and adolescents: the histocompatibility and alternate stem cell source working committee of the international bone marrow transplant registry. J Clin Oncol. 2004;22:4872–80.
27. Shinzato A, Tabuchi K, Atsuta Y, et al. PBSCT is associated with poorer survival and increased chronic GvHD than BMT in Japanese paediatric patients with acute leukaemia and an HLA-matched sibling donor. Pediatr Blood Cancer. 2013;60:1513–9.
28. Keesler DA, St Martin A, Bonfim C, Seber A, Zhang MJ, Eapen M. Bone marrow versus peripheral blood from unrelated donors for children and adolescents with acute leukemia. Biol Blood Marrow Transplant. 2018;24:2487–92.
29. American Academy of Pediatrics. Committee on, B. Children as hematopoietic stem cell donors. Pediatrics. 2010;125:392–404.
30. Sakaguchi H, Watanabe N, Matsumoto K, et al. Comparison of donor sources in hematopoietic stem cell transplantation for childhood acute leukemia: a nationwide retrospective study. Biol Blood Marrow Transplant. 2016;22:2226–34.
31. Peters C, Schrappe M, von Stackelberg A, et al. Stem-cell transplantation in children with acute lymphoblastic leukemia: a prospective international multicenter trial comparing sibling donors with matched unrelated donors-the ALL-SCT-BFM-2003 trial. J Clin Oncol. 2015;33:1265–74.
32. Eapen M, Rubinstein P, Zhang MJ, et al. Outcomes of transplantation of unrelated donor umbilical cord blood and bone marrow in children with acute leukaemia: a comparison study. Lancet. 2007;369:1947–54.
33. Rocha V, Cornish J, Sievers EL, et al. Comparison of outcomes of unrelated bone marrow and umbilical cord blood transplants in children with acute leukemia. Blood. 2001;97:2962–71.
34. Wagner JE Jr, Eapen M, Carter S, et al. One-unit versus two-unit cord-blood transplantation for hematologic cancers. N Engl J Med. 2014;371:1685–94.

35. O'Donnell PV, Luznik L, Jones RJ, et al. Nonmyeloablative bone marrow transplantation from partially HLA-mismatched related donors using posttransplantation cyclophosphamide. Biol Blood Marrow Transplant. 2002;8:377–86.
36. Luznik L, O'Donnell PV, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. Biol Blood Marrow Transplant. 2008;14:641–50.
37. Sugita J, Kawashima N, Fujisaki T, et al. HLA-haploidentical peripheral blood stem cell transplantation with post-transplant cyclophosphamide after busulfan-containing reduced-intensity conditioning. Biol Blood Marrow Transplant. 2015;21:1646–52.
38. Berger M, Lanino E, Cesaro S, et al. Feasibility and outcome of haploidentical hematopoietic stem cell transplantation with post-transplant high-dose cyclophosphamide for children and adolescents with hematologic malignancies: an AIEOP-GITMO retrospective multicenter study. Biol Blood Marrow Transplant. 2016;22:902–9.
39. Dietrich S, Finel H, Martinez C, et al. Post-transplant cyclophosphamide-based haplo-identical transplantation as alternative to matched sibling or unrelated donor transplantation for non-Hodgkin lymphoma: a registry study by the European society for blood and marrow transplantation. Leukemia. 2016;30:2086–9.
40. Bertaina A, Zecca M, Buldini B, et al. Unrelated donor vs HLA-haploidentical alpha/beta T-cell- and B-cell-depleted HSCT in children with acute leukemia. Blood. 2018;132:2594–607.
41. Davies SM, Ramsay NK, Klein JP, et al. Comparison of preparative regimens in transplants for children with acute lymphoblastic leukemia. J Clin Oncol. 2000;18:340–7.
42. Kebriaei P, Anasetti C, Zhang MJ, et al. Intravenous busulfan compared with total body irradiation pretransplant conditioning for adults with acute lymphoblastic leukemia. Biol Blood Marrow Transplant. 2018;24:726–33.
43. Kato M, Takahashi Y, Tomizawa D, et al. Comparison of intravenous with oral busulfan in allogeneic hematopoietic stem cell transplantation with myeloablative conditioning regimens for pediatric acute leukemia. Biol Blood Marrow Transplant. 2013;19:1690–4.
44. Koh K, Tomizawa D, Moriya Saito A, et al. Early use of allogeneic hematopoietic stem cell transplantation for infants with MLL gene-rearrangement-positive acute lymphoblastic leukemia. Leukemia. 2015;29:290–6.
45. Brochstein JA, Kernan NA, Groshen S, et al. Allogeneic bone marrow transplantation after hyperfractionated total-body irradiation and cyclophosphamide in children with acute leukemia. N Engl J Med. 1987;317:1618–24.
46. Gassas A, Sung L, Saunders EF, Doyle JJ. Comparative outcome of hematopoietic stem cell transplantation for pediatric acute lymphoblastic leukemia following cyclophosphamide and total body irradiation or VP16 and total body irradiation conditioning regimens. Bone Marrow Transplant. 2006;38:739–43.
47. Kato M, Ishida H, Koh K, et al. Comparison of chemotherapeutic agents as a myeloablative conditioning with total body irradiation for pediatric acute lymphoblastic leukemia: a study from the pediatric ALL working group of the Japan Society for Hematopoietic Cell Transplantation. Pediatr Blood Cancer. 2015;62:1844–50.
48. Tracey J, Zhang MJ, Thiel E, Sobocinski KA, Eapen M. Transplantation conditioning regimens and outcomes after allogeneic hematopoietic cell transplantation in children and adolescents with acute lymphoblastic leukemia. Biol Blood Marrow Transplant. 2013;19:255–9.
49. Fujino H, Ishida H, Iguchi A, et al. High rates of ovarian function preservation after hematopoietic cell transplantation with melphalan-based reduced intensity conditioning for pediatric acute leukemia: an analysis from the Japan Association of Childhood Leukemia Study (JACLS). Int J Hematol. 2019;109:578–83.
50. Kato K, Kato M, Hasegawa D, et al. Comparison of transplantation with reduced and myeloablative conditioning for children with acute lymphoblastic leukemia. Blood. 2015;125:1352–4.

Part III
Supportive Issues in Pediatric ALL

Chapter 16
Supportive Therapy

Michihiro Yano

Abstract Proper supporting therapy for malignant patients is important for pain relief, safety of treatment, and improvement of treatment efficacy. This chapter focuses on the following three areas of supporting therapy.

1. Tumor lysis syndrome.
2. Chemotherapy-induced nausea and vomiting.
3. Cancer pain management.

Keywords Supporting therapy · Supportive care · Tumor lysis syndrome Hyperuricemia · Antiemetic agents · Persistent cancer pain · Analgesics · Opioids

16.1 Tumor Lysis Syndrome

Tumor lysis syndrome (TLS) is a major metabolic oncogenic emergency in pediatric acute lymphoblastic leukemia (ALL). Both active proliferation and rapid destruction of leukemia cells before and after initiation of treatment may cause laboratory abnormal findings, such as hyperuricemia, hyperkalemia, hyperphosphatemia, and hypocalcemia. Because these conditions can lead to a lethal condition, their appropriate management is crucial. Immediate patient risk classification is particularly important. The TLS diagnostic criteria established by Cairo et al. are available for objective assessment of TLS status [1]. These criteria use two components to allow clear evaluation of TLS: laboratory TLS and clinical TLS. To prevent the onset and progression of TLS, serum electrolytes and biochemical markers related to the kidney and liver functions should be monitored repeatedly. Before starting treatment for a leukemia patient, a sufficient volume of drip infusion is needed (100–125 mL/m^2/h). Make sure that the infusion solution does not contain potassium, phosphorus,

M. Yano (✉)
Department of Pediatrics, Akita University Hospital, Akita, Japan
e-mail: yanomi@doc.med.akita-u.ac.jp

M. Kato (ed.), *Pediatric Acute Lymphoblastic Leukemia*,
https://doi.org/10.1007/978-981-15-0548-5_16

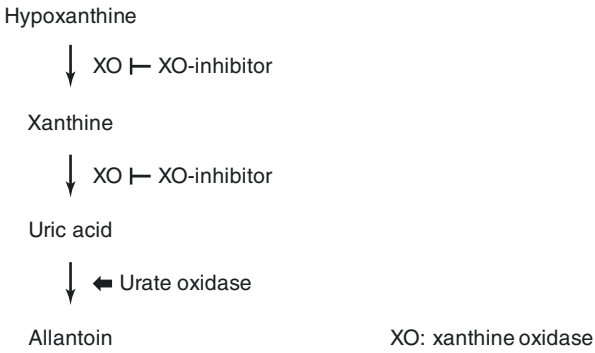


Fig. 16.1 Pathway of purine catabolism (after hypoxanthine) and sites of action for antihyperuricemic agents

or calcium. The alkalization of urine, which had been performed in the past, is not supposed to be done now. The patient's urine volume should be brought up to 2–3 mL/kg/h. If the patient is at a high risk for developing TLS, preventing hyperuricemia is essential. Two types of hyperuricemia-preventing agents can be administered to prevent TLS: xanthine oxidase inhibitors (XO-inhibitors) and urate oxidase (Fig. 16.1). XO-inhibitors (typically allopurinol and febuxostat) inhibit the oxidation-effect step converting hypoxanthine into xanthine and xanthine into uric acid. As XO-inhibitors do not affect already generated uric acid, they should be administered as soon as possible if TLS onset is suspected. Urate oxidase (rasburicase) can promote the conversion of uric acid to water-soluble allantoin. Since rasburicase is a recombinant agent, its re-administration cannot be confirmed to be safe. The administration of rasburicase to G6PD-deficient patient is contraindicated. When a patient is hyperleukocytic (leukocyte count, 100–300 $\times$ 10^9/L), leukapheresis should also be performed in combination with administration of these agents.

16.2 Chemotherapy-Induced Nausea and Vomiting

Chemotherapy-induced nausea and vomiting (CINV) in one of the major adverse effects experienced by patients undergoing chemotherapy. Preventing CINV is thus an important step in maintaining treatment quality for patients. The emetic risk of chemotherapeutic agents can be roughly classified (Table 16.1, modification of Ref. [2], but the degree of symptoms varies depending on the dose, their combination, and the individual variations. CINV is induced through stimulation of the chemoreceptor trigger zone (CTZ) located in the floor of the fourth ventricle. CINV can be well controlled by reducing the stimulation of neurotransmitters such as serotonin and substance P. Serotonin secreted from enterochromaffine cells following stimulation by anticancer agents binds to 5-HT_3 receptors in the digestive tract and CTZ, but 5-HT_3 receptor antagonists (Table 16.2) competitively bind to these receptors at both sites. Although first-generation 5-HT_3 receptor antagonists can suppress CINV in the acute phase, they lack sufficient efficacy in the late phase. However, second-generation

Table 16.1 Emetic risk of anticancer agents in ALL

High
Cyclophosphamide (high dose)
Cytarabine (high dose)
Doxorubicin
Moderate
Cyclophosphamide
Cytarabine
Methotrexate
Daunorubicin
Mild
Vincristine
Etoposide
L-asparaginase
6-mercaptopurine

Table 16.2 Two types of antiemetic agents

5-HT_3 receptor antagonists
Granisetron, ondansetron, ramosetron, azasetron (1st generation) palonosetron (2nd generation)
NK_1 receptor antagonists
Aprepitant, fosaprepitant

5-HT_3 receptor antagonists (palonosetron) can ameliorate late emesis well. Neurokinin-1 (NK1) receptors, present in the CTZ and vomiting center, provide another CINV trigger point. Because substance P is a ligand for the NK1 receptor and is a major neurotransmitter involved in CINV, its antagonists can suppress unpleasant symptoms. NK1 receptor antagonists, such as aprepitant and fosaprepitant, have a superior antiemetic effect when combined with 5-HT_3 receptor antagonists in both the acute and late phases of CINV. In order to obtain better control of CINV, concomitant administration of other drugs such as corticosteroids, sedatives, and dopamine-receptor antagonists (e.g., metoclopramide) may be useful.

16.3 Cancer Pain Management

Cancer patients experience various types of pain after their disease onset, and pediatric patients in particular suffer pain that they have never experienced in their short life. Because expressing the detail characteristics of such pains is difficult, medical staffs must listen closely to their choice of words and complaints and organize the implied characteristics objectively. In assessing young patients' pain, it is necessary to understand the characteristics of children and evaluate their complaints among multidisciplinary medical teams. The correct assessment of pain is essential for planning and implementing appropriate pain management. Cancer pain is classifiable

into nociceptive pain and neuropathic pain, according to the disease condition. Nociceptive pain consists of somatic and visceral components and is often associated with tumor metastasis. Neuropathic pain, on the other hand, shows central and peripheral types and is caused by nerve damage or inflammation due to an advanced tumor. Some anticancer agents (e.g., vincristine) may cause peripheral-type neuropathic pain. With appropriate assessments of pain, a pharmacological treatment plan can be formulated. In 2012, the WHO recommended meaningful guidelines for managing persistent pain in children. In those guidelines, the following four key concepts were presented regarding the correct use of analgesics [3].

– Use of a two-step strategy,
– Provision doses at regular intervals,
– Use of an appropriate route of administration,
– Adaptation of treatment to the individual child.

In the previous WHO guideline (1986), these points were introduced as a "three-step strategy," "by the clock," "by the mouth," and "by the individual," respectively. With the introduction of the two-step strategy, recommended medications are indicated according to the severity of pain (Table 16.3). In this approach, the first step targets mild pain, and the administration of NSAID, such as paracetamol (acetaminophen) or ibuprofen, is recommended (Table 16.4). If the patient has moderate to severe pain, a strong opioid (morphine, fentanyl, or oxycodone) is then introduced as a second-step agent. It is necessary to fully understand the side effects of opioids and respond promptly if any such effects are observed. Because opioid-induced constipation is common, some laxatives should be administered for prophylactically. Although many types of analgesic adjuvant medicines can support the effects of main analgesics, careful use is required for infant patients, in terms of their safety.

Table 16.3 Two-step analgesic strategy

	Level of pain	Type of anagesics	Medicine
1st step	Mild	NSAIDs	Paracetamol, Ibuprofen
2nd step	Moderate to severe	Strong opioids	Morphine, Fentanyl, Oxycodone

Table 16.4 Dosage of first-step analgesics

	Dose (oral route)			
Medicine	Neonates from 0 to 29 days	Infants from 30 days to 3 months	Infants from 3 to 12 months or Child from 1 to 12 years	Maximum daily dose
Paracetamol	5–10 mg/kg every 6–8 h	10 mg/kg every 4–6 h	10–15 mg/kg every 4–6 h	Neonates, infants and children: 4 doses/day
Ibuprofen	–	–	5–10 mg/kg every 6–8 h	Child: 40 mg/kg/day

References

1. Cairo MS, Coiffier B, Reiter A, Younes A, Expert Panel TLS. Recommendations for the evaluation of risk and prophylaxis of tumour lysis syndrome (TLS) in adults and children with malignant diseases: an expert TLS panel consensus. Br J Med. 2010;149:578–86.
2. Hesketh PJ, Kris MG, Grungerg SM, Hainsworth JD, Harker G, Aapro MS, Gandara D, Lindley CM. Proposal for classifying the acute emetogenicity of cancer chemotherapy. J Clin Oncol. 1997;15:103–9.
3. WHO guidelines on the pharmacological treatment of persisting pain in children with medical illness. 2012. http://apps.who.int/medicinedocs/documents/s19116en/s19116en.pdf.

Chapter 17
Late Effects in Pediatric Acute Lymphoblastic Leukemia

Motohiro Kato

Abstract Overall survival probability of pediatric acute lymphoblastic leukemia is currently 80–90%. Considering this dramatic improvement, it has become increasingly important to recognize the occurrence of long-term late effects. Severe late effects—including secondary malignant neoplasms, cardiotoxicity, osteonecrosis, neurocognitive sequelae, and infertility—affect quality of life of childhood leukemia survivors. Cooperative groups have provided essential information about the long-term effects, giving recommendations for long-term follow-up.

Keywords Secondary malignant neoplasms · Cardiotoxicity · Osteonecrosis Neurocognitive dysfunction

17.1 Introduction

In the past two decades, the survival probability of children with acute lymphoblastic leukemia (ALL) has dramatically improved up to 80–90%. Thus, consideration for quality of life status in survivors is as important as further reduction of relapse risk. In general, children can tolerate more intensive therapy than adults, but chemotherapy and irradiation during infancy and childhood potentially cause late effects [1–3], defined as physical or psychological problems that persist or develop after 5-year from leukemia treatment.

Numerous reports demonstrated that long-term survivors of childhood leukemia are at a risk of developing various late effects [1, 2], such as secondary malignant neoplasms (SMNs), organ dysfunction, growth retardation, and decreased fertility (Table 17.1). Childhood leukemia survivors also often face social and psychological barriers, including schooling and job problems. To minimize the risk for late effects, ALL therapy has evolved substantially over time, particularly with the elimination

M. Kato (✉)
Department of Transplantation and Cell Therapy, Children's Cancer Center, National Center for Child Health and Development, Setagaya-ku, Tokyo, Japan
e-mail: kato-mt@ncchd.go.jp

M. Kato (ed.), *Pediatric Acute Lymphoblastic Leukemia*,
https://doi.org/10.1007/978-981-15-0548-5_17

Table 17.1 Late effects of pediatric acute lymphoblastic leukemia

Late effects	Risk factors
Secondary malignant neoplasms	Irradiation, alkylating agents, topoisomerase II inhibitors
Cardiotoxicity	High cumulative dose of anthracycline (≥250 mg/m^2 of doxorubicin equivalent), younger age at treatment (<5 years old)
Osteonecrosis	Steroid (dexamethasone), older age, female
Neurocognitive sequelae	Cranial irradiation, intrathecal therapy (>20 times)
Dental problem	Irradiation, busulfan, younger age
Infertility	Irradiation, busulfan, older age (female)

of prophylactic cranial irradiation and the risk-adjusted use of chemotherapy [4]. Long-term medical follow-up based on current knowledge of late effects is required to maintain survivors' health and quality of life [3, 5]. The long-term follow-up guidelines for childhood cancer survivors by the Children's Oncology Group are found in http://www.survivorshipguidelines.org/.

17.2 Secondary Malignant Neoplasms

SMNs are one of the most serious complications and leading causes of late mortality of childhood cancer survivors [5–7]. DNA-damaging effect of chemotherapeutic agents and irradiation causes secondary malignancies in ALL patients treated with these modalities [7, 8]. Strikingly, the risk of developing SMNs remains elevated for more than 20 years from end of treatment. To avoid SMNs, recent clinical trials challenged to eliminate cranial irradiation by replacing with intrathecal therapy, and it successfully reduced an incidence of secondary brain tumor less than 2% [3, 9, 10].

Therapy-related solid tumors have a strong association with irradiation. Follow-up screening and surveillance of brain tumors should be performed after cranial irradiation, and thyroid cancer is also observed after stem cell transplantation with total body irradiation. As SMNs, hematologic malignancies are also observed after leukemia treatment. "Therapy-related" leukemia is mainly myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML). Previous studies showed that alkylating agents such as cyclophosphamide caused MDS/AML associated with chromosome abnormality involving deletion of chromosomes 5 and/or 7, while MDS/AML induced by topoisomerase II inhibitor is frequently associated with *MLL* rearrangement [11].

Recently, several studies demonstrated importance of germline pathogenic variants in cancer-predisposing genes in pediatric cancer. Familial cancer history can be a risk factor for developing SMNs [12], suggesting that genetic susceptibility also confers prevalence of SMNs. Actually, a large-scale study reported that loss-of-function germline *TP53* variants increased a risk of second malignant neoplasms [13].

17.3 Cardiotoxicity

Anthracyclines are widely used anticancer agents, not limited to pediatric ALL, but these are well-known causes of late cardiomyopathy, caused by myocardial injury due to formation of free radicals. In childhood cancer survivors, the reported incidence of anthracycline-associated clinical heart failure (HF) has been as high as 2% by 20 years after treatment [14], and incidence continues to increase with extended follow-up [15].

High cumulative dose of anthracyclines is a strong risk factor for heart problems, and leukemia survivors with ≥250 mg/m^2 of doxorubicin or the equivalent doses of other anthracyclines should be followed by annual echocardiogram and electrocardiogram. Dexrazoxane has a cardioprotective effect against anthracycline-induced heart failure [16].

17.4 Osteonecrosis

Avascular necrosis (AVN) of bone is an important musculoskeletal complication affecting activity of daily life of leukemia survivors. The diagnosis of AVN should be confirmed by magnetic resonance imaging. Low grade AVN is asymptomatic which can be found only by MRI [17], but severe AVN causes pain, and surgical procedure including total joint replacement is required in the most severe cases [18].

AVN is caused by reduced blood supply to the bones, and the older age (≥10 years), female, and dexamethasone usage are the risk factor for developing osteonecrosis. Alternate-week dexamethasone during delayed intensification phases reduced the risk of AVN without increasing relapse risk for children of older age [19]. A genome-wide association study identified an association between osteonecrosis and inherited variants in genes encoding glutamate receptors [20].

17.5 Neurocognitive Sequelae

Typically, neurocognitive sequelae develop as a result of irradiation for the whole brain [21]. Previous studies showed that risk factors for this late effect is higher dose of irradiation, younger age, and concomitant use of intrathecal therapy [22]. Childhood ALL survivors have a greater likelihood of being placed in special education or learning programs than their siblings, but most are able to overcome these problems [23].

To avoid neurocognitive deficit and SMNs, recent regimens omit prophylactic irradiation; intensive intrathecal injection and high-dose methotrexate also potentially causes long-term neurocognitive deficits and neurobehavioral problems [24, 25]. Younger patients and females are risk factors for these late effects [26].

17.6 Infertility

Compared with their siblings, childhood cancer survivors had an increased risk of clinical infertility [27]. Irradiation and high cumulative dose of alkylating agents potentially cause permanent infertility of childhood leukemia survivors. Myeloablative irradiation (≥8 Gy) and busulfan (>8 mg/kg) were associated with infertility due to gonadal dysfunction, and more than 90% of infertility was reported [28]. Age at receiving transplantation seems to be important in determining gonadal dysfunction, 50% of prepubertal girls who received total body irradiation will enter puberty spontaneously and achieve menarche at a normal age [28].

17.7 Dental Sequelae

Dental abnormalities can also occur in childhood leukemia survivors. Risk factors for aberrant dental development are irradiation and alkylating agents. Especially, children who received hematopoietic stem cell transplantation at younger age had many disturbances in dental development [29]. Regular dental examination is required.

References

1. Oeffinger KC, Mertens AC, Sklar CA, et al. Chronic health conditions in adult survivors of childhood cancer. N Engl J Med. 2006;355:1572–82.
2. Gibson TM, Mostoufi-Moab S, Stratton KL, et al. Temporal patterns in the risk of chronic health conditions in survivors of childhood cancer diagnosed 1970–99: a report from the childhood cancer survivor study cohort. Lancet Oncol. 2018;19:1590–601.
3. Essig S, Li Q, Chen Y, et al. Risk of late effects of treatment in children newly diagnosed with standard-risk acute lymphoblastic leukaemia: a report from the childhood cancer survivor study cohort. Lancet Oncol. 2014;15:841–51.
4. Hudson MM, Neglia JP, Woods WG, et al. Lessons from the past: opportunities to improve childhood cancer survivor care through outcomes investigations of historical therapeutic approaches for pediatric hematological malignancies. Pediatr Blood Cancer. 2012;58:334–43.
5. Armstrong GT, Liu Q, Yasui Y, et al. Late mortality among 5-year survivors of childhood cancer: a summary from the childhood cancer survivor study. J Clin Oncol. 2009;27:2328–38.
6. Loning L, Zimmermann M, Reiter A, et al. Secondary neoplasms subsequent to Berlin-Frankfurt-Munster therapy of acute lymphoblastic leukemia in childhood: significantly lower risk without cranial radiotherapy. Blood. 2000;95:2770–5.
7. Schmiegelow K, Levinsen MF, Attarbaschi A, et al. Second malignant neoplasms after treatment of childhood acute lymphoblastic leukemia. J Clin Oncol. 2013;31:2469–76.
8. Ishida Y, Maeda M, Urayama KY, et al. Secondary cancers among children with acute lymphoblastic leukaemia treated by the Tokyo Children's cancer study group protocols: a retrospective cohort study. Br J Haematol. 2014;164:101–12.
9. Walter AW, Hancock ML, Pui CH, et al. Secondary brain tumors in children treated for acute lymphoblastic leukemia at St Jude Children's research hospital. J Clin Oncol. 1998;16:3761–7.

10. Pui CH, Campana D, Pei D, et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. N Engl J Med. 2009;360:2730–41.
11. Pedersen-Bjergaard J, Philip P. Balanced translocations involving chromosome bands 11q23 and 21q22 are highly characteristic of myelodysplasia and leukemia following therapy with cytostatic agents targeting at DNA-topoisomerase II. Blood. 1991;78:1147–8.
12. Andersson A, Enblad G, Tavelin B, et al. Family history of cancer as a risk factor for second malignancies after Hodgkin's lymphoma. Br J Cancer. 2008;98:1001–5.
13. Qian M, Cao X, Devidas M, et al. TP53 germline variations influence the predisposition and prognosis of B-cell acute lymphoblastic leukemia in children. J Clin Oncol. 2018;36:591–9.
14. van der Pal HJ, van Dalen EC, van Delden E, et al. High risk of symptomatic cardiac events in childhood cancer survivors. J Clin Oncol. 2012;30:1429–37.
15. Chow EJ, Chen Y, Kremer LC, et al. Individual prediction of heart failure among childhood cancer survivors. J Clin Oncol. 2015;33:394–402.
16. Asselin BL, Devidas M, Chen L, et al. Cardioprotection and safety of dexrazoxane in patients treated for newly diagnosed T-cell acute lymphoblastic leukemia or advanced-stage lymphoblastic non-hodgkin lymphoma: a report of the children's oncology group randomized trial pediatric oncology group 9404. J Clin Oncol. 2016;34:854–62.
17. Schmiegelow K, Attarbaschi A, Barzilai S, et al. Consensus definitions of 14 severe acute toxic effects for childhood lymphoblastic leukaemia treatment: a Delphi consensus. Lancet Oncol. 2016;17:e231–9.
18. Mattano LA Jr, Sather HN, Trigg ME, Nachman JB. Osteonecrosis as a complication of treating acute lymphoblastic leukemia in children: a report from the Children's cancer group. J Clin Oncol. 2000;18:3262–72.
19. Mattano LA Jr, Devidas M, Nachman JB, et al. Effect of alternate-week versus continuous dexamethasone scheduling on the risk of osteonecrosis in paediatric patients with acute lymphoblastic leukaemia: results from the CCG-1961 randomised cohort trial. Lancet Oncol. 2012;13:906–15.
20. Karol SE, Yang W, Van Driest SL, et al. Genetics of glucocorticoid-associated osteonecrosis in children with acute lymphoblastic leukemia. Blood. 2015;126:1770–6.
21. Waber DP, Turek J, Catania L, et al. Neuropsychological outcomes from a randomized trial of triple intrathecal chemotherapy compared with 18 Gy cranial radiation as CNS treatment in acute lymphoblastic leukemia: findings from Dana-Farber Cancer Institute ALL consortium protocol 95-01. J Clin Oncol. 2007;25:4914–21.
22. Campbell LK, Scaduto M, Sharp W, et al. A meta-analysis of the neurocognitive sequelae of treatment for childhood acute lymphocytic leukemia. Pediatr Blood Cancer. 2007;49:65–73.
23. Haupt R, Fears TR, Robison LL, et al. Educational attainment in long-term survivors of childhood acute lymphoblastic leukemia. JAMA. 1994;272:1427–32.
24. Krull KR, Hardy KK, Kahalley LS, Schuitema I, Kesler SR. Neurocognitive outcomes and interventions in long-term survivors of childhood cancer. J Clin Oncol. 2018;36:2181–9.
25. Iyer NS, Balsamo LM, Bracken MB, Kadan-Lottick NS. Chemotherapy-only treatment effects on long-term neurocognitive functioning in childhood ALL survivors: a review and meta-analysis. Blood. 2015;126:346–53.
26. Cheung YT, Sabin ND, Reddick WE, et al. Leukoencephalopathy and long-term neurobehavioural, neurocognitive, and brain imaging outcomes in survivors of childhood acute lymphoblastic leukaemia treated with chemotherapy: a longitudinal analysis. Lancet Haematol. 2016;3:e456–66.
27. Barton SE, Najita JS, Ginsburg ES, et al. Infertility, infertility treatment, and achievement of pregnancy in female survivors of childhood cancer: a report from the childhood cancer survivor study cohort. Lancet Oncol. 2013;14:873–81.
28. Sanders JE, Hawley J, Levy W, et al. Pregnancies following high-dose cyclophosphamide with or without high-dose busulfan or total-body irradiation and bone marrow transplantation. Blood. 1996;87:3045–52.
29. Ruyssinck L, Toulouse K, Bordon Cueto de Braem V, Cauwels R, Dhooge C. Impact of hematopoietic stem cell transplantation on dental development. Biol Blood Marrow Transplant. 2019;25:107–13.