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Chromosome abnormalities in advanced stage T-cell lymphoblastic lymphoma of children and adolescents: a report from Japanese Paediatric Leukaemia/Lymphoma Study Group (JPLSG) and review of the literature

Masahiro Sekimizu,[1] Shosuke Sunami,[2] Atsuko Nakazawa,[3] Yasuhide Hayashi,[4] Yuri Okimoto,[5] Akiko M. Saito,[6] Keizo Horibe,[1,7] Masahito Tsurusawa[8] and Tetsuya Mori[9]

[1]Department of Paediatrics, National Hospital Organization Nagoya Medical Centre, Aichi, [2]Department of Paediatrics, Narita Red Cross Hospital, Chiba, [3]Department of Pathology, National Centre for Child Health and Development, Tokyo, [4]Department of Haematology/Oncology, Gunma Children's Medical Centre, Gunma, [5]Division of Haematology and Oncology, Chiba Children's Hospital, Chiba, [6]Department of Clinical Research Promotion, Clinical Research Centre, National Hospital Organization Nagoya Medical Centre, [7]Clinical Research Centre, National Hospital Organization Nagoya Medical Centre, [8]Department of Paediatrics, Aichi Medical University, Aichi, and [9]Division of Paediatric Oncology, National Centre for Child Health and Development, Tokyo, Japan

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Correspondence: Masahiro Sekimizu, Department of Paediatrics, National Hospital Organization Nagoya Medical Centre, 4-1-1 Sannomaru, Naka-ku, Nagoya, Aichi 460-0001, Japan. E-mail: sekimizu@nnh.hosp.go.jp

Summary

T-cell acute lymphoblastic leukaemia (T-ALL) and T-cell lymphoblastic lymphoma (T-LBL) are combined into one category as T lymphoblastic leukaemia/lymphoma in the current World Health Organization (WHO) classification. However, there is still ongoing discussion on whether T-ALL and T-LBL are two separate entities or represent two variant phenotypes of the same disease. Cytogenetic analysis has been used to identify the molecular background of haematological malignancies. To compare the distribution of chromosomal abnormalities of T-ALL and T-LBL, large series of cytogenetic data are required, but are absent in T-LBL in contrast to the abundant data in T-ALL. Among 111 T-LBL cases in our clinical trial, we obtained complete cytogenetic data from 56 patients. The comparison between our cytogenetic findings and those from three published T-LBL studies revealed no significant difference. However, meta-analysis showed that translocations involving chromosome region 9q34 were significantly more common in T-LBL than in T-ALL. In particular, four out of the 92 T-LBL cases, but none of the 523 paediatric T-ALL cases, showed translocation t(9;17)(q34;q22–23) ($P = 0{\cdot}0004$). Further studies are needed for the possible linkage between abnormal expression of genes located at 9q34 and/or 17q22–23 and the unique 'lymphoma phenotype' of T-LBL.

Keywords: T-cell lymphoma, child, non-Hodgkin lymphoma, cancer cytogenetics, leukaemia.

In children and adolescents, precursor T lymphoblastic neoplasms have been classified into two diseases: T-cell acute lymphoblastic leukaemia (T-ALL) and T-cell lymphoblastic lymphoma (T-LBL). Although the current World Health Organization (WHO) classification designates both malignancies as T lymphoblastic leukaemia/lymphoma (Borowitz & Chan, 2008), there is continuing discussion on whether T-ALL and T-LBL are two separate entities or whether they represent two different clinical presentations of the same disease. They show overlapping clinical, pathological and immunophenotypic features. In general, the word 'lymphoma' is used if there is a bulky mass in the mediastinum or elsewhere, with less peripheral blood and bone marrow (BM) involvement. Most study groups distinguish between leukaemia and lymphoma on the basis of the extent of BM involvement: patients with <25% lymphoblasts in the BM are diagnosed with lymphoblastic lymphoma; in cases

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of 25% or more BM blasts, the diagnosis is leukaemia. While this distinction may appear somewhat arbitrary, a notable observation is that T-LBL patients with large mediastinal masses frequently exhibit little, if any, evidence of tumour dissemination and BM involvement, but the molecular background for this difference is unknown.

Chromosomal analysis has been widely used as a primary step that is required to narrow down the responsible genes that define a disease entity. For instance, discovery of Ph chromosome led to the identification of the chimeric *BCR/ABL1* gene, which is responsible for and defines chronic myeloid leukaemia. Compared with T-ALL, chromosomal abnormalities in T-LBL are not well defined. Reports in the literature and current textbooks claim that the typical chromosomal aberrations reported in T-ALL can also be found in T-LBL (Borowitz & Chan, 2008). However, there are no large series of cytogenetic data on T-LBL (Burkhardt, 2010).

This study aimed to fill the gap regarding cytogenetic data in T-LBL and compare the cytogenetic findings of T-ALL and T-LBL, which may lead to identification of the molecular background behind phenotypical differences between the two disease entities.

Study patients

From November 2004 to October 2010, 154 eligible children (aged 1–18 years) with newly diagnosed advanced stage LBL (Murphy stages III and IV) (Murphy, 1980) were entered in the Japanese Paediatric Leukaemia/Lymphoma Study Group (JPLSG) ALB-NHL03 study (UMIN000002212, http://www.umin.ac.jp/ctr/index-j.htm). Patients with primary immunodeficiencies, Down syndrome and T-cell diseases as second malignancies were excluded. The ethics committee of each participating institute approved the study protocol.

Cytogenetic analysis

Cytogenetic analysis was performed on cell suspensions obtained from 31 tumour/lymph nodes, 19 pleural effusions and six bone marrow samples. The methods of chromosome preparation for cytogenetic analysis are described elsewhere (Sanger *et al*, 1987; Horsman *et al*, 2001). Karyotypes are described according to the International System for Human Cytogenetic Nomenclature (ISCN) (Shaffer & Tommerup, 2005). Only those cases with abnormal cytogenetic study results, defined as two or more cells with the same structural abnormality or the same numerical gain, three or more cells with the same numerical loss or isolated cells with disease-associated abnormalities, were eligible for inclusion in this study.

Statistical methods

Two-tailed Fisher's exact test was used to analyse the patients' characteristics and the frequency of each chromosome abnormality. Significant differences in the analysis of he frequency of each chromosome abnormality were determined by the two-tailed Fisher's exact test with Bonferroni correction comparison. The P value threshold for inclusion of a new variable was chosen to be $P < 0{\cdot}003$ in this analysis (0·05/17, after Bonferroni correction). A review of T-LBL and T-ALL karyotypes reported in the literature was obtained from a PubMed search and information on chromosome abnormalities and gene fusions was obtained from Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer (http://cgap.nci.nih.gov/Chromosomes/Mitelman).

Results

Patient characteristics

A total of 154 children were enrolled on JPLSG ALB-NHL03 protocols; 111 cases were T-LBL. Among 111 T-LBL cases, the study population for the current analysis included 56 patients for whom complete cytogenetic data were obtained. With respect to presenting features, patients with reviewed and accepted cytogenetic data were similar to both those without accepted cytogenetic data and the entire cohort of concurrently enrolled T-lineage LBL patients (Table S1).

Frequency of chromosomal abnormalities

Multiple chromosome abnormalities were identified in 31 patients (45%). Structural chromosome abnormalities were identified in 29 patients (52%), and numerical chromosome abnormalities were identified in 18 patients (32%). Ploidy results included pseudodiploid in 14 patients (25%), hypodiploid in three patients (5%), hyperdiploid with 47–50 chromosomes in 10 patients (18%), hyperdiploid with more than 50 chromosomes in four patients (7%) and diploid in 25 patients (45%) (Table S2).

All of the hypodiploid cases had 43–45 chromosomes; none had a near-haploid karyotype. Of the four cases with more than 50 chromosomes, two had near-tetraploid karyotypes. The frequencies of ploidy groups in this series are compared with those reported in other series of karyotyped T-LBL patients and paediatric T-ALL (Table S2). Structural chromosome abnormalities were identified in 29 patients (52%). In the current study, seven patients (13% of those with abnormal karyotypes) exhibited a rearrangement at one or more of the chromosome bands (7p15, 7q32–36 and/or 14q11–13) that are the locations of T-cell receptor chain genes. Rearrangements in the 14q11–13 region, in which the T-cell receptor α/δ chain genes are located, were present in three patients (5%) of the karyotypically abnormal cases in this series (Table S2). Structural abnormalities involving chromosome region 9q34 were identified in nine patients (16%). Translocations involving chromosome region 9q34 were identified in three patients (5%) (t(9;17)(q34;q22), t(7;9)(q34;q34) and t(2;9)(q23;q34)). In comparison between cytogenetic findings in the current data and combined data of three published reports (Burkhardt

et al, 2006; Lones *et al*, 2007; Uyttebroeck *et al*, 2007; Table S1), the frequencies of numerical and structural cytogenetic abnormalities in T-LBL and T-ALL had no significant difference (Table S2).

We compared the cytogenetic findings in the current study with the published reports from the three largest-scale studies on T-LBL (Burkhardt *et al*, 2006; Lones *et al*, 2007; Uyttebroeck *et al*, 2007; Table S3) and those from the two largest-scale studies on T-ALL combined (Heerema *et al*, 1998; Schneider *et al*, 2000; Table S3) (Table I). The frequencies of almost all of the cytogenetic abnormalities in T-LBL and T-ALL had no significant difference, but translocation involving chromosome region 9q34 was significantly more common in T-LBL than in T-ALL ($P = 0{\cdot}0004$, Table S3) and translocation t(9;17) was also more common in T-LBL (4%, 4/92) than in T-ALL (0%, 0/523, $P = 0{\cdot}0004$) (Table I).

The current study included a patient with translocation t(9;17)(q34;q22). As far as we could tell from the consulted published reports, all T-LBL patients with translocation t(9;17) presented with a mediastinal mass and without any bone marrow involvement (Kaneko *et al*, 1988; Shikano *et al*, 1992) (Table II).

Table I. Comparison of cytogenetic findings between T-LBL and T-ALL.

	T-LBL		T-ALL		
	n	%	*n*	%	*P* value
Total	92		523		
Normal karyotype†	36	39	219	42	0·6478
Abnormal karyotype	56	61	304	58	0·6478
Hypodiploid	4	4	20	4	0·9999
Pseudodiploid	30	33	204	39	0·2000
Hyperdiploid(47–50)	18	20	64	12	0·0328
Hyperdiploid(>50)	4	4	16	3	0·5217
Any translocation	26	28	177	34	0·3367
Any del chrome.	19	21	160	31	0·0328
Any der chrome.	4	4	58	11	0·0583
del(6q)	6	7	69	13	0·0833
Loss of 9p	10	11	44	8	0·5487
Any 14q11–13 abnormality	10	11	72	14	0·5100
Any 7q32–36 abnormality	7	8	35	7	0·8220
Any translocation including 9q34	8	9	7	1	0·0004*
t(7;10)	1	1	2	0	0·3855
t(10;11)	1	1	8	2	0·9999
t(9;17)	4	4	0	0	0·0004*

†Includes one Klinefelter syndrome, and one inv(9) without other abnormality in current report.

The *P* value threshold for inclusion of a new variable was chosen to be 0·003 (0·05/17, after Bonferroni correction). *$P < 0{\cdot}003$.

T-LBL: current study (JPLSG ALB-NHL03) combined with three published reports(Burkhardt *et al*, 2006; Lones *et al*, 2007; Uyttebroeck *et al*, 2007).

T-ALL: combined two published reports (Heerema *et al*, 1998; Schneider *et al*, 2000).

Discussion

This is the largest study involving cytogenetic analysis of T-LBL and the first study to directly compare cytogenetic findings of T-LBL and T-ALL. The frequencies of almost all of the cytogenetic abnormalities in both entities were found to have no significant difference, but translocation involving chromosome region 9q34 was significantly more common in T-LBL than in T-ALL. The current study included a patient with unique translocation t(9;17)(q34;q22). Interestingly, four out of the 92 T-LBL cases, but none of the 523 paediatric T-ALL cases, showed this translocation ($P = 0{\cdot}0004$) (Table I). Translocation t(9;17) has been reported in several haematological diseases, such as precursor B-cell ALL (Coyaud *et al*, 2010), acute myeloid leukaemia (Mrózek *et al*, 2001), chronic myeloid leukaemia (DeAngelo *et al*, 2004), chronic lymphocytic leukaemia (Michaux *et al*, 2005), diffuse large B-cell lymphoma (Hammond *et al*, 1992) and follicular lymphoma (Aamot *et al*, 2007), but these breakpoints, 9q34 and 17q22–23, are limited in the cases of T-LBL (http://cgap.nci.nih.gov/Chromosomes/Mitelman). These results imply a linkage between abnormal expression of genes located at 9q34 and/or 17q22–23 and the unique phenotypes of the T-LBL mentioned above.

Cytogenetic analysis has been used to identify the molecular background of haematological malignancies. To compare the distribution of chromosomal abnormalities of T-ALL and T-LBL, large series of cytogenetic data are required, but are absent in T-LBL in contrast to the abundant data in T-ALL. Three recent series of cytogenetic data on paediatric T-LBL have been published, reporting the cytogenetic findings in 13, 11 and 12 paediatric T-LBL cases (Burkhardt *et al*, 2006; Lones *et al*, 2007; Uyttebroeck *et al*, 2007). Thus, this study can play a role to fill the gap of cytogenetic data on T-LBL.

Translocation involving chromosome region 9q34 was found to be significantly more common in T-LBL than in T-ALL (Table I). Among genes located in the 9q34 region, *SET, PKN3, ABL1, NUP214* and *NOTCH1* have previously been implicated in malignancy, with *SET, ABL1, NUP214* and *NOTCH1* being implicated in leukemogenesis (Ellisen *et al*, 1991; van Vlierberghe *et al*, 2008; Hagemeijer & Graux, 2010).

An oncogenic *SET-NUP214* fusion gene has been reported in a case of acute undifferentiated leukaemia with a reciprocal translocation t(9;9)(q34; q34) (von Lindern *et al*, 1992) and NK adult acute myeloid leukaemia as a result of a cryptic deletion of 9q34 (Rosati *et al*, 2007). van Vlierberghe *et al* (2008) identified the *SET-NUP214* fusion gene in three patient samples out of 92 paediatric cases of T-cell leukaemia. *SET-NUP214* may contribute to T-ALL pathogenesis by inhibition of T-cell maturation through the transcriptional activation of the *HOXA* genes (van Vlierberghe *et al*, 2008). However, the frequency of this mutation in T-LBL is unknown.

NOTCH1, previously termed *TAN1*, was discovered as a partner gene in T-ALL with a translocation t(7;9)(q34;q34.3), and was found in <1% of T-ALLs (Ellisen *et al*, 1991). Several

Table II. Clinical characteristics and detailed karyotype data in T-LBL patients with t(9;17).

	Age (years)	Sex	Tumour site	Stage	BM blast %	Karyotype
Kaneko *et al* (1988)	14	F	Mediastinum	III	0	46,XX,t(9;17)(q34;q23)
	15	M	Mediastinum	III	0	46,XY,-9,del(6)(q13q21),t(9;17)(q34;q23),+der(9)t(9;17)(q34;q23)
	10	M	Mediastinum	III	0	47,XY,+19,t(9;17)(q34;q23)
Shikano *et al* (1992)	14	F	Mediastinum	III	0	46,XX,t(9;17)(q34;q23)
	7	M	Mediastinum	III	0	49,XY-l,+der(l)t(l;?)(p36;?),t(9;17)(q34;q23),+14,+marl,+mar2
	5	F	Mediastinum	III	0	47,XX,t(9;17)(q34;q23),+der(17)t(9;17)(q34;q23)
Burkhardt *et al* (2006)	ND	ND	ND	ND	ND	46,XX,del(6)(q1?2q1?6),t(9;17)(q34;q22)
	ND	ND	ND	ND	ND	47,XX,t(9;17)(q34;q22),+20
Lones *et al* (2007)	8	M	Mediastinum	III	0	47,XY,t(9;17)(q3?4;q2?3),+20
Current study	7	M	Mediastinum	III	0	46,XY,t(9;17)(q34;q22)

ND, no data available.

study groups reported *NOTCH1* mutations in 31–62% of T-ALL patients (Weng *et al*, 2004; Breit *et al*, 2006; van Grotel *et al*, 2006; Zhu *et al*, 2006; Malyukova *et al*, 2007; Asnafi *et al*, 2009; Gedman *et al*, 2009; Park *et al*, 2009). In contrast, only two studies reported *NOTCH1* mutation analyses in T-LBL: Park *et al* (2009) reported *NOTCH1* mutations in six out of 14 paediatric T-LBL patients (43%), and Baleydier *et al* (2008) reported mutations in six out of nine paediatric T-LBL (66%), with 32 adult patients with *NOTCH1* mutations in 16 cases (54% in all patients) (Baleydier *et al*, 2008). According to these reports, the frequencies of *NOTCH1* mutation were not significantly different between T-LBL and T-ALL.

ABL1 fusion genes have been identified that provide proliferation and survival advantage to lymphoblasts. *NUP214-ABL1*, *EML1-ABL1*, *BCR-ABL1* and *ETV6-ABL1* chimeric genes have been reported. The most frequent one in T-ALL is the *NUP214-ABL*1 fusion gene, which has been identified in 6% of cases, in both children and adults (Graux *et al*, 2009). In addition, using an oligonucleotide microarray, *ABL1* overexpression was identified in 8% of cases in T-ALL (Chiaretti *et al*, 2007). Our review of these published reports indicated that the frequency of ABL1 mutation in T-LBL is unknown.

Raetz *et al* (2006) analysed the gene expression profiles of ten T-ALL BM samples and nine T-LBL samples using a microarray. They identified 133 genes for which the expression levels differed between T-LBL and T-ALL. *ZNF79* (encoding zinc finger protein 79) and *ABL1*, both located in chromosome region 9q34, were included in these genes and showed at least twofold higher overexpression in T-LBL than that in T-ALL. Additionally, *MED13* (previously termed *THRAP1*), which is located in 17q22-q23, also showed at least twofold higher overexpression in T-LBL than that in T-ALL (Raetz *et al*, 2006). Taking these findings together, it is possible that *ZNF79*, *ABL1* or *THRAP1* as well as other genes at 9q34 and 17q22–23 are involved in the 'lymphoma phenotype' such as a bulky mass in the mediastinum and minimal BM involvement. These findings need further study to determine if this linkage constitutes a unique 'lymphoma phenotype'.

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Authorship

MS designed the study, prepared the data file, performed the analysis, interpreted data and wrote the manuscript. SS is a lead principal investigator for the JPLSG ALB-NHL03 study. AN contributed to pathological diagnosis. YH contributed to chromosome analysis. YO is a principal investigator contributing a patient to this study. AMS contributed to statistical analysis. KH received a research grant from the Ministry of Health, Labour and Welfare of Japan. MT is a chairperson of JPLSG. TM is a chairperson of JPLSG lymphoma committee. SS, KH, MT and TM were primarily responsible for the study design, data analysis and interpretation of the data. All authors approved the final manuscript.

Disclosure

The authors declare no competing financial interests.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Respective clinical characteristics with and without karyotype data in 111 T-LBL patients in the current study.

Table S2. Comparison of cytogenetic findings in T-LBL between current study and combined data of three published reports.

Table S3. Published data of cytogenetic findings in T-LBL and T-ALL.

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Safety Assessment of Intensive Induction Therapy in Childhood Anaplastic Large Cell Lymphoma: Report of the ALCL99 Randomised Trial

Grazyna Wrobel, MD,[1] Audrey Mauguen, MSc,[2]* Angelo Rosolen, MD,[3] Alfred Reiter, MD,[4] Denise Williams, MD,[5] Keizo Horibe, MD,[6] Laurence Brugières, MD,[2] Marie-Cécile Le Deley, MD, PhD[2,7] on behalf of European Inter-Group for Childhood, Non-Hodgkin Lymphoma (EICNHL)

Background. ALCL99 protocol including six courses of chemotherapy derived from the NHL-BFM protocol is widely used for the treatment of paediatric anaplastic large-cell lymphoma. In the ALCL99 trial, patients were randomised to receive MTX 1 g/m^2 in 24 hr with intrathecal injection (MTX1) versus MTX 3 g/m^2 in 3 hr without intrathecal (MTX3); then to receive or not vinblastine (high-risk patients). The present study provides information about the acute adverse reactions (ARs) during the six courses of the ALCL99 treatment, assesses risk factors for ARs and evaluates the risk of overweight related to treatment. ***Methods.*** Data concerning ARs were assessed using CTCv2 and analysed overall and according to the type of course. ***Results.*** Between 1999 and 2005, 352 patients were recruited. Toxicity assessed after 2050 courses included grade 4 neutropaenia (70% of courses), grade 3–4 stomatitis (13%), grade 3–4 transaminase elevation (10%) and grade 3–4 infection (5%). Four patients (1%) died of toxicity. The toxicity profile differed between courses-A (significantly more haematological toxicity) and courses-B (significantly more stomatitis). The percentage of ARs was higher after the first course than after subsequent courses. Severe toxicity was more frequent after MTX1 than after MTX3 courses but did not differ between courses with or without vinblastine. Overall 20% of patients had a weight gain exceeding 20%. ***Conclusions.*** The high rate of acute toxicity should be considered when using the ALCL99 protocol. Chemotherapy including MTX 3 g/m^2 in 3 hr was less toxic than the same regimen with MTX 1 g/m^2 in 24 hr. Adding vinblastine did not increase the risk of toxicity. Pediatr Blood Cancer2011;56:1071–1077.

Key words: ALCL99 protocol; anaplastic large cell lymphoma; child; chemotherapy; overweight; toxicity

INTRODUCTION

Anaplastic large-cell lymphoma (ALCL) accounts for 10%–15% of childhood non-Hodgkin lymphomas (NHL) [1]. The optimal treatment for paediatric ALCL in terms of efficacy and safety is still under investigation. While most European countries recommend an intensive, short chemotherapy regimen based on treatment of B-NHL [2–5], several other paediatric groups treated patients with less intensive but prolonged chemotherapy [6,7]. A strategy based on NHL-BFM 90, with six courses of polychemotherapy including high dose of methotrexate (HD MTX) given at 3-week interval has proven to be efficient for treating patients with ALCL [4,8]. Furthermore, this protocol including low cumulative doses of alkylating agents and anthracyclines is expected to be associated with a low risk of long term toxicity. A large international trial on childhood ALCL, the ALCL99 trial, was undertaken to compare the efficacy and safety of two doses and modes of administration of MTX and to study the impact of adding vinblastine in patients at high risk of failure. This trial could show the high efficiency of this protocol with a 2-year event-free survival of 74.1% (95% CI, 69.2% to 78.4%) on the whole population of the study with no impact of the dose and administration of MTX on the outcome [9]. Given the good results of this study, ALCL99 protocol is now widely used, not only in centres which participated to the trial, but also outside Europe and in adults. Knowledge of the toxicity profile of this protocol may help to monitor toxicity for further patients. In addition to comparison of the toxicity between the two doses and modes of administration of MTX [9], the objective of this paper is to provide a complete information about the observed acute adverse reactions (ARs) during the six courses of the ALCL99 treatment, to asses risk factors for acute toxicity and to evaluate the risk of overweight related to treatment.

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MATERIALS AND METHODS

Study Population

The present report is based on the analysis of the data concerning patients randomised in the ALCL99-R1 trial between November 1999 and December 2005. This trial involved 175 centres across 11 European countries plus Japan.

[1]Department of Pediatric Bone Marrow Transplantation, Oncology and Haematology, Medical University of Wroclaw, Wroclaw, Poland; [2]Institut Gustave-Roussy (IGR), Villejuif, France; [3]University Hospital, Padova, Italy; [4]Justus Liebig University, Giessen, Germany; [5]Addenbrookes Hospital, Cambridge, UK; [6]National Hospital Organization Nagoya Medical Center, Nagoya, Japan; [7]Université Paris-Sud, Le Kremlin Bicêtre, France

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*Correspondence to: Audrey Mauguen, MSc, Service de Biostatistique et d'Epidémiologie, Institut de Cancérologie Gustave-Roussy, 114 rue Edouard Vaillant, 94805 Villejuif cedex, France.
E-mail: audrey.mauguen@igr.fr

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TABLE I. Chemotherapy Doses and Schedule by Type of Course (2050 Induction Courses Analysed)

Chemotherapy doses & schedule	Course A n = 420	Course AM n = 423	Course AV n = 95	Course AMV n = 94	Course B n = 366	Course BM n = 372	Course BV n = 144	Course BMV n = 136
Dexamethasone	10 mg/m^2 D 1–5							
Ifosfamide	800 mg/m^2 D 1–5					0		
Cytarabine	150 mg/m^2 × 2 D 4 and 5					0		
Etoposide	100 mg/m^2 D 4 and 50					0		
Cyclophosphamide	0					200 mg/m^2 D 1–5		
Doxorubicin	0					25 mg/m^2 D 4 and 5		
MTX	1 g/m^2 D 1[a]	3 g/m^2 D 1[b]	1 g/m^2 D 1[a]	3 g/m^2 D 1[b]	1 g/m^2 D 1[a]	3 g/m^2 D 1[b]	1 g/m^2 D 1[a]	3 g/m^2 D 1[b]
Triple IT injection[c]	D 1	0	D 1	0	D 1	0	D 1	0
Vinblastine	0	0	6 mg/m^2 D 1	6 mg/m^2 D 1	0	0	6 mg/m^2 D 1	6 mg/m^2 D 1

[a]Arm MTX1: Methotrexate 1 g/m^2 in a 24-hr infusion with triple IT at day 1 and leucovorin rescue (15 mg/m^2) at H42, 48 and 54. [b]Arm MTX3: Methotrexate 3 g/m^2 in a 3-hr infusion with no IT and leucovorin rescue (15 mg/m^2 every 6 hr) starting at H24 and ending when the MTX level was <0.15 μm/L. [c]Triple IT injection include Methotrexate, Cytarabine, and HydroCortisone Hemi-Succinate, with dose adapted to patient age. D = day.

Treatment

The treatment based on the NHL-BFM 90 protocol has previously been described [9]. After a 5-day cytoreductive prephase, patients received six alternating courses A and B (Table I). The MTX dose and administration schedule in courses A and B were randomly allocated before the first course-A either MTX 1 g/m^2 over 24 hr with intrathecal (IT) chemotherapy injections (MTX1-arm) or MTX 3 g/m^2 over 3 hr, without IT therapy (MTX3-arm). In the MTX1-arm, leucovorin rescue was given at a dose of 15 mg/m^2, with only 3 doses at hour 42, 48 and 54, whereas in the MTX3-arm, it started at hour 24 and was given every 6 hr, until the MTX level was <0.15 μm/L. Additionally, high-risk patients (defined as patients with mediastinal, skin or visceral involvement) could receive, or not receive, vinblastine (6 mg/m^2) during each induction course (VLB randomised trial). According to the protocol, the courses were started as soon as the peripheral counts had recovered from the previous course, with absolute neutrophils count (ANC) ≥0.5 × 10^9/L and platelets ≥50 × 10^9/L.

Toxicity Assessment

Acute toxicity was assessed after each course using a standardised form including a checklist of 21 items selected from the cancer therapy evaluation program (CTEP) common toxicity criteria, CTC-V2.0 [10], grade 0–4 for severity and including: haemoglobin level, white blood cell, granulocyte and platelet counts, fever, infection, stomatitis, vomiting, diarrhoea, dermatologic toxicity, creatinine level, proteinuria, haematuria, glomerular filtration rate, bilirubin, transaminases, cardiac impairment, left ventricular-shortening fraction (LV-SF), central and peripheral neurotoxicity and anaphylaxis. A free text area was available to document other ARs. We did not perform site monitoring of toxicity data but we cross-checked that all severe adverse events reported to the pharmaco-vigilance unit were also reported on the toxicity forms. Grade 4 haematological toxicity and grade 3 or 4 non-haematological toxicity were classified as severe toxicity. In addition, the patient's weight and height were recorded at the first and the last chemotherapy courses and the body mass index (BMI) was computed.

Statistical Analysis

Descriptive statistics were used to report course modifications, the interval between courses and safety data per course. Missing data were excluded from the denominator, when calculating percentages. The number of courses with severe toxicity was calculated for each patient. Endpoints considered successively in the risk factor analysis of toxicity were grade 4 haematological toxicity, grade 3–4 infection, grade 3–4 stomatitis and grade 3–4 liver toxicity. The evaluated risk factors for ARs were the type of course (A versus B, MTX1 versus MTX3, without versus with vinblastine), the number of the course (first versus subsequent), patient age (less than 3 years, 3–16 years, 16 years or more), gender and risk group (standard versus high-risk). Mixed logistic models with unstructured correlation matrix were used, taking into account the correlation between the repeated courses per patient. Models were adjusted on cooperative groups to control for possible differences in ARs reporting between countries. Adjusted odds-ratios (OR) were estimated with their 95% confidence intervals (95%CI). For BMI analysis, we used the standard definition for childhood overweight and obesity proposed by Cole et al.; these age and sex specific cut-off points of a BMI are based on pooled international data and linked to the widely accepted adult cut-off points of a BMI of 25 and 30 kg/m^2 [11]. The mean relative variation of weight between start and end of treatment was compared between patients who experienced episode of severe toxicity after ≥2 courses and those who experienced episode of toxicity after ≤1 course using *t*-test. Data were entered and checked with the PIGAS software and analysed with the SAS software (version 9.1; SAS Software, Cary, NC).

RESULTS

Patient and Course Characteristics, Overall Outcome

Overall 352 patients were analysed, 211 males and 141 females. The median age of the patients was 10.5 years at diagnosis (range 3 months to 19.5 years). Baseline characteristics of the study population have been described elsewhere [9]. Only 15 patients received less than six courses (4%). Detailed information on all courses was available for all but one patient, leading to a total of 2056 courses.

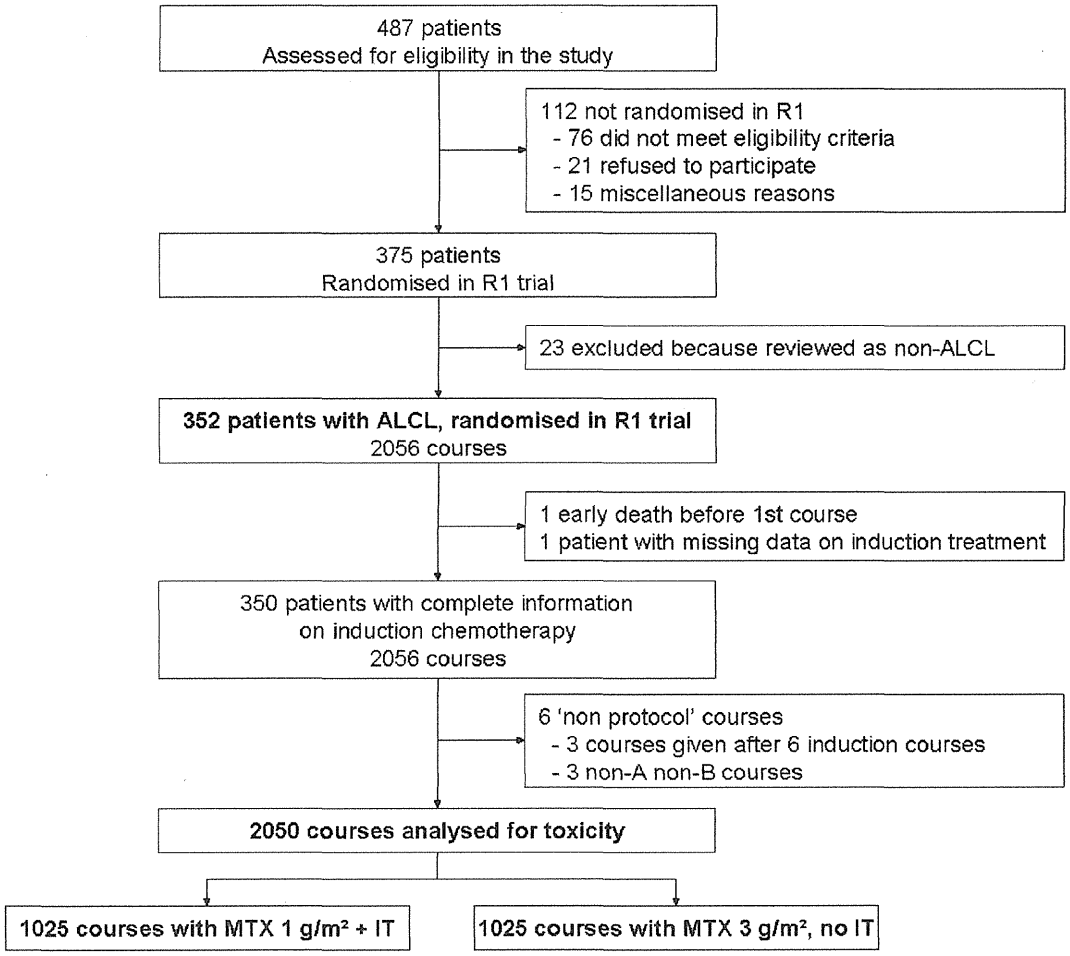

Fig. 1. Participant flow chart.

After exclusion of 3 courses other than courses A and B and 3 courses given after the 6 protocol courses, the 2050 analysed courses were 1032 courses-A and 1018 courses-B; 1025 courses with MTX 1 g/m^2 + IT (MTX1 course) and 1025 courses with MTX 3 g/m^2 and no IT (MTX3 course); 469 courses with vinblastine and 1581 courses without vinblastine (797 in standard risk patients and 784 in high risk patients). The participant flow chart is available on Figure 1. The overall treatment outcome in the MTX1 arm and MTX3 arm has been previously reported [9]. As shown 2-year EFS was 73.6% and 74.5% in the MTX1 and MTX3 arms, respectively.

Toxic Deaths

Four treatment-related deaths were reported among the 352 patients (1%): one tumour lysis syndrome during the prephase, two due to sepsis (after the first and fifth course) and one due to a macrophagic activation syndrome, occurring 1 month after the end of chemotherapy, with polymerase chain reaction (PCR) positivity for HHV6 virus and no evidence of relapse.

Time Interval Between Courses and Treatment Modifications

The median interval between each of the six courses was 23 days, ranging from 12 to 54 days, with only 35% of courses within the expected 21-day interval. The interval between courses was greater than 26 days after 26% of courses. When controlling for toxicity, the risk of a delayed course was significantly higher after courses-A than after courses-B (OR = 2.0, 95%CI, 1.5 to 2.5, $P < 10^{-4}$) and increased from the first to the last course ($P < 10^{-4}$) (cf. Supplemental Table I). The percentage of delayed courses varied also according to the national or cooperative group, from 16.3% to 51.8%. The differences of risk of delayed courses between national or cooperative groups remained significant in multivariate analysis when adjusted on the other risk factors ($P < 10^{-4}$).

A dose modification (20% or more dose reduction of at least one drug) was reported after 58/2050 courses (3%), 0.8% for dexamethasone (16/2050), 1.6% for ifosfamide (16/1032 courses-A), 1.1% for cytarabine (11/1032 courses-A), 1.0% for etoposide (10/1032 courses-A), 0.4% for cyclophosphamide (4/1018 courses-B), 0.6% for doxorubicin (6/1018 courses-B), 0.8% for MTX (13/1025 MTX1 courses and 3/1025 MTX3 courses) and 0.6% for vinblastine (3/469 courses with vinblastine). The reasons for the dose reductions were mainly gut toxicity and hemato-infectious toxicity. In addition, 30 courses (11 patients) were different from that allocated by randomisation (switch between MTX1 and MTX3 courses in 22 courses, omission of vinblastine in 8 courses), mainly due to secondary refusal of the

TABLE II. Description of Toxicity Reported by Course (2050 courses)

Toxicity item	No. of missing data[a]	No toxicity N (%)	Grade 1–2 toxicity N (%)	Grade 3 toxicity N (%)	Grade 4 toxicity N (%)
Haematological toxicity					
Anaemia	3	243 (12)	1025 (50)	646 (32)	133 (6)
Neutropenia	3	225 (11)	138 (7)	251 (12)	1433 (70)
Thrombocytopenia	5	938 (46)	401 (20)	368 (18)	338 (16)
Infection[b]	10	1201 (59)	729 (36)	97 (5)	13 (<1)
Gut toxicity					
Stomatitis	6	1239 (61)	536 (26)	172 (8)	97 (5)
Diarrhoea	14	1820 (89)	184 (9)	15 (1)	17 (1)
Dermatologic toxicity	21	1839 (91)	163 (8)	27 (1)	0 (0)
Renal toxicity					
Creatinine elevation	23	1964 (97)	62 (3)	1 (<1)	0 (0)
Proteinuria	164	1845 (98)	41 (2)	0 (0)	0 (0)
Haematuria	145	1872 (98)	32 (2)	0 (0)	1 (<1)
Liver toxicity					
Hyperbilirubinemia	113	1830 (95)	76 (4)	24 (1)	7 (<1)
SGOT/SGPT[c] elevation	53	1208 (61)	587 (29)	175 (9)	27 (1)
Cardiac toxicity					
Cardiac function impairment	343	1697 (99)	9 (1)	0 (0)	1 (<1)
Echocardiogram abnormality (LV-SF)	633	1392 (98)	17 (1)	7 (<1)	1 (<1)
Neurological toxicity					
Central neurotoxicity	13	2024 (99)	8 (<1)	4 (<1)	1 (<1)
Peripheral neurotoxicity	20	1993 (98)	35 (2)	2 (<1)	0 (0)
Anaphylaxia	16	2015 (99)	16 (1)	2 (<1)	1 (<1)

[a]Missing data were excluded from the denominator when calculating percentages. [b]Fever of unknown origin quoted as grade 2 infection. [c]Serum Glutamic Oxaloacetic Transaminase (SGOT) / Serum Glutamic Pyruvic Transaminase (SGPT).

randomised treatment (15/30 courses). Only two patients stopped induction treatment prematurely due to toxicity.

Adverse Reactions

The main ARs obtained from the checklist and reported per course are listed in Table II. The most frequent toxicity was haematological, with grade 4 neutropenia occurring after 70% of courses. Infections were reported after 41% of courses, including only 5% of courses with grade 3–4 infection. Liver toxicity and stomatitis were also frequent (transaminase elevation occurrence was 39%, stomatitis: 39%), but grade 3–4 occurred after less than 15% of courses. For each of the other items listed on the checklist, grade 3–4 toxicity occurred in less than 3% of the courses. In the free text areas for other ARs, 9 thromboses were reported, occurring in 7 patients (2%): 2 cerebral thromboses, 3 lower-limb venous thromboses, 1 pulmonary embolism and 3 central venous line-related thromboses.

During induction treatment, 95% of patients experienced at least one episode of grade 4 haematological toxicity, whereas the percentage of patients who experienced severe extra-haematological toxicity after at least one course was 21% for grade 3–4 infection, 42% for grade 3–4 stomatitis and 38% for grade 3–4 liver toxicity. Information on transfusions was available for 263 patients. Among them, 147 patients (56%) received at least one platelet transfusion, mostly after 1–3 courses, and 199 patients (76%) received at least one red cell transfusion, including 20 patients (8%), who received red cell transfusions after each of the six courses. As it was not stated in the protocol, the level of red cells and platelets for transfusion was not only related to the severity of anemia and thrombopenia but also to the guidelines for transfusion in each cooperative group. Thus the number of transfusions by patient varied from a country group to another ($P = 0.04$ for red cell and $P < 10^{-4}$ for platelets).

Risk Factors for Severe Toxicity

The rate of severe toxicity was significantly different from a cooperative group to another, varying from 68.6% to 87.7% ($P = 0.03$). However, the impact of the group varied for each specific toxicity. As detailed in Table III, severe haematological toxicity and stomatitis were significantly more frequent after MTX1 courses than after MTX3 courses, whereas none of the toxicity criteria were found to be associated with the administration of vinblastine. The toxicity profile differed considerably between courses-A (significantly more haematological toxicity) and courses-B (significantly more stomatitis). The percentage of ARs was significantly higher after the first course compared to subsequent courses for all toxicity criteria, even after controlling for the type of course (A versus B) and for disease extension (standard risk versus high risk group).

An increased risk of haematological toxicity and stomatitis was observed in females as compared to males. Age was not associated with a risk of toxicity except for liver toxicity, which appeared to occur more frequently in young children, as compared to older ones. None of the studied toxicity criteria was associated with disease extension at diagnosis.

TABLE III. Multivariate Analysis of Risk Factors for Severe Toxicity

		Grade 4 haematological *4 missing values*				Grade 3–4 infection *10 missing values*				Grade 3–4 stomatitis *6 missing values*				Grade 3–4 liver toxicity *118 missing values*				Any severe toxicity			
Characteristics	N	%	OR	95% IC	*P*-value	%	OR	95% IC	*P*-value	%	OR	95% IC	*P*-value	%	OR	95% IC	*P*-value	%	OR	95% IC	*P*-value
MTX	—	—	—	—	$<10^{-4}$	—	—	—	0.29	—	—	—	$<10^{-4}$	—	—	—	0.04	—	—	—	$<10^{-4}$
MTX 1 g/m^2	1025	79	2.2	[1.6–3.0]	—	6	1.3	[0.8–2.1]	—	21	5.9	[3.9–8.8]	—	13	1.5	[1.0–2.2]	—	83	2.3	[1.7–3.2]	—
MTX 3 g/m^2	1025	64	1[a]	—	—	5	1[a]	—	—	6	1[a]	—	—	10	1[a]	—	—	68	1[a]	—	—
Vinblastine	—	—	—	—	0.25	—	—	—	0.47	—	—	—	0.40	—	—	—	0.87	—	—	—	0.27
No vinblastine	1581	72	1[a]	—	—	6	1[a]	—	—	13	1[a]	—	—	13	1[a]	—	—	76	1[a]	—	—
Vinblastine	469	72	1.3	[0.8–1.9]	—	4	0.8	[0.4–1.5]	—	12	1.2	[0.8–2.0]	—	7	1.1	[0.6–1.9]	—	74	1.3	[0.8–1.9]	—
Type of course	—	—	—	—	$<10^{-4}$	—	—	—	0.85	—	—	—	$<10^{-4}$	—	—	—	0.11	—	—	—	$<10^{-4}$
A	1032	80	2.2	[1.8–2.7]	—	6	1.0	[0.6–1.5]	—	11	0.2	[0.1–0.3]	—	15	0.8	[0.6–1.1]	—	83	2.0	[1.6–2.4]	—
B	1018	63	1[a]	—	—	5	1[a]	—	—	16	1[a]	—	—	8	1[a]	—	—	68	1[a]	—	—
N° of the course	—	—	—	—	0.007	—	—	—	0.009	—	—	—	$<10^{-4}$	—	—	—	$<10^{-4}$	—	—	—	$<10^{-4}$
First	350	83	1[a]	—	—	9	1[a]	—	—	22	1[a]	—	—	31	1[a]	—	—	89	1[a]	—	—
Subsequent	1700	69	0.7	[0.5–0.9]	—	5	0.5	[0.3–0.8]	—	11	0.1	[0.1–0.2]	—	8	0.2	[0.1–0.2]	—	73	0.5	[0.3–0.6]	—
Gender	—	—	—	—	0.01	—	—	—	0.28	—	—	—	0.02	—	—	—	0.43	—	—	—	0.06
Male	1242	68	1[a]	—	—	5	1[a]	—	—	12	1[a]	—	—	12	1[a]	—	—	73	1[a]	—	—
Female	808	77	1.5	[1.1–2.1]	—	6	1.3	[0.8–2.2]	—	15	1.6	[1.1–2.4]	—	11	0.8	[0.6–1.3]	—	79	1.4	[1.0–1.9]	—
Age	—	—	—	—	0.84	—	—	—	0.81	—	—	—	0.47	—	—	—	0.03	—	—	—	0.81
<3 years	101	66	0.8	[0.3–2.0]	—	5	0.9	[0.3–3.0]	—	11	1.8	[0.7–4.6]	—	25	3.8	[1.2–11.4]	—	69	0.8	[0.3–2.1]	—
3–16 years	1803	72	1.0	[0.6–1.8]	—	5	0.8	[0.3–1.7]	—	13	1.2	[0.6–2.5]	—	11	1.5	[0.6–3.7]	—	76	1.0	[0.6–1.9]	—
≥16 years	146	73	1[a]	—	—	7	1[a]	—	—	17	1[a]	—	—	10	1[a]	—	—	78	1[a]	—	—
Risk group	—	—	—	—	0.77	—	—	—	0.98	—	—	—	0.53	—	—	—	0.70	—	—	—	0.53
Standard risk	798	72	1[a]	—	—	5	1[a]	—	—	13	1[a]	—	—	13	1[a]	—	—	76	1[a]	—	—
High risk	1252	71	0.9	[0.7–1.4]	—	6	1.0	[0.6–1.7]	—	13	0.9	[0.6–1.3]	—	11	0.9	[0.6–1.4]	—	75	0.9	[0.6–1.3]	—

For each toxicity criterion considered separately, the relative model included all variables in the table. [a]Reference category.

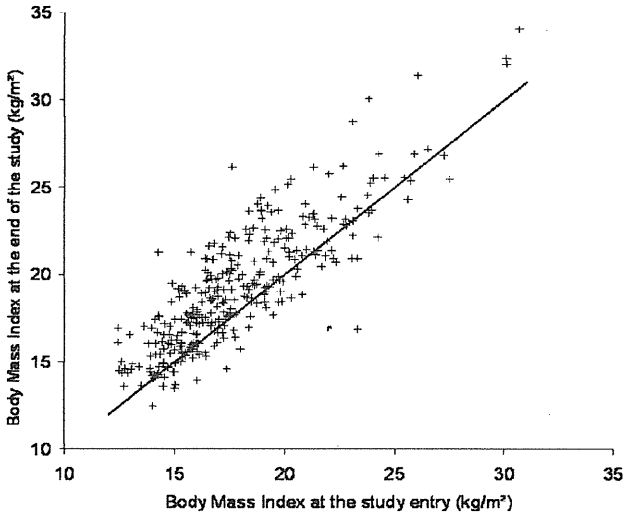

Fig. 2. Variation of patient Body Mass Index (BMI) between the start and the end of induction treatment. Plus symbols represent patients. The black line represents a constant BMI during treatment.

Weight Changes

During treatment, 211/337 informative patients (63%) had more than a 5% weight gain, including 66 patients (20%), in whom it exceeded 20% of the initial weight. Overall, the mean weight gain was 3.5 kg (std = 4.6), corresponding to a relative gain of 10.0% (std = 11.1%). For most of the patients, the BMI increased over the treatment period, with a mean BMI gain of 8.3% (cf. Fig. 2). The proportion of patients with overweight has doubled between the start and the end of induction treatment (4% versus 8%, $P < 10^{-4}$). Weight changes were mainly associated with toxicity: the relative increase of weight was less important in the 147 patients who experienced episode of severe toxicity after ≥ 2 courses than in the 190 who experienced episode of severe toxicity after ≤ 1 course (+0.07 versus +0.12, $P < 10^{-4}$).

DISCUSSION

Most of patients treated for cancers experience chemotherapy toxicity. The development and use of chemotherapy regimens with similar efficacy, but less toxicity would be clinically relevant. Recently, we demonstrated that there was no significant difference in terms of event-free survival between both groups of ALCL patients, randomly allocated to MTX1 or MTX3 arm of ALCL99 regimen, while severe toxicity was more frequent in MTX1 as compared to MTX3-arm [9].

In the present study we determined the toxicity profile and assessed the risk of toxicity of the different courses used in the ALCL99 regimen. The use of a standardised form including a pre-established list of items allowed us to collect the toxic events of interest after each course of chemotherapy. The heterogeneity in the incidence of severe toxicity between cooperative groups may reflect a difference in the reporting process from a group to another. However all analyses were adjusted on the cooperative groups allowing us to assess risk factors through the different groups.

As previously reported [9], the ALCL99 protocol was associated with a high incidence of severe haematological toxicity and mucositis, nevertheless the toxicity-related death rate was low (less than 1%). In addition a large number of treated centres recruiting patients in this trial were not familiar with the BFM protocol. A widespread use of the BFM-based ALCL99 protocol can thus be considered feasible.

The three main risk factors for severe toxicity were: the type of course (A versus B), the number of the course and the MTX dose and schedule. As previously discussed, the relationship between the risk of toxicity and this latter factor was probably linked to a longer exposure to MTX due to the prolonged infusion and to late leucovorin rescue in the MTX1-arm [9]. This finding corroborates the data reported in the NHL-BFM 95 study, comparing toxicity of two regimen with different durations of perfusion but same doses of MTX [12].

We did not observe any impact of the addition of vinblastine on the risk of toxicity. The pattern of toxicity differed after course-A and course-B: haematological toxicity occurred more frequently after course-A than after course-B, whereas mucositis and liver toxicity, probably related to doxorubicin, occurred more frequently after course B. The incidence of grade 3–4 toxicity after the first course was higher than after subsequent courses, probably due to the poor condition of the patients at presentation. A similar finding was reported concerning infections after induction chemotherapy in Ewing's sarcoma tumour treated in the Euro-E.W.I.N.G protocol [13].

We also observed a slight increase in haematological toxicity and stomatitis in females. A difference between genders in tolerance to chemotherapy has already been shown and has been attributed to a higher clearance of doxorubicin in males than in females [14]. Except for liver toxicity, which occurred more frequently in younger patients, no significant impact of age or risk group was observed.

Despite the high toxicity rate, the feasibility of delivering the protocol appears to be satisfactory: treatment was prematurely stopped in only two patients due to toxicity and a dose modification was required in less than 3% of courses. However, the expected 21-day interval between courses was achieved in only 35% of the courses and the interval between courses was longer than 26 days after 26% of the courses. It is notable that in several national/cooperative groups, this percentage remained lower than 20%. Differences between national groups probably reflect the impact of institutional variations in clinical practices since it was very significant when controlling for the type of course and the observed toxicity.

A high prevalence of weight gain was observed during induction treatment, with an increase of at least 20% of the initial weight in more than 20% of the patients. This weight gain is probably related to the high doses of dexamethasone administered in each course of the protocol. The high percentage of patients with an abnormal BMI at the end of induction therapy, may herald a risk of obesity in long-term survivors, as already reported in acute lymphoblastic leukemia (ALL) patients [15]. Early detection of patients at risk and implementation of preventive measures (appropriate diet, physical activity), should be considered at the initiation of treatment. The risk associated with the overweight should be taken into account, when planning the follow-up of late effects in this population.

Apart from the risk of becoming overweighted, the risk of long-term side effects due to this protocol is probably low, since the cumulative doses of drugs associated with late toxicity, such as anthracyclines and/or alkylating agents are low (doxorubicin 150 mg/m^2; cyclophosphamide 3.4 mg/m^2 and ifosfamide 12 g/m^2). The low risk of long-term side effects makes this protocol attractive, despite the high rate of acute haematological toxicity. Indeed the risk of disease failure observed with this protocol [9] compares favourably with that of other protocols, associated with less acute toxicity such as the APO and CHOP regimens, but containing a higher cumulative dose of doxorubicin (300 mg/m^2 in the APO protocol [6] and 300–400 mg/m^2 in the 6–8 courses of CHOP administered to treat adult large-cell NHL [16]).

Despite the high incidence of acute toxicity, the percentage of life-threatening events caused by this protocol was acceptable. The significantly lower risk of toxicity in the MTX3-arm compared to the MTX1-arm prompted the EICNHL group to recommend the use of 3 g/m^2 of MTX as a 3-hr infusion without IT prophylaxis in patients with ALCL [9].

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Brief report

Limited stage I disease is not necessarily indicative of an excellent prognosis in childhood anaplastic large cell lymphoma

Andishe Attarbaschi,[1] Georg Mann,[1] Angelo Rosolen,[2] Denise Williams,[3] Anne Uyttebroeck,[4] Ildiko Marky,[5] Laurence Lamant,[6] Keizo Horibe,[7] Grazyna Wrobel,[8] Auke Beishuizen,[9] Wilhelm Wössmann,[10] Alfred Reiter,[10] Audrey Mauguen,[11] Marie-Cecile Le Deley,[11,12] and Laurence Brugières,[13] for the European Intergroup for Childhood Non-Hodgkin Lymphoma (EICNHL) ALCL99 Trial

[1]Paediatric Hematology and Oncology, St Anna Children's Hospital, Vienna, Austria; [2]Paediatric Hematology and Oncology, University of Padova, Padova, Italy; [3]Paediatric Hematology and Oncology, Cambridge University Hospitals Foundation Trust, Addenbrooke's Hospital, Cambridge, United Kingdom; [4]Paediatric Hematology and Oncology, University of Leuven, Leuven, Belgium; [5]Paediatric Hematology and Oncology, University of Goteborg, Goteborg, Sweden; [6]Pathology, Hôpital Purpan, Toulouse, France; [7]National Hospital Organization Nagoya Medical Center, Aichi, Japan; [8]Department of Bone Marrow Transplantation and Childhood Oncology and Hematology, Medical University of Wroclaw, Wroclaw, Poland; [9]Paediatric Hematology and Oncology, Erasmus MC–Sophia Children's Hospital, Rotterdam, The Netherlands; [10]NHL-BFM Study Centre, Paediatric Hematology and Oncology, Justus Liebig University, Giessen, Germany; [11]Biostatistics and Epidemiology Unit, Institute Gustave-Roussy, Villejuif, France; [12]Université Paris-Sud 11, Paris, France; and [13]Paediatric Hematology and Oncology, Institute Gustave-Roussy, Villejuif, France

Data on incidence, characteristics, and prognosis in stage I childhood anaplastic large cell lymphoma are scarce. Of 463 patients enrolled in the international ALCL99 trial, 36 (8%) had stage I disease and were treated with a prephase chemotherapy, followed by either 3 chemotherapy courses in case of initial complete resection (6 patients) or otherwise by 6 courses of chemotherapy (30 patients). Disease localization was to the peripheral lymph nodes in 26, soft tissue mass in 8, and solitary bone and bronchial disease in 1 patient each. Of the 6 patients with complete resection, none experienced relapse, whereas of the 30 remaining stage I patients, 9 (30%) relapsed, including in all cases a new site of disease involvement and including 3 of 5 anaplastic lymphoma kinase–negative patients. In summary, the failure rate for incompletely resected stage I disease was similar to that for patients with stage II and stage III/IV disease. Whether anaplastic lymphoma kinase negativity contributed to this moderate outcome has to be proven prospectively. This study was registered at www.clinicaltrials.gov as NCT00006455. (*Blood.* 2011;117(21): 5616-5619)

Introduction

Anaplastic large cell lymphoma (ALCL) is a distinct clinicopathologic entity of non-Hodgkin lymphoma (NHL) and accounts for 10%-15% of childhood NHL.[1,2] Clinical presentation is heterogeneous and includes the frequent involvement of peripheral lymph nodes and extranodal sites, as well as the presence of B-symptoms.[3] Optimal treatment of pediatric ALCL has not yet been defined, and therapeutic strategies differ considerably, with some groups adapting chemotherapy according to the extent of disease at diagnosis and others treating all patients with the same regimen.[4-9] Regardless of the type of chemotherapy, failure-free survival rates are 70%-75%.[4-9]

More than 70% of children with ALCL present with advanced-stage disease, so that systematic data are scarce regarding stage I disease, defined as a single tumor (extranodal) or anatomic area (nodal) involved with the exclusion of the mediastinum and abdomen.[10] In 1999, the European Intergroup for Childhood NHL (EICNHL) designed a prospective multinational trial for childhood ALCL (ALCL99). Herein, we report on the incidence, clinical characteristics, and outcome of stage I patients included in that trial.

Methods

Between 1999 and 2006, 463 children and adolescents 22 years old or younger with systemic ALCL were enrolled into the ALCL99 trial, including patients from 10 study groups and 12 countries. The diagnosis of ALCL was based on morphologic and immunophenotypic criteria according to the World Health Organization classification system and centrally reviewed in 96% of the patients included in the trial.[11] All cases were tested for expression of anaplastic lymphoma kinase (ALK). Staging procedures were performed as described elsewhere.[3,10,12-14] All patients were treated with informed consent from the patients, patient's parents, or legal guardians. Studies were conducted in accordance with the Declaration of Helsinki, and approval was given by the ethic committees of all participating centers.

Details concerning eligibility criteria, stratification, and therapy of the ALCL99 trial, as well as overall outcome, have been published previously.[12,14] In brief, patients were stratified into 3 subgroups (low-, standard-, and high-risk group) and received prephase chemotherapy followed by pulselike chemotherapy with 5-day courses. The number of courses was based on stage of disease and risk factors as established by the EICNHL.[3] There were no strict criteria outlined in the protocol to determine in which patients a complete resection should be performed; however, our guidelines suggested that patients were candidates for complete surgical resection (1) if they had localized disease, (2) if the operation could be performed

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easily and safely without any significant delay in beginning chemotherapy, and (3) most importantly, if the operation could be performed without resulting in any functional impairment. In patients without completely resectable disease, initial surgery included the least invasive procedure to establish the diagnosis.

Patients included in the low-risk group had complete resection of a stage I tumor (except for completely resected isolated skin disease, which underwent a wait-and-see strategy) and received 3 courses of chemotherapy for a total treatment duration of up to 10 weeks. Patients enrolled in the high-risk group had at least 1 risk factor (skin and/or mediastinal and/or visceral involvement), whereas all others were included in the standard-risk group. Treatment of the standard- and high-risk groups consisted of 6 chemotherapy courses that involved 2 randomized questions with respect to the dosage and type of administration of high-dose methotrexate (standard- and high-risk groups) and the addition of vinblastine to standard chemotherapy and for maintenance therapy (high-risk group only).[12,14] Total treatment duration was 4-5 months for patients who did not receive vinblastine and 12 months for the others.

Event-free and overall survival were estimated with Kaplan-Meier curves, and comparisons were performed with log-rank tests. All but 2 patients were followed up for > 2 years (median, 5.7 years).

Table 1. Comparison of clinical and laboratory features at initial diagnosis of 463 ALCL99 patients according to stage of disease

	Stage I (*n* = 36)	Stage II (*n* = 78)	Stage III/IV (*n* = 349)	*P*, I vs II vs III/IV
Male sex, n (%)	20 (56)	49 (63)	213 (61)	.76
Mean age at biopsy,y (±SD)	11.2 ± 4.1	11.0 ± 3.9	10.2 ± 4.5	.21
Histologic subtype: LH/SC/mixed ALCL, n (%)	8 (23)	15 (21)	106 (33)	.08
Immunophenotype, n (%)				
ALK+	31 (86)	75 (96)	335 (96)	.05
Cell lineage, n (%)				
Null	5 (14)	12 (15)	39 (11)	.49
T	31 (86)	66 (85)	310 (89)	
B-symptoms, n (%)	4 (11)	21 (27)	234 (68)	< .0001
Localization, n (%)				
Lymph node	26 (72)	72 (92)	301 (86)	.02
Soft tissue	8 (22)	15 (19)	51 (15)	.34
Bone lesion	4* (11)	6 (8)	67 (23)	.008
LDH (before treatment), U/L	314 (±232)	366 (±216)	563 (±651)	.004

LH indicates lymphohistiocytic variant; SC, small cell variant; mixed ALCL, LH and/or SC component; and LDH, lactate dehydrogenase.

*Of the 4 patients,1 had solitary bone disease and 3 had a concomitant soft tissue mass.

Results and discussion

Among the 463 patients, we identified 36 children (8%) with stage I disease (6 with and 30 without initial complete resection). Twenty-six patients (72%) had peripheral lymph node involvement only, 8 (22%) presented with a soft tissue mass, and 1 patient each (2.5%) had solitary bone and bronchial disease, respectively. Of the 8 cases with a soft tissue tumor, 3 had underlying bone disease, and 3 had contiguous skin involvement.

All 36 cases were CD30+, 31 (86%) were ALK+, and 31 (86%) expressed at least 1 T-cell marker. Histologic subtyping demonstrated classic ALCL in 26 (72%); mixed ALCL in 6 (17%), including a small cell or lymphohistiocytic component in each of them; small cell ALCL in 2 (6%); and Hodgkin-like and not further specified ALCL in 1 patient each (2.5%). Of note, all 5 ALK− patients showed classic histopathology, including 4 with a T-cell phenotype. Sites of involvement were soft tissue in 3 patients and peripheral lymph nodes in 2. All 5 ALK− patients were assigned to the group without initial complete resection.

The incidence of peripheral lymph node involvement, B-symptoms, ALK positivity, and high levels of lactate dehydrogenase was significantly lower in stage I than in stage II or stage III/IV ALCL99 patients (Table 1). All 6 patients with complete resection were included in the low-risk group and neither experienced relapse nor died, whereas the other 30 patients were treated in the standard-risk group, with 9 of them (30%; 95% confidence interval 14%-46%) having recurrent disease (new localization, n = 7; new localization associated with local relapse, n = 2; Figure 1A). Seven of these cases occurred among the 26 patients with initial peripheral lymph node involvement, whereas the other 2 cases had soft tissue and bone/soft tissue disease, respectively. The histologic subtype of the relapsed patients was classic ALCL in 6 patients and mixed, small cell, and unspecified ALCL in 1 patient each. Three relapses occurred among the 5 ALK− patients. Seven of the relapsed patients are still alive in second remission. Three-year event-free and overall survival of the 36 patients with stage I disease was not superior to that of the 78 stage II and 349 stage III/IV patients (Figure 1B). Among the entire cohort of 36 stage I patients, histologic subtype ($P = .583$), ALK expression ($P = .066$), and peripheral lymph node involvement ($P = .689$) were not significantly associated with outcome.

Childhood ALCL inherently is a rare disease, with disease-free survival rates (70%-75%) lagging behind the good outcome achieved in mature B-cell and lymphoblastic lymphoma (80%-85%).[2,15,16] While two-thirds of patients present with disseminated disease at initial diagnosis, patients with limited-stage disease are rarely observed and reported.[4,5,7-9,17,18] Before designing the ALCL99 trial, the EICNHL undertook an analysis on 225 ALCL patients to identify risk factors at diagnosis that predicted treatment failure.[3] That study demonstrated that the St Jude staging system did not adequately address the high proportion of extranodal involvement in childhood ALCL. Moreover, the St Jude classification also lost prognostic value when the identified risk factors (skin, mediastinal, and visceral involvement) were considered for analysis of outcome. Nevertheless, completely resected stage I disease remained as an excellent prognostic subgroup. Thus, in the ALCL99 trial, treatment stratification did not rely primarily on the St Jude staging criteria (except for patients with completely resected stage I) but on the newly established risk factors.

To the best of our knowledge, the ALCL99 trial, which covers a 6-year time period, represents the largest series of uniformly treated children with ALCL. Thus, incidence rates and distribution of disease stages can be considered reproducible. The present data show that limited stage I ALCL disease has an incidence of 8%, which is in the same range as in other available studies.[4,5,7-9,19]

Although the number of patients identified was rather low, the present study showed that initially completely resected stage I ALCL has a good prognosis with 3 courses of chemotherapy; however, patients with stage I disease and no complete resection had a relapse rate of 30% at 5 years, which was no better than the rate observed in patients with stage II or stage III/IV disease. Because a new site of disease involvement was found in all cases at

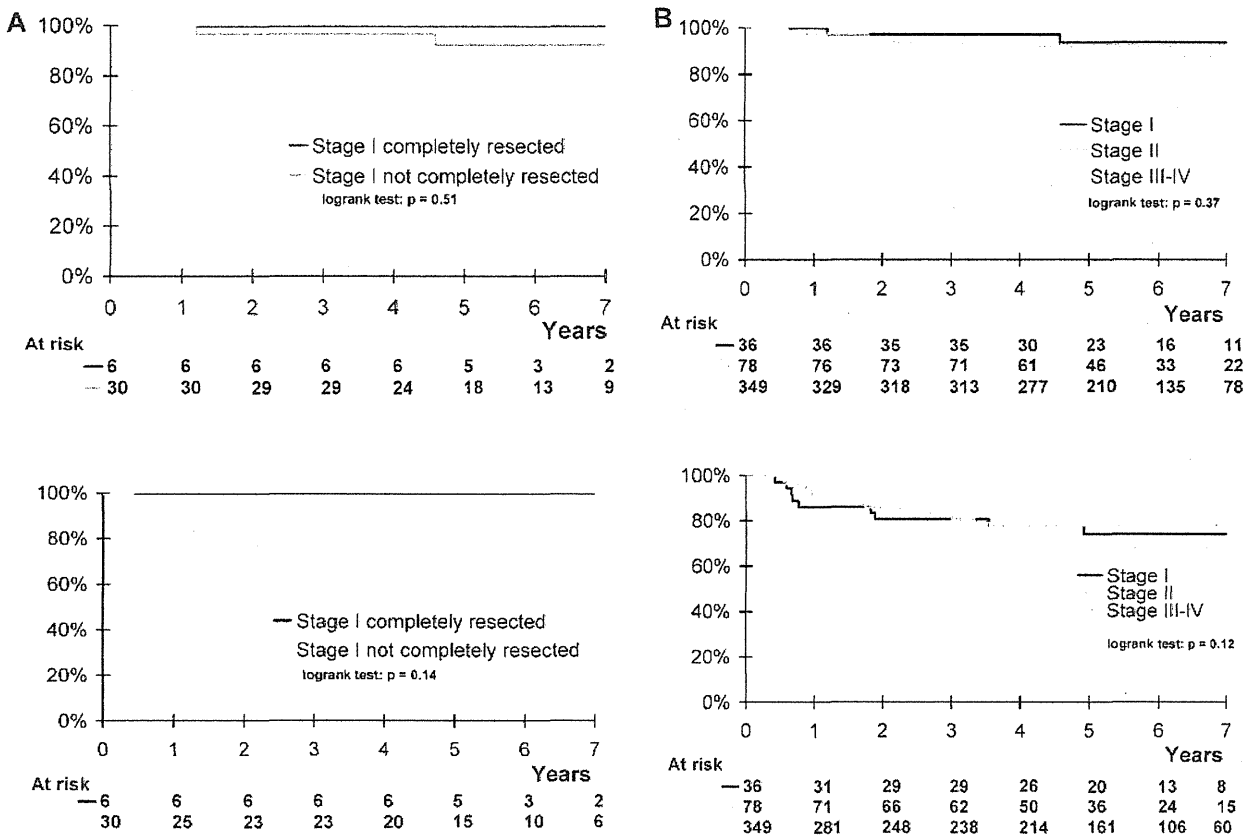

Figure 1. Overall and event-free survival according to initial resection and stage of disease. (A) Overall and event-free survival of 36 ALCL99 patients with stage I disease, according to initial resection. Three-year overall survival: patients with complete resection, 100%; patients without complete resection, 97% (83%-99%). Three-year event-free survival: patients with complete resection, 100%; patients without complete resection, 77% (59%-88%). (B) Overall and event-free survival of 463 ALCL99 patients* according to stage of disease. Three-year overall survival: stage I patients, 97% (86%-100%); stage II patients, 94% (86%-97%); stage III/IV patients, 91% (87%-93%). Three-year event-free survival: stage I patients, 81% (65%-90%); stage II patients, 82% (72%-89%); stage III/IV patients, 69% (64%-74%). *All but 2 patients were followed up for > 2 years (median, 5.7 years)

the time of relapse, there appeared to be a lack of systemic disease control in an apparently limited-stage lymphoma. Whether this fairly good outcome for pediatric stage I ALCL could be improved by intensification of conventional contemporary chemotherapy, refinement of risk stratification with new prognostic markers, or new treatment approaches such as additional immunotherapy (ie, anti-CD30 antibody) or long-term vinblastine monotherapy, as used in relapsed patients, is an issue of ongoing debate for future clinical trials.

Moreover, because several recent studies showed that the extent of submicroscopic disease in bone marrow or peripheral blood at diagnosis (as assessed by polymerase chain reaction for *NPM1-ALK*) correlated with the stage of disease and allowed the identification of patients who had an increased risk of relapse, we can only speculate that the relapses in stage I disease may have occurred because of high minimal disseminated disease at diagnosis.[20,21] Although not statistically significant, the moderate outcome of stage I ALCL may also be explained by the adverse biologic factor of ALK negativity, with 3 of 5 ALK^- patients and 6 of 31 ALK^+ patients having a relapse ($P = .066$). In addition, except for the higher proportion of ALK^- patients among the group with no complete resection, reasons explaining the difference in outcome between completely resected and nonresected stage I disease remain elusive. However, this observation should in no way suggest that physicians should aim for complete resection in a disease that responds readily to polychemotherapy, because this leaves patients at risk for mutilation and functional impairment.

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Authorship

Contribution: A.A., M.L.D., and L.B. wrote the manuscript; A. Rosolen, D.W., A. Reiter, M.L.D., and L.B. designed and planned the study; M.L.D. was in charge of data pooling, data checking, and statistical analysis; A.M. revised the data and performed statistical analyses; and A.A., G.M., A. Rosolen, D.W., A.U., I.M., L.L., K.H., G.W., A.B., W.W., A. Reiter, and L.B. served as principal or coinvestigators in their study groups and institutions, coordinated the study in their countries, provided study materials, and recruited patients. All authors read and approved the final version of the manuscript.

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Correspondence: Andishe Attarbaschi, Associate Professor, MD, St Anna Children's Hospital, Kinderspitalgasse 6, 1090 Vienna, Austria; e-mail: andishe.attarbaschi@stanna.at.

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Distinct Impact of Imatinib on Growth at Prepubertal and Pubertal Ages of Children with Chronic Myeloid Leukemia

Haruko Shima, MD, PhD, Mika Tokuyama, MD, Akihiko Tanizawa, MD, PhD, Chikako Tono, MD, Kazuko Hamamoto, MD, Hideki Muramatsu, MD, PhD, Akihiro Watanabe, MD, Noriko Hotta, MD, Masaki Ito, MD, PhD, Hidemitsu Kurosawa, MD, PhD, Koji Kato, MD, PhD, Masahito Tsurusawa, MD, PhD, Keizo Horibe, MD, PhD, and Hiroyuki Shimada, MD, PhD

Objective To determine the extent of growth impairment resulting from imatinib treatment in children with chronic myeloid leukemia (CML).

Study design Clinical records of 48 chronic-phase CML children administered imatinib as the first-line therapy between 2001 and 2006 were analyzed retrospectively. Cumulative change in height was assessed using the height height-SDS and converted height data from age- and sex-adjusted Japanese norms.

Results A decrease in height-SDS was observed in 72.9% of children, with a median maximum reduction in height-SDS of 0.61 during imatinib treatment. Median follow-up time was 34 months (range, 10-88 months). Growth impairment was seen predominantly in children who started imatinib at a prepubertal age compared with those who started at pubertal age. Growth velocity tended to recuperate in prepubertal children with growth impairment, as they reached pubertal age, suggesting that imatinib had little impact on growth during puberty.

Conclusions Growth impairment was a major adverse effect of long-term imatinib treatment in children with CML. We report the distinct inhibitory effect of imatinib on growth in prepubertal and pubertal children with CML. We should be aware of growth deceleration in children, especially in young children given imatinib before puberty and subjected to prolonged exposure. *(J Pediatr 2011;159:676-81).*

Since the introduction of imatinib, the treatment of chronic myeloid leukemia (CML) has changed from cure by allogeneic stem cell transplantation to maintenance of the best achievable treatment response (hematologic, cytogenetic, and molecular responses). Various side effects, including nausea, vomiting, diarrhea, skin rash, edema, elevated liver enzyme values, and cytopenia, are known to be common during imatinib treatment, but generally are mild to moderate.[1] However, the long-term side effects of imatinib therapy remain unknown, and its effects on growth are a major concern when treating children. Growth deceleration has been reported in 3 children as well as in a cohort given imatinib.[2-5] The present study was conducted to evaluate the effect of imatinib on growth in children and adolescents with CML.

Methods

In Japan, imatinib was approved and became available for treatment of CML in December 2001. The Japanese Pediatric Leukemia/Lymphoma Study Group's CML Committee reviewed records of 99 Japanese children under age 18 years diagnosed with chronic-phase CML between 2001 and 2006. Among these children, 76 who received imatinib as first-line therapy were eligible for the study. Concurrent hydroxyurea administration was permitted. Exclusion criteria were as follows: (1) reached final height at the time of diagnosis (n = 3); (2) afflicted by a chronic disease (eg, schistorrhachis) or on any treatment that could affect growth (n = 4); and (3) a follow-up period of <10 months while receiving imatinib (n = 21). Forty-eight children (21 girls, 27 boys) met these criteria and were enrolled in the study. The study design was approved by the Keio University School of Medicine's Ethics Committee.

BSA	Body surface area
CML	Chronic myeloid leukemia
GH	Growth hormone
PDGF	Platelet-derived growth factor

From the Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan (H. Shima, H. Shimada); Molecular Oncology Division, National Cancer Center Research Institute, Tokyo, Japan (H. Shima); Department of Pediatrics, Toho University Sakura Medical Center, Chiba, Japan (M. Tokuyama); Department of Pediatrics, University of Fukui Faculty of Medical Sciences, Fukui, Japan (A.T.); Department of Pediatrics, Aomori Rosai Hospital, Aomori, Japan (C.T.); Department of Pediatrics, Hiroshima Red Cross Hospital and Atomic Bomb Survivors Hospital, Hiroshima, Japan (K.H.); Department of Pediatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan (H.M.); Department of Pediatrics, Niigata Cancer Center Hospital, Niigata, Japan (A.W.); Department of Pediatrics, Tokuyama Central Hospital, Tokuyama, Japan (N.H.); Department of Pediatrics, Fukushima Medical University School of Medicine, Fukushima, Japan (M.I.); Department of Pediatrics, Dokkyo Medical University, Tochigi, Japan (H.K.); Department of Pediatrics, Aichi Medical University, Aichi, Japan (K.K.); Division of Pediatric Hematology/Oncology, Children's Medical Center, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan (K.K.); and Clinical Research Center, National Hospital Organization Nagoya Medical Center, Nagoya, Japan (K.H.)

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Height-Growth Evaluation

As part of the medical examination, height was measured by experienced medical workers at the start of imatinib treatment and at follow-up visits. Height data were converted to numbers with SDs using age- and sex-adjusted Japanese norms to give SDSs. Growth while on imatinib therapy was assessed using cumulative change in height-SDS (ΔSDS) from the start of imatinib treatment to the annual follow-up time points. Minimum height-ΔSDS was determined as the lowest value of annually calculated height-ΔSDS in each patient. Average dose of imatinib d_{ave} (mg/m^2) for an individual during the administration period (i) from 1 through n during l-year treatment was calculated using the following formulas:

$$\overline{d} = \frac{\sum_{i=1}^{n} d_i m_i}{\sum_{i=1}^{n} m_i}, \overline{BSA} = \frac{\sum_{j=1}^{l} BSAj}{\sum_{j=1}^{l} k_j}, \text{ and } d_{ave} = \frac{\overline{d}}{\overline{BSA}},$$

where d is the dose of imatinib, m is the number of days of imatinib administration, and BSA is body surface area (BSA). BSA in the jth year (BSA_j) was calculated from data obtained at the observation time point closest to the jth full-year point within 6 months. The value of k_j is 1 if BSA_j is available at the jth year and 0 otherwise. The data after reaching final height were censored for 2 patients. The final height was defined as the maximum height measured when height increase velocity slowed to <1 cm per year. In this study, age threshold equivalent to the onset of puberty was defined as 9 years for girls and 11 years for boys, as generally agreed upon by pediatricians.

Statistical Analyses

Statistical differences in height-SDS between 2 time points—at the commencement of imatinib treatment and at final follow up—within the cohort were assessed using the Wilcoxon signed-rank test. Statistical differences between the 2 subgroups classified according to minimum height-ΔSDS were assessed using the Mann-Whitney U test. The statistical differences among the 3 subgroups classified according to the average imatinib dose were evaluated using the Steel-Dwass test. The statistical differences among all annually calculated height-ΔSDS values during imatinib therapy in prepubertal and pubertal children at the commencement of imatinib treatment were assessed using the Tukey-Kramer honestly significant difference test.

Results

The median age at diagnosis was 9 years (range, 2-15 years). The median average imatinib dose was 287 mg/m^2 (range, 161-543 mg/m^2), and median follow-up was 34 months (range, 10-88 months). The overall median height of the 48 children was nearly normal at the start of imatinib treatment (median height-SDS, 0.01; range, −2.30 to 1.50), but was decreased significantly at the final measurement, with a median height-SDS of −0.85 (range, −2.80 to 1.30) ($P < .001$, Wilcoxon signed-rank test), indicating that imatinib adversely affected growth (**Figure 1**, A and B). Height <−2 SD at the last follow-up was observed in 6 children (12.5%), excluding 1 child whose height was <−2 SD at the start of imatinib treatment. A decrease in height-SDS of >0.5 SD was observed in 25 children (52.1%), including 16 (33.3%) with a decrease of >1 SD during imatinib treatment. The median minimum annually calculated height-ΔSDS during follow-up was −0.61 (range, −2.20 to 0.60) (**Figure 1**, C).

We next divided the study cohort according to their minimum height-ΔSDS into 2 subgroups: <−0.5 (n = 25) and ≥−0.5 (n = 23). Sex distribution, average imatinib dose, and proportion of patients with hydroxyurea administration were comparable between the 2 subgroups (**Table**). The greatest significant difference observed between the 2 subgroups was age at initiation of imatinib treatment. The proportion of prepubertal children was significantly higher in the minimum height-ΔSDS <−0.5 subgroup than in the ≥−0.5 subgroup. In contrast, the ≥−0.5 subgroup consisted mainly of children at pubertal age at the start of imatinib treatment.

To evaluate the relationship between administered imatinib dose and growth impairment, we divided the cohort according to the average administered dose for each individual and

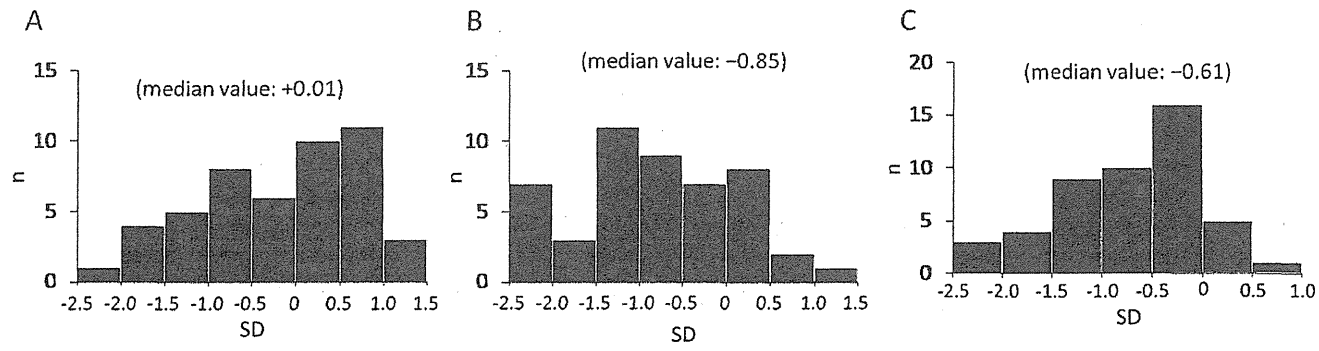

Figure 1. Change in height-SDS during imatinib treatment. Height-SDS is shown at **A,** the commencement of imatinib treatment and **B,** at the last follow-up. **C,** Minimum height-ΔSDS during imatinib treatment. The median value is indicated above each plot. n, number of patients.

Table. Patient characteristics

	Minimum height-ΔSDS		
	<−0.5 (n = 25)	≥−0.5 (n = 23)	*P* value
Age at the commencement of imatinib			
Median, years	7	12	<.001
Range, years	2-12	4-15	
Prepubertal age, n (%)*	23 (92.0)	4 (17.4)	<.001
Pubertal age, n (%)†	2 (8.0)	19 (82.6)	<.001
Male sex, n (%)	14 (56.0)	13 (56.5)	.9808
Duration of imatinib treatment, months, median (range)			
Prepubertal age*	42 (19-88)	14 (10-22)	.009
Pubertal age†	41 (21-60)	26 (10-61)	.406
Average imatinib dose, mg/m^2			
Median	293	282	.272
Range	161-543	197-376	
Hydroxyurea administration, n (%)	2 (8.0)	3 (13.0)	.577

*Prepubertal age: males, <11 years; females, <9 years.
†Pubertal age: males, ≥11 years; females, ≥9 years.

recommended pediatric doses for treating chronic-phase CML (260-340 mg/m^2)[6] into 3 subgroups: <260 mg/m^2 (n = 17), 260-340 mg/m^2 (n = 19), and >340 mg/m^2 (n = 12). The median minimum height-ΔSDS of these 3 subgroups was −0.6 (median dose, 222 mg/m^2), −0.48 (median dose, 293 mg/m^2), and −0.85 (median dose, 360 mg/m^2), respectively, indicating no significant difference among the 3 subgroups.

Representative growth charts of children at various ages at the start of imatinib treatment are shown in **Figure 2**. Growth impairment was particularly significant in children who were prepubertal at the start of imatinib treatment (**Figure 2**, A and B), and only mild growth impairment or no impairment was seen in most of the children who were pubertal at the start of imatinib treatment (**Figure 2**, C and D). However, the prepubertal children with growth impairment regained growth velocity as they reached pubertal age (**Figure 2**, E-H).

Mariani et al[2] reported a 9-year-old boy who demonstrated impaired growth shortly after the start of imatinib treatment but experienced catch-up growth with the onset of puberty. Thus, to evaluate whether children at pubertal age evade growth deceleration, we dichotomized the study cohort into 2 subgroups: children who started imatinib at prepubertal age (n = 27) and those who did so at pubertal age (n = 21). In the former group, height-ΔSDS began to decline during the first year of imatinib treatment, resulting in significant deceleration in growth. In the latter group, height-ΔSDS remained steady through imatinib treatment, suggesting that imatinib has little effect on growth in pubertal children (**Figure 3**).

Collectively, our data show a high frequency of growth impairment and >0.5 SD of cumulative decrease in height-SDS in children given imatinib for chronic-phase CML. This growth impairment was seen predominantly in young children who were started imatinib at prepubertal age.

Discussion

Imatinib is now a major option as the first-line therapy for childhood CML.[6-9] Thus, it is important for clinicians to be aware of its possible long-term effects. Imatinib inhibits several tyrosine kinases, including c-abl, c-kit, c-fms, and platelet-derived growth factor (PDGF) receptors.[7,10,11] Several studies in adults have suggested that inhibition of c-kit, c-fms, and PDGF receptors results in modulation of bone metabolism.[12-15] Inhibition of osteoclasts and osteoblasts may result in dysregulated bone remodeling.[11,15-17] Three recently published case reports indicated growth impairment as an adverse effect of long-term imatinib treatment in children.[2-4] In addition, a French group reported a significant decrease in height-SDS in 22 children, with a median difference of −0.37 (range, −1.09 to 0.14; $P < .0001$) during the first year of imatinib treatment.[5] Although the impact of imatinib on growth was noticeable in children in these previous studies, it has not yet been fully elucidated.

In our study of 48 children with chronic-phase CML, the severity of growth impairment was related to age at the start of imatinib treatment. Growth impairment was observed predominantly in children at prepubertal age compared with children at pubertal age. In children who started imatinib at prepubertal age, height-ΔSDS decreased during treatment, and in most cases, more than 2 years of continuous treatment was necessary to exhibit a reduction in height-SDS of >0.5 SD (**Figure 3**). Although 4 children who started imatinib at prepubertal age were included in the height-ΔSDS ≥−0.5 subgroup, these children were receiving imatinib for <2 years (**Table**), possibly indicating a high risk for developing severe growth impairment thereafter. We compared the distinct impact of long-term imatinib treatment on growth in prepubertal and pubertal children with CML.

Because the average imatinib dose varied among patients in our cohort, analysis was also performed according to the administered dose of imatinib. Although not significant, children exposed to imatinib doses >340 mg/m^2 showed a greater decrease in height-SDS compared with those exposed to lower doses, suggesting the need for further analysis to determine the correlation between imatinib dose and severity of growth impairment.