Cancer Science
The official journal of the Japanese Cancer Association JCA

Review Article

Oncogenic mutations of *ALK* in neuroblastoma

Seishi Ogawa,[1,2,6] Junko Takita,[3,4] Masashi Sanada[1] and Yasuhide Hayashi[5]

[1]Cancer Genomics Project, The University of Tokyo, Tokyo; [2]Core Research for Evolutional Science and Technology, Exploratory Research for Advanced Technology, Japan Science and Technology Agency, Saitama; [3]Department of Pediatrics, The University of Tokyo, Tokyo; [4]Cell Therapy and Transplantation Medicine, The University of Tokyo, Tokyo; [5]Gunma Children's Medical Center, Gunma, Japan

(Received October 21, 2010/Revised November 26, 2010/Accepted November 28, 2010/Accepted manuscript online December 9, 2010)

Neuroblastoma is one of the most common solid cancers among children. Prognosis of advanced neuroblastoma is still poor despite the recent advances in chemo/radiotherapies. In view of improving the clinical outcome of advanced neuroblastoma, it is important to identify the key molecules responsible for the pathogenesis of neuroblastoma and to develop effective drugs that target these molecules. Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase, initially identified through the analysis of a specific translocation associated with a rare subtype of non-Hodgkin's lymphoma. Recently it was demonstrated that *ALK* is frequently mutated in sporadic cases with advanced neuroblastoma. Moreover, germline mutations of *ALK* were shown to be responsible for the majority of hereditary neuroblastoma. ALK mutants found in neuroblastoma show constitutive active kinase activity and oncogenic potentials. Inhibition of ALK in neuroblastoma cell lines carrying amplified or mutated *ALK* alleles results in compromised downstream signaling and cell growth, indicating potential roles of small molecule ALK inhibitors in the therapeutics of neuroblastoma carrying mutated ALK kinases. (*Cancer Sci* 2011; 102: 302–308)

Neuroblastoma is a malignant embryonal neoplasm arising from developing neural crest tissues.[1] It commonly affects younger children, where the median age of diagnosis is 17 months and approximately 90% of the patients are <4 years old. In the United States, the incidence of neuroblastoma is estimated to be one in 7000 births, although the incidence calculated from the mass screening program in Japan was as high as 29.80 cases per 100 000 births, which is significantly higher than the estimation in the prescreening cohort (11.56 cases per 100 000 births).[2] It is the third most common cancer in childhood after leukemia and brain tumors, accounting for 7–11% of all pediatric cancers.[3] The presentation and following clinical courses of neuroblastoma are highly variable, ranging from a solitary localized mass with no apparent clinical symptoms to widely disseminated diseases presenting with severe systemic illness.[1] While some tumors undergo spontaneous regression without therapy, approximately 60–70% of high-risk neuroblastoma patients are resistant to any therapies currently available and succumb to death,[4–6] even though a substantial improvement in 5-year survival rates has been obtained for a subset of advanced tumors through the development of multimodal chemo/radiotherapies during the past several decades.[1] Thus, one of the urgent problems in the current neuroblastoma treatment would be to develop rational and effective therapeutic strategies for the high-risk neuroblastoma cases based on their molecular pathogenesis.

On the other hand, during the past three decades, little advancement has been made in the understanding of neuroblastoma pathogenesis in terms of critical gene targets, except for the identification of frequent *MYCN* amplification.[7] Amplification of the *MYCN* gene is found in approximately 20% of neuroblastoma, especially in advanced diseases, and has been consistently associated with poor prognosis.[8,9] Although *MYCN* amplification is a critical genetic event in neuroblastoma development,[10] it encodes a transcription factor and thus may not be a plausible pharmacological target for therapeutics. Recently, several groups independently discovered activating mutations of the *ALK* gene in the majority of familial neuroblastoma and also in a subset of sporadic neuroblastoma cases.[11–14] Given that the mutated ALK kinases are well-tractable targets for small-molecule kinase inhibitors, the discovery draws attention in the field of neuroblastoma research. In this review, we provide a brief overview of the role of *ALK* mutations in neuroblastoma pathogenesis and their implication in future therapeutics.

Genetic analysis of familial neuroblastoma

One of the first clues to identifying the novel genetic target of neuroblastoma was obtained from a linkage study of neuroblastoma-prone families. It was recognized that approximately 1–2% of newly diagnosed neuroblastoma cases occur within families (familial/hereditary neuroblastoma), indicating the existence of dominantly acting neuroblastoma susceptibility gene(s),[15–19] although previous linkage studies, in an attempt to identify the susceptibility locus, failed to provide a reproducible result due to the insufficient power of the studies.[20–22] Germline mutations of the paired-like homeobox 2B (*PHOX2B*) gene at 4p12 was reported to be responsible for neuroblastoma predisposition, but they were mostly related to a rare form of familial neuroblastoma associated with congenital central hypoventilation syndrome (CCHS) and/or Hirschsprung disease, with rare somatic mutations.[23–26] Recently, researchers at the Pennsylvania University analyzed 20 neuroblastoma pedigrees for linkage using approximately 6000 genetic markers, and mapped a candidate neuroblastoma susceptibility locus to the 2p region between rs18621106 and rs2008535, which contains 104 genes including *MYCN* and *ALK*.[11] Through a resequencing analysis of the *ALK* exons within the pedigrees they identified germline mutations of the *ALK* gene in >90% of the pedigrees that co-segregated with neuroblastoma development within the families, clearly demonstrating that the germline *ALK* mutations are responsible for the susceptibility to the development of hereditary neuroblastoma in the majority of the cases.[11,12] Moreover, the subsequent analysis of *ALK* mutations in sporadic neuroblastoma cases identified a subset of sporadic neuroblastoma cases carrying acquired/germline mutations of *ALK*, which was also reported independently by other groups.[12–14,27]

[6]To whom correspondence should be addressed.
E-mail: sogawa-tky@umin.ac.jp

doi: 10.1111/j.1349-7006.2010.01825.x

Genome-wide copy number scanning of neuroblastoma

These groups conducted genome-wide copy number analyses of neuroblastoma using comparative genomic hybridization (CGH) arrays[12] or single nucleotide polymorphism (SNP) arrays.[11,14,27,28] With thousands to half-a-million genetic probes, both platforms enabled high-throughput detection of subtle genetic changes occurring in tumor genomes.[29,30] Neuroblastoma genomes show characteristic copy number changes that involve large chromosomal segments, including gains of 17q, 1q, 2p and 11p, and losses of 1p, 3p and 11q, which, like other human cancers, collectively comprise a unique genomic profile of neuroblastoma.[11,12,14] High-level amplifications, which usually involve discrete chromosomal regions <1 Mb in length, occurred in approximately 30% of neuroblastoma cases. Approximately 90% of the high-level amplifications in neuroblastoma were centered on the *MYCN* locus at 2p24, whereas other amplicons rarely mutually overlapped, except for the amplifications at 2p23, which exclusively involved the *ALK* locus in common[12,14,28] (Fig. 1).

High-level amplification of the *ALK* gene and aberrantly activated ALK signaling in neuroblastoma was first described by Osajima-Hakomori *et al.*[31] in two neuroblastoma-derived cell lines and a single case of primary neuroblastoma. The genome-wide copy number studies confirmed their finding, in which the frequency of *ALK* amplifications is reported to occur in 3–5% of primary neuroblastoma cases.[11,12,14] Subsequent resequencing studies of *ALK* coding exons disclosed non-synonymous nucleotide substitutions of *ALK* in a subset of sporadic neuroblastoma cases and also of neuroblastoma-derived cell lines with mutation rates of approximately 6–11% and approximately 30%, respectively. Amplified *ALK* alleles, as a rule, did not harbor additional mutations, although in rare cases mutated ALK alleles were amplified.

Genetic abnormalities of the *ALK* gene in human cancers

ALK was initially isolated as a partner of the fusion gene generated by t(2;5)(q23;q35) translocation, which is characteristic of anaplastic large cell lymphoma (ALCL), a rare subtype of non-Hodgkin's lymphoma.[32,33] *ALK* encodes an orphan receptor tyrosine kinase with an apparent molecular mass of 220 kDa. Jelly belly,[34] and pleiotrophin[35] and midkine[36] have been postulated as putative ALK ligands in *Drosophila* and mammalians, respectively, but a dispute about the authentic ligands of ALK still remains. ALK has an extracellular domain that is highly similar to LTK and, together with IGF-1R and c-Ros kinases, belongs to the insulin family of proteins.[37] Expression of ALK is largely restricted to neural tissues and is most abundant in the neonatal brain and, to a lesser extent, in the adult brain.[38–41] In the developing brain, the highest expression was found in the thalamus, mid-brain, olfactory bulb and selected parts of cranial and dorsal ganglia.[38,39] It is of particular note that high frequencies of ALK expression were reported in primary neuroblastoma specimens (22 out of 24 samples) and in other tumor cell lines derived from neuroectdermal tumors including neuroblastoma (13 out of 29 cell lines).[42] These expression patterns of ALK suggest its primary role in normal neural development as well as the pathogenesis of neuroblastoma, although *ALK*-deficient mice seem to show apparently normal develop.[37]

In t(2;5)(q23;q35) translocation, the carbonyl terminal of ALK that contains a kinase domain is fused with nucleophosmin (NPM), generating NPM/ALK fusion protein. *ALK* was also shown to participate in the generation of different fusion genes with a variety of partner genes in ALCL,[43–47] inflammatory fibroblastic tumor,[43,48–52] squamous cell carcinoma of the esophagus[53] and non-small-cell lung cancers (NSCLC).[54,55] In NSCLC, *ALK* was reported to be fused with EML4 to generate EML4–ALK fusion protein as a result of inv(2)(p21p23), which is found in 6% of the NSCLC cases[55] (Fig. 2).

These ALK-containing fusion proteins invariably show constitutive kinase activity and transform NIH3T3 cells and/or confer growth factor independence to 32D and/or Ba/F3 cells.[56–58] When bone marrow cells were retrovirally transduced with *NPM–ALK* and transplanted into mice, they developed B-cell lymphoma within 4 months.[58] The critical role of ALK fusion proteins in neoplastic evolution has been further demonstrated

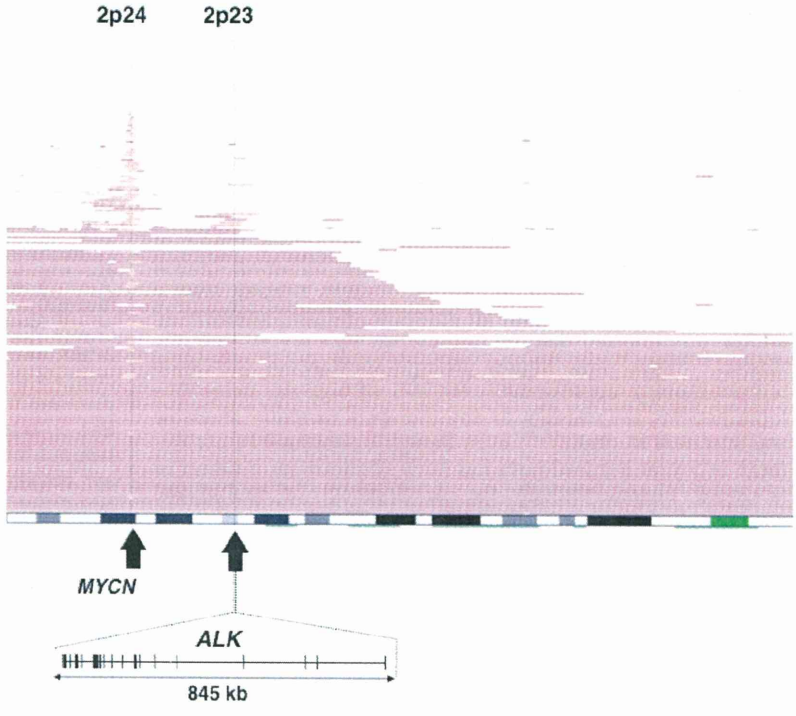

Fig. 1. Copy number gains and high-level amplifications in the short arm of chromosome 2 in neuroblastoma. Each horizontal line indicates a region showing a simple copy number (CN) gain (CN < 5; thick red) and high-level amplification (CN > 5; thin red) in each case. The majority of high-level amplifications involved the *MYCN* locus at 2p24, while the other group of amplicons is found at 2p23, which exclusively contains the *ALK* locus.

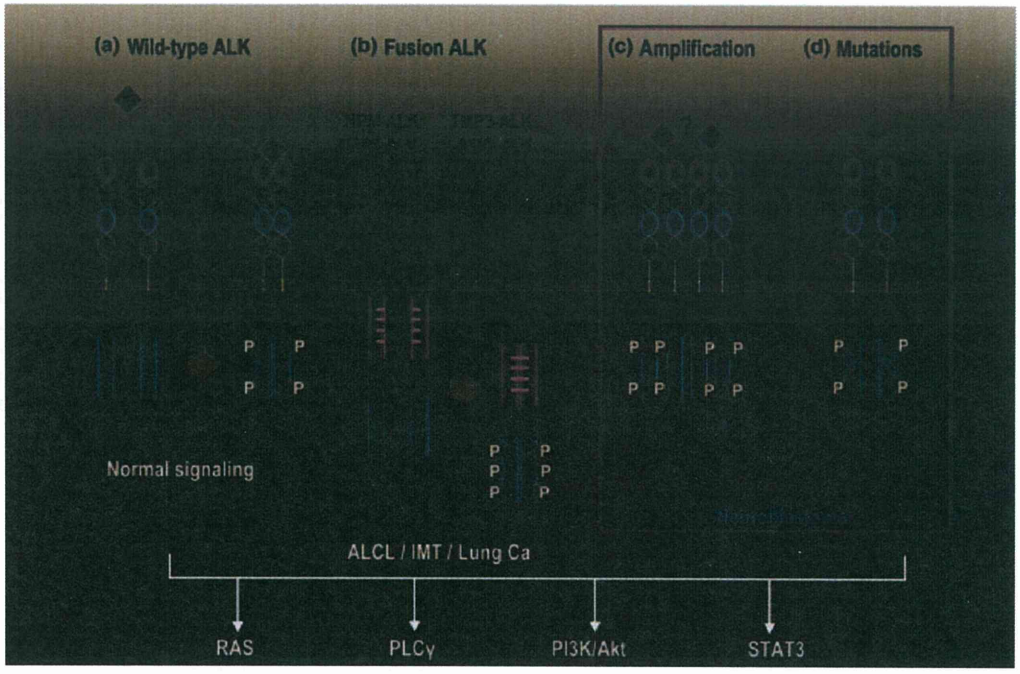

Fig. 2. Aberrant activation of ALK in human cancers. (a) Ligand-dependent physiological activation of wild-type ALK. (b) Fusion ALK kinases found in anaplastic large cell lymphoma (ALCL) and non-small-cell lung cancer (Lung Ca), such as NPM–ALK and EML4–ALK, self-dimerize through their N-terminal domains derived from fusion partners, leading to their transphosphorylation and constitutive activation of the kinase. In a subset of neuroblastoma, aberrant activation of ALK occurs by gene amplification (c) or somatic/germline mutations (d). Activated ALK transmits constitutive signals through downstream pathways, which is thought to be important for tumorigenesis. IMT indicates inflammatory myofibroblastic tumor.

using transgenic mouse models with *ALK* fusion genes; mice carrying *NPM–ALK* or *EML4–ALK* transgenes under *Vav* or *CD4*, or *surfactant protein C* promoter develop aggressive lymphoma or adenocarcinoma of the lung, respectively.[59–61] The aberrant kinase activity of these ALK-fusion proteins is thought to be caused by transphosphorylation upon self-dimerization through their N-terminal domain derived from the fusion partners. Mutations or deletions of the dimerization domain of NPM–ALK and EML4–ALK result in loss of the transforming capacity of the fusion kinases.[55,57] The constitutive active fusion kinases transmit signals through activation of a variety of signal transducers, including PLCγ, PI3K/AKT, STAT3 and RAS.[62–67]

In neuroblastoma, on the other hand, aberrant activation of ALK kinase is caused by gene amplification[31] or mutations.[11–14] Thus, ALK represents a unique type of oncogenic kinase, in that it is deregulated either by gene fusions, or by gene amplification or mutations, depending on the tumor type.

Biological consequences of ALK mutations

Most reported *ALK* mutations occurred within the kinase domain, in which three highly conserved amino acid positions, F1174, F1245 and R1275, were predominantly affected, suggesting their functional importance for the regulation of kinase activity[11–14] (Figs 3,4). The F1174 residue is located at the end of the Cα1 helix and corresponds to equivalent positions mutated in EGFR (V769) and ERBB2 (V769), while the F1245 lies in the catalytic domain and corresponds to the L833 residue of EGFR, a mutation of which is reported to be associated with gefitinib resistance in lung cancer (Fig. 5).[13] The R1275 position lies within the activation loop and is invariably changed to glutamine, and amino acid substitution at this position to a positively charged one would displace the loop to positions that permit autophosphorylation and autoactivation of the kinase (Fig. 5).[68,69] However, the distributions of these mutations were different between sporadic cases and familial cases; R1275 mutations are commonly found in both sporadic and familial cases, while no germline mutations involving the F1174 or F1245 position have been reported.[11–14] Because not all mutant *ALK* carriers develop neuroblastoma (i.e. incomplete penetrance), a germline *ALK* mutation is not fully oncogenic and additional genetic events are thought to be required for neuroblastoma development. *ALK* mutations tend to be associated with advanced diseases and also with *MYCN* amplification in sporadic neuroblastoma cases, although the trend was not clear for germline *ALK* mutations.[11–14]

When expressed in NIH3T3 cells, the predominant kinase domain mutant (F1174L) and a juxtamembrane mutant (K1062M) are shown to have transforming capacity; mutant-transduced cells display increased colony formation in soft agar and tumor generation in nude mice, whereas the mutant kinases show increased autophosphorylation and *in vitro* kinase activity compared with wild-type ALK.[14] In addition, when introduced into an IL-3-dependent cell line, BaF3, the two major kinase domain mutants (F1174L and R1275Q), render the cell line independent of IL-3.[13] Expression of the F1174L mutant in NIH3T3 and Ba/F3 cells leads to constitutive activation of the downstream signaling pathways of the ALK kinase, as demonstrated by increased levels of phosphorylated ERK1/2, STAT3 and AKT.[13,14] These functional and biochemical studies together indicate that these ALK mutants are actually oncogenic and could be responsible for the pathogenesis of neuroblastoma.

doi: 10.1111/j.1349-7006.2010.01825.x

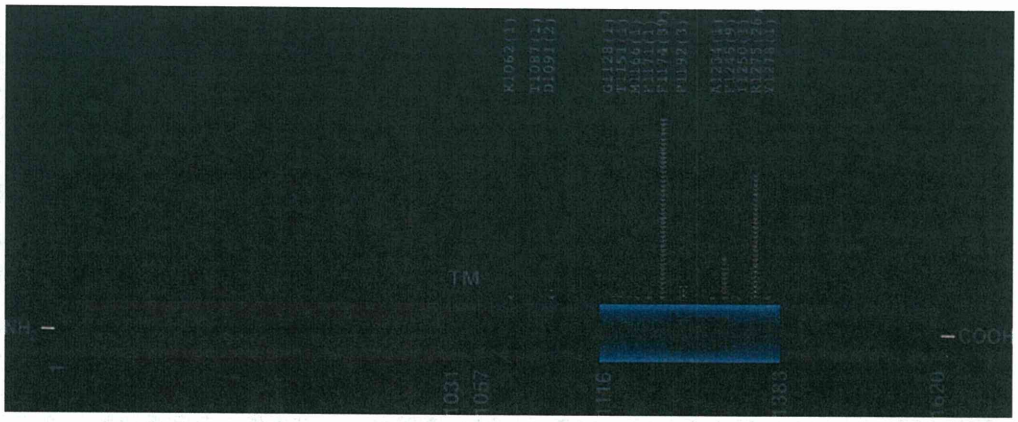

Fig. 3. Frequency and distribution of *ALK* mutations reported in familial and sporadic cases of neuroblastoma.[11–14,27] Locations of somatic and germline mutations of *ALK* in each case or family are depicted by filled and open arrows, respectively. The exact positions and amino acids involved are indicated on the top, where the number of reported mutations is indicated in parenthesis.

```
                    1174
HUMAN ALK     ...ALIISKFNHQNIVR..
HUMAN LTK     ...ALIISKFRHQNIVR..
HUMAN INSR    ...ASVMKGFTCHHVVR..
HUMAN IGF1R   ...ASVMKEFNCHHVVR..

                    1245
HUMAN ALK     ...EENHFIHRDIAARN..
HUMAN LTK     ...EENHFIHRDIAARN..
HUMAN INSR    ...NAKKFVHRDLAARN..
HUMAN IGF1R   ...NANKFVHRDLAARN..

                    1275
HUMAN ALK     ...GDFGMARDIYRASY..
HUMAN LTK     ...GDFGMARDIYRASY..
HUMAN INSR    ...GDFGMTRDIYETDY..
HUMAN IGF1R   ...GDFGMTRDIYETDY..
```

Fig. 4. Alignment of amino acids of ALK among different species. Conserved amino-acids among different insulin receptor family kinases are shown by gray boxes and the mutated positions are shown in red.

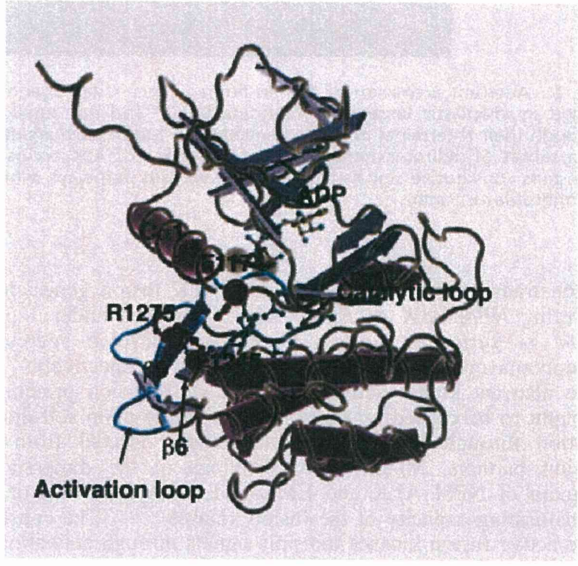

Fig. 5. A 3-D structure of the kinase domain of ALK kinase predicted from that solved for IGF-1R, where the positions of three major mutations are indicated by light blue spheres. Activation and catalytic loops are depicted by red and pink wires.

Effects of ALK inhibition on ALK fusion kinases

The critical role of *ALK* mutations in neuroblastoma development is further supported by the experiments using inhibition of mutant ALK. Tumor suppressive effects of ALK inhibition have been well documented in *NPM–ALK*-positive ALCL and *EML4–ALK*-positive NSCLC. NVP–TAE684 is a highly potent and selective small molecule ALK inhibitor, which blocks the growth of ALCL-derived cell lines with very low IC_{50} values between 2 and 10 nM.[70] NVP–TAE684 treatment of ALCL-derived cell lines induces rapid and sustained inhibition of phosphorylation of NPM–ALK and its downstream signaling, leading to cell cycle arrest and apoptosis.[70] NVP–TAE684 also induces varying degrees of growth suppression in *EML4–ALK*-bearing lung cancer cell lines, including NCI-H3112, NCI-H2228 and DFCI032.[67,71] PF-2341066 was another compound, which was initially identified as an orally available c-Met inhibitor in biochemical enzymatic screens, but was subsequently found to show selective inhibition of ALK.[72,73] It is highly selective for both ALK and c-Met kinases, being almost 20-fold selective for ALK and c-Met compared with 120 other kinases.[73] PF-2341066 inhibited cell growth of NPM–ALK-positive ALCL-derived cell lines, as well as EML4–ALK-positive NSCLC-derived cell lines with decreased downstream signaling pathways, although their IC_{50} values were significantly higher than those of NVP–TAE684.[71,72] Recently, Soda *et al.* generated transgenic mice, in which the *EML4–ALK*-transgene was selectively expressed in the developing lung under the *surfactant protein C* promoter.[61] All mice developed multiple lung adenocarcinomas soon after birth, which were successfully treated with a 2,4-pyrimidinediamine derivative that specifically inhibits ALK kinase.[61] These observations strongly support that aberrant ALK activity of ALK-fusion proteins is central to the development of ALCL and NSCLC.

Effects of ALK inhibition on *ALK*-mutated neuroblastoma cell lines

In neuroblastoma, the predominant mechanism of ALK activation should be some conformational change caused by a point mutation typically involving the kinase domain, which potentially affects the kinetics of ALK inhibitors on the mutated kinase. However, as long as major ALK mutants are concerned, their kinase activity seems to be successfully inhibited by the currently available ALK inhibitors. Ba/F3 cells transduced with the F1174L or R1275Q ALK mutant were effectively killed by NVP–TAE684 or PF-2341066, whereas the cells transduced with a constitutive active FLT3 mutant or wild-type ALK were not.[13] Thus, both compounds specifically inhibit the kinase activity of these ALK mutants, although the inhibition is more efficient for F1174F than for R1275Q. In fact, many, if not all, neuroblastoma cell lines carrying mutated or amplified *ALK* alleles are shown to be sensitive to these ALK inhibitors.[12,13,71] Interestingly, the sensitivity of some neuroblastoma cell lines to small molecule ALK inhibitors was recognized prior to the discovery of *ALK* mutations in neuroblastoma. McDermott *et al.* tested more than 600 cancer cell lines for their sensitivity to NVP-TAE684 and/or PF-2341066 and found that neuroblastoma cell lines, as well as cell lines derived from ALCL and lung cancer, frequently show sensitivity to these inhibitors.[71] The dependence of *ALK*-mutated neuroblastoma to ALK inhibition is further confirmed by ALK knockdown experiments; shRNA-mediated knockdown of ALK in *ALK*-mutated neuroblastoma cell lines results in the suppression of cell growth, indicating that the major effect of ALK inhibitors on *ALK*-mutated neuroblastoma cell lines are mediated by their activity on ALK rather than off-target effects on other kinases.

As mentioned above, the sensitivity of *ALK*-mutated neuroblastoma cell lines to ALK inhibitors seems to substantially differ among cell lines, depending on the type of *ALK* mutations. The F1174L mutant seems to be more sensitive to NVP–TAE684 than the R1275Q mutant.[13] Some *ALK*-mutated cell lines were resistant to ALK inhibition; SMS–KCNR harbors the R1275Q mutation, but was not killed by NVP–TAE684 or shRNA, indicating that this cell line acquired some additional mutations, escaping from its dependence on ALK signaling.

Concluding remarks

Genetic analyses of neuroblastoma have revealed that aberrant activation of ALK kinase in human cancer is not only caused by gene fusions but also by gene amplification or germline/somatic mutations. However, probably the most significant impact of the discovery of *ALK* mutations in neuroblastoma would be the possibility of successful treatment of *ALK*-mutated neuroblastoma with small molecule ALK-inhibitors, which are now under development in several pharmaceutical companies. Because ALK expression is restricted to developing neural tissues and ALK-deficient mice develop normally,[37] mutated ALK is likely to be a plausible therapeutic target. Although the enthusiasm for *ALK*-targeted therapy for advanced neuroblastoma seems to be too early at this moment, an encouraging result was reported from a clinical trial of crizotinib (PF-2341066) for NSCLC carrying the *EML4–ALK* fusion gene. A total of 50 patients were evaluable for response, where 64% of the overall response rate and 90% of the disease control rate were obtained[74] with minimum adverse reactions. Nevertheless, the result in NSCLC is not easily translated into neuroblastoma cases. For example, while some ALK mutants are shown to be inhibited by the available ALK inhibitors *in vitro*, the impact of different mutation types on the action of inhibitors should be further evaluated. The effect of frequent co-existence of *MYCN* amplification with *ALK* mutations on sensitivity to ALK inhibitors is still elusive, although a cell line, KELLY, which carries both the F1174L mutation and *MYCN* amplification, was reported to be sensitive to NVP–TAE684.[13,71] Finally, the role of ALK inhibitors in *ALK*-non-mutated neuroblastoma is another interest. Some neuroblastoma cell lines (NBEBC1 and NB1771) were shown to be sensitive to shRNA-mediated ALK knockdown, even though they were reported to have no mutated *ALK* alleles.[11] Interestingly, ALK is phosphorylated in these cell lines at lower levels. Considering the frequent expression of ALK in neuroblastoma cells, it may be postulated that regardless of its mutation status, ALK play a positive role during the initiation and promotion of neuroblastoma, even though established tumors may or may not depend on the ALK activity. Clearly, much more work is required before the clinical role of ALK inhibitors in the treatment of advanced neuroblastoma is established.

Disclosure Statement

The authors have no conflict of interest.

References

1 Maris JM, Hogarty MD, Bagatell R, Cohn SL. Neuroblastoma. *Lancet* 2007; **369**: 2106–20.
2 Hiyama E, Iehara T, Sugimoto T *et al.* Effectiveness of screening for neuroblastoma at 6 months of age: a retrospective population-based cohort study. *Lancet* 2008; **371**: 1173–80.
3 Brodeur GM. Neuroblastoma: biological insights into a clinical enigma. *Nat Rev Cancer* 2003; **3**: 203–16.
4 Pearson AD, Pinkerton CR, Lewis IJ, Imeson J, Ellershaw C, Machin D. High-dose rapid and standard induction chemotherapy for patients aged over 1 year with stage 4 neuroblastoma: a randomised trial. *Lancet Oncol* 2008; **9**: 247–56.
5 De Bernardi B, Nicolas B, Boni L *et al.* Disseminated neuroblastoma in children older than one year at diagnosis: comparable results with three consecutive high-dose protocols adopted by the Italian Co-Operative Group for Neuroblastoma. *J Clin Oncol* 2003; **21**: 1592–601.
6 Matthay KK, Villablanca JG, Seeger RC *et al.* Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. Children's Cancer Group. *N Engl J Med* 1999; **341**: 1165–73.
7 Brodeur GM, Seeger RC, Schwab M, Varmus HE, Bishop JM. Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. *Science* 1984; **224**: 1121–4.
8 Seeger RC, Brodeur GM, Sather H *et al.* Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. *N Engl J Med* 1985; **313**: 1111–6.
9 Katzenstein HM, Bowman LC, Brodeur GM *et al.* Prognostic significance of age, MYCN oncogene amplification, tumor cell ploidy, and histology in 110 infants with stage D(S) neuroblastoma: the pediatric oncology group experience – a pediatric oncology group study. *J Clin Oncol* 1998; **16**: 2007–17.
10 Hansford LM, Thomas WD, Keating JM *et al.* Mechanisms of embryonal tumor initiation: distinct roles for MycN expression and MYCN amplification. *Proc Natl Acad Sci U S A* 2004; **101**: 12664–9.
11 Mosse YP, Laudenslager M, Longo L *et al.* Identification of ALK as a major familial neuroblastoma predisposition gene. *Nature* 2008; **455**: 930–5.
12 Janoueix-Lerosey I, Lequin D, Brugieres L *et al.* Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma. *Nature* 2008; **455**: 967–70.
13 George RE, Sanda T, Hanna M *et al.* Activating mutations in ALK provide a therapeutic target in neuroblastoma. *Nature* 2008; **455**: 975–8.

doi: 10.1111/j.1349-7006.2010.01825.x

14 Chen Y, Takita J, Choi YL *et al.* Oncogenic mutations of ALK kinase in neuroblastoma. *Nature* 2008; **455**: 971–4.
15 Friedman DL, Kadan-Lottick NS, Whitton J *et al.* Increased risk of cancer among siblings of long-term childhood cancer survivors: a report from the childhood cancer survivor study. *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 1922–7.
16 Shojaei-Brosseau T, Chompret A, Abel A *et al.* Genetic epidemiology of neuroblastoma: a study of 426 cases at the Institut Gustave-Roussy in France. *Pediatr Blood Cancer* 2004; **42**: 99–105.
17 Maris JM, Kyemba SM, Rebbeck TR *et al.* Molecular genetic analysis of familial neuroblastoma. *Eur J Cancer* 1997; **33**: 1923–8.
18 Kushner BH, Gilbert F, Helson L. Familial neuroblastoma. Case reports, literature review, and etiologic considerations. *Cancer* 1986; **57**: 1887–93.
19 Knudson AG Jr, Strong LC. Mutation and cancer: neuroblastoma and pheochromocytoma. *Am J Hum Genet* 1972; **24**: 514–32.
20 Longo L, Panza E, Schena F *et al.* Genetic predisposition to familial neuroblastoma: identification of two novel genomic regions at 2p and 12p. *Hum Hered* 2007; **63**: 205–11.
21 Maris JM, Weiss MJ, Mosse Y *et al.* Evidence for a hereditary neuroblastoma predisposition locus at chromosome 16p12-13. *Cancer Res* 2002; **62**: 6651–8.
22 Perri P, Longo L, Cusano R *et al.* Weak linkage at 4p16 to predisposition for human neuroblastoma. *Oncogene* 2002; **21**: 8356–60.
23 Amiel J, Laudier B, Attie-Bitach T *et al.* Polyalanine expansion and frameshift mutations of the paired-like homeobox gene PHOX2B in congenital central hypoventilation syndrome. *Nat Genet* 2003; **33**: 459–61.
24 Mosse YP, Laudenslager M, Khazi D *et al.* Germline PHOX2B mutation in hereditary neuroblastoma. *Am J Hum Genet* 2004; **75**: 727–30.
25 Trochet D, Bourdeaut F, Janoueix-Lerosey I *et al.* Germline mutations of the paired-like homeobox 2B (PHOX2B) gene in neuroblastoma. *Am J Hum Genet* 2004; **74**: 761–4.
26 Raabe EH, Laudenslager M, Winter C *et al.* Prevalence and functional consequence of PHOX2B mutations in neuroblastoma. *Oncogene* 2008; **27**: 469–76.
27 Caren H, Abel F, Kogner P, Martinsson T. High incidence of DNA mutations and gene amplifications of the ALK gene in advanced sporadic neuroblastoma tumours. *Biochem J* 2008; **416**: 153–9.
28 George RE, Attiyeh EF, Li S *et al.* Genome-wide analysis of neuroblastomas using high-density single nucleotide polymorphism arrays. *PLoS ONE* 2007; **2**: e255.
29 Nannya Y, Sanada M, Nakazaki K *et al.* A robust algorithm for copy number detection using high-density oligonucleotide single nucleotide polymorphism genotyping arrays. *Cancer Res* 2005; **65**: 6071–9.
30 Yamamoto G, Nannya Y, Kato M *et al.* Highly sensitive method for genomewide detection of allelic composition in nonpaired, primary tumor specimens by use of affymetrix single-nucleotide-polymorphism genotyping microarrays. *Am J Hum Genet* 2007; **81**: 114–26.
31 Osajima-Hakomori Y, Miyake I, Ohira M, Nakagawara A, Nakagawa A, Sakai R. Biological role of anaplastic lymphoma kinase in neuroblastoma. *Am J Pathol* 2005; **167**: 213–22.
32 Shiota M, Fujimoto J, Takenaga M *et al.* Diagnosis of t(2;5)(p23;q35)-associated Ki-1 lymphoma with immunohistochemistry. *Blood* 1994; **84**: 3648–52.
33 Morris SW, Kirstein MN, Valentine MB *et al.* Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. *Science* 1994; **263**: 1281–4.
34 Lee HH, Norris A, Weiss JB, Frasch M. Jelly belly protein activates the receptor tyrosine kinase Alk to specify visceral muscle pioneers. *Nature* 2003; **425**: 507–12.
35 Stoica GE, Kuo A, Aigner A *et al.* Identification of anaplastic lymphoma kinase as a receptor for the growth factor pleiotrophin. *J Biol Chem* 2001; **276**: 16772–9.
36 Stoica GE, Kuo A, Powers C *et al.* Midkine binds to anaplastic lymphoma kinase (ALK) and acts as a growth factor for different cell types. *J Biol Chem* 2002; **277**: 35990–8.
37 Duyster J, Bai RY, Morris SW. Translocations involving anaplastic lymphoma kinase (ALK). *Oncogene* 2001; **20**: 5623–37.
38 Iwahara T, Fujimoto J, Wen D *et al.* Molecular characterization of ALK, a receptor tyrosine kinase expressed specifically in the nervous system. *Oncogene* 1997; **14**: 439–49.
39 Morris SW, Naeve C, Mathew P *et al.* ALK, the chromosome 2 gene locus altered by the t(2;5) in non-Hodgkin's lymphoma, encodes a novel neural receptor tyrosine kinase that is highly related to leukocyte tyrosine kinase (LTK). *Oncogene* 1997; **14**: 2175–88.
40 Pulford K, Lamant L, Morris SW *et al.* Detection of anaplastic lymphoma kinase (ALK) and nucleolar protein nucleophosmin (NPM)-ALK proteins in normal and neoplastic cells with the monoclonal antibody ALK1. *Blood* 1997; **89**: 1394–404.
41 Shiota M, Fujimoto J, Semba T, Satoh H, Yamamoto T, Mori S. Hyperphosphorylation of a novel 80 kDa protein-tyrosine kinase similar to Ltk in a human Ki-1 lymphoma cell line, AMS3. *Oncogene* 1994; **9**: 1567–74.
42 Lamant L, Pulford K, Bischof D *et al.* Expression of the ALK tyrosine kinase gene in neuroblastoma. *Am J Pathol* 2000; **156**: 1711–21.
43 Cools J, Wlodarska I, Somers R *et al.* Identification of novel fusion partners of ALK, the anaplastic lymphoma kinase, in anaplastic large-cell lymphoma and inflammatory myofibroblastic tumor. *Genes Chromosom Cancer* 2002; **34**: 354–62.
44 Colleoni GW, Bridge JA, Garicochea B, Liu J, Filippa DA, Ladanyi M. ATIC–ALK: a novel variant ALK gene fusion in anaplastic large cell lymphoma resulting from the recurrent cryptic chromosomal inversion, inv(2)(p23q35). *Am J Pathol* 2000; **156**: 781–9.
45 Ma Z, Cools J, Marynen P *et al.* Inv(2)(p23q35) in anaplastic large-cell lymphoma induces constitutive anaplastic lymphoma kinase (ALK) tyrosine kinase activation by fusion to ATIC, an enzyme involved in purine nucleotide biosynthesis. *Blood* 2000; **95**: 2144–9.
46 Trinei M, Lanfrancone L, Campo E *et al.* A new variant anaplastic lymphoma kinase (ALK)-fusion protein (ATIC–ALK) in a case of ALK-positive anaplastic large cell lymphoma. *Cancer Res* 2000; **60**: 793–8.
47 Touriol C, Greenland C, Lamant L *et al.* Further demonstration of the diversity of chromosomal changes involving 2p23 in ALK-positive lymphoma: 2 cases expressing ALK kinase fused to CLTCL (clathrin chain polypeptide-like). *Blood* 2000; **95**: 3204–7.
48 Griffin CA, Hawkins AL, Dvorak C, Henkle C, Ellingham T, Perlman EJ. Recurrent involvement of 2p23 in inflammatory myofibroblastic tumors. *Cancer Res* 1999; **59**: 2776–80.
49 Bridge JA, Kanamori M, Ma Z *et al.* Fusion of the ALK gene to the clathrin heavy chain gene, CLTC, in inflammatory myofibroblastic tumor. *Am J Pathol* 2001; **159**: 411–5.
50 Ma Z, Hill DA, Collins MH *et al.* Fusion of ALK to the Ran-binding protein 2 (RANBP2) gene in inflammatory myofibroblastic tumor. *Genes Chromosom Cancer* 2003; **37**: 98–105.
51 Panagopoulos I, Nilsson T, Domanski HA *et al.* Fusion of the SEC31L1 and ALK genes in an inflammatory myofibroblastic tumor. *Int J Cancer* 2006; **118**: 1181–6.
52 Debelenko LV, Arthur DC, Pack SD, Helman LJ, Schrump DS, Tsokos M. Identification of CARS–ALK fusion in primary and metastatic lesions of an inflammatory myofibroblastic tumor. *Lab Invest* 2003; **83**: 1255–65.
53 Jazii FR, Najafi Z, Malekzadeh R *et al.* Identification of squamous cell carcinoma associated proteins by proteomics and loss of beta tropomyosin expression in esophageal cancer. *World J Gastroenterol* 2006; **12**: 7104–12.
54 Takeuchi K, Choi YL, Togashi Y *et al.* KIF5B–ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. *Clin Cancer Res* 2009; **15**: 3143–9.
55 Soda M, Choi YL, Enomoto M *et al.* Identification of the transforming EML4–ALK fusion gene in non-small-cell lung cancer. *Nature* 2007; **448**: 561–6.
56 Fujimoto J, Shiota M, Iwahara T *et al.* Characterization of the transforming activity of p80, a hyperphosphorylated protein in a Ki-1 lymphoma cell line with chromosomal translocation t(2;5). *Proc Natl Acad Sci U S A* 1996; **93**: 4181–6.
57 Bischof D, Pulford K, Mason DY, Morris SW. Role of the nucleophosmin (NPM) portion of the non-Hodgkin's lymphoma-associated NPM-anaplastic lymphoma kinase fusion protein in oncogenesis. *Mol Cell Biol* 1997; **17**: 2312–25.
58 Kuefer MU, Look AT, Pulford K *et al.* Retrovirus-mediated gene transfer of NPM–ALK causes lymphoid malignancy in mice. *Blood* 1997; **90**: 2901–10.
59 Chiarle R, Gong JZ, Guasparri I *et al.* NPM–ALK transgenic mice spontaneously develop T-cell lymphomas and plasma cell tumors. *Blood* 2003; **101**: 1919–27.
60 Turner SD, Tooze R, Maclennan K, Alexander DR. Vav-promoter regulated oncogenic fusion protein NPM–ALK in transgenic mice causes B-cell lymphomas with hyperactive Jun kinase. *Oncogene* 2003; **22**: 7750–61.
61 Soda M, Takada S, Takeuchi K *et al.* A mouse model for EML4–ALK-positive lung cancer. *Proc Natl Acad Sci U S A* 2008; **105**: 19893–7.
62 Bai RY, Dieter P, Peschel C, Morris SW, Duyster J. Nucleophosmin-anaplastic lymphoma kinase of large-cell anaplastic lymphoma is a constitutively active tyrosine kinase that utilizes phospholipase C-gamma to mediate its mitogenicity. *Mol Cell Biol* 1998; **18**: 6951–61.
63 Bai RY, Ouyang T, Miething C, Morris SW, Peschel C, Duyster J. Nucleophosmin-anaplastic lymphoma kinase associated with anaplastic large-cell lymphoma activates the phosphatidylinositol 3-kinase/Akt antiapoptotic signaling pathway. *Blood* 2000; **96**: 4319–27.
64 Nieborowska-Skorska M, Slupianek A, Xue L *et al.* Role of signal transducer and activator of transcription 5 in nucleophosmin/anaplastic lymphoma kinase-mediated malignant transformation of lymphoid cells. *Cancer Res* 2001; **61**: 6517–23.

65 Slupianek A, Nieborowska-Skorska M, Hoser G *et al.* Role of phosphatidylinositol 3-kinase-Akt pathway in nucleophosmin/anaplastic lymphoma kinase-mediated lymphomagenesis. *Cancer Res* 2001; **61**: 2194–9.
66 Amin HM, McDonnell TJ, Ma Y *et al.* Selective inhibition of STAT3 induces apoptosis and G(1) cell cycle arrest in ALK-positive anaplastic large cell lymphoma. *Oncogene* 2004; **23**: 5426–34.
67 Koivunen JP, Mermel C, Zejnullahu K *et al.* EML4–ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. *Clin Cancer Res* 2008; **14**: 4275–83.
68 Hubbard SR, Mohammadi M, Schlessinger J. Autoregulatory mechanisms in protein-tyrosine kinases. *J Biol Chem* 1998; **273**: 11987–90.
69 Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell* 2010; **141**: 1117.
70 Galkin AV, Melnick JS, Kim S *et al.* Identification of NVP–TAE684, a potent, selective, and efficacious inhibitor of NPM–ALK. *Proc Natl Acad Sci U S A* 2007; **104**: 270–5.
71 McDermott U, Iafrate AJ, Gray NS *et al.* Genomic alterations of anaplastic lymphoma kinase may sensitize tumors to anaplastic lymphoma kinase inhibitors. *Cancer Res* 2008; **68**: 3389–95.
72 Christensen JG, Zou HY, Arango ME *et al.* Cytoreductive antitumor activity of PF-2341066, a novel inhibitor of anaplastic lymphoma kinase and c-Met, in experimental models of anaplastic large-cell lymphoma. *Mol Cancer Ther* 2007; **6**: 3314–22.
73 Zou HY, Li Q, Lee JH *et al.* An orally available small-molecule inhibitor of c-Met, PF-2341066, exhibits cytoreductive antitumor efficacy through antiproliferative and antiangiogenic mechanisms. *Cancer Res* 2007; **67**: 4408–17.
74 Bang Y, Kwak EL, Shaw AT *et al.* Clinical activity of the oral ALK inhibitor PF-02341066 in ALK-positive patients with non-small cell lung cancer (NSCLC). *J Clin Oncol* 2010; **28**(Suppl): abstr3.

doi: 10.1111/j.1349-7006.2010.01825.x

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.)

Supported by a grant for clinical cancer research from the Ministry of Health, Labor, and Welfare, Japan. The authors declare no conflicts of interest.

0022-3476/$ - see front matter. 10.1016/j.jpeds.2011.03.046

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

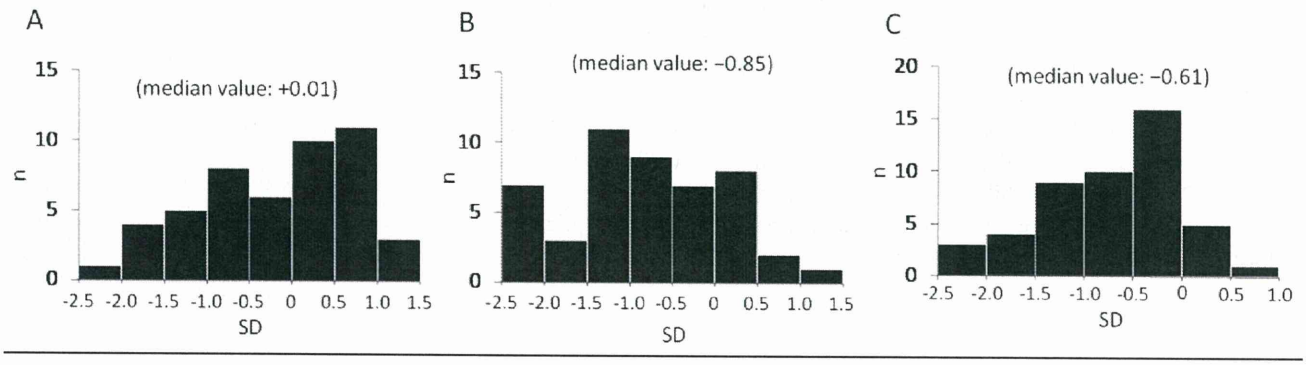

Figure 1. Change in height-SDS during imatinib treatment. Height-SDS is shown at **A,** the commencement of imatinib treatment and **B,** at the last follow-up. **C,** Minimum height-ΔSDS during imatinib treatment. The median value is indicated above each plot. n, number of patients.

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.

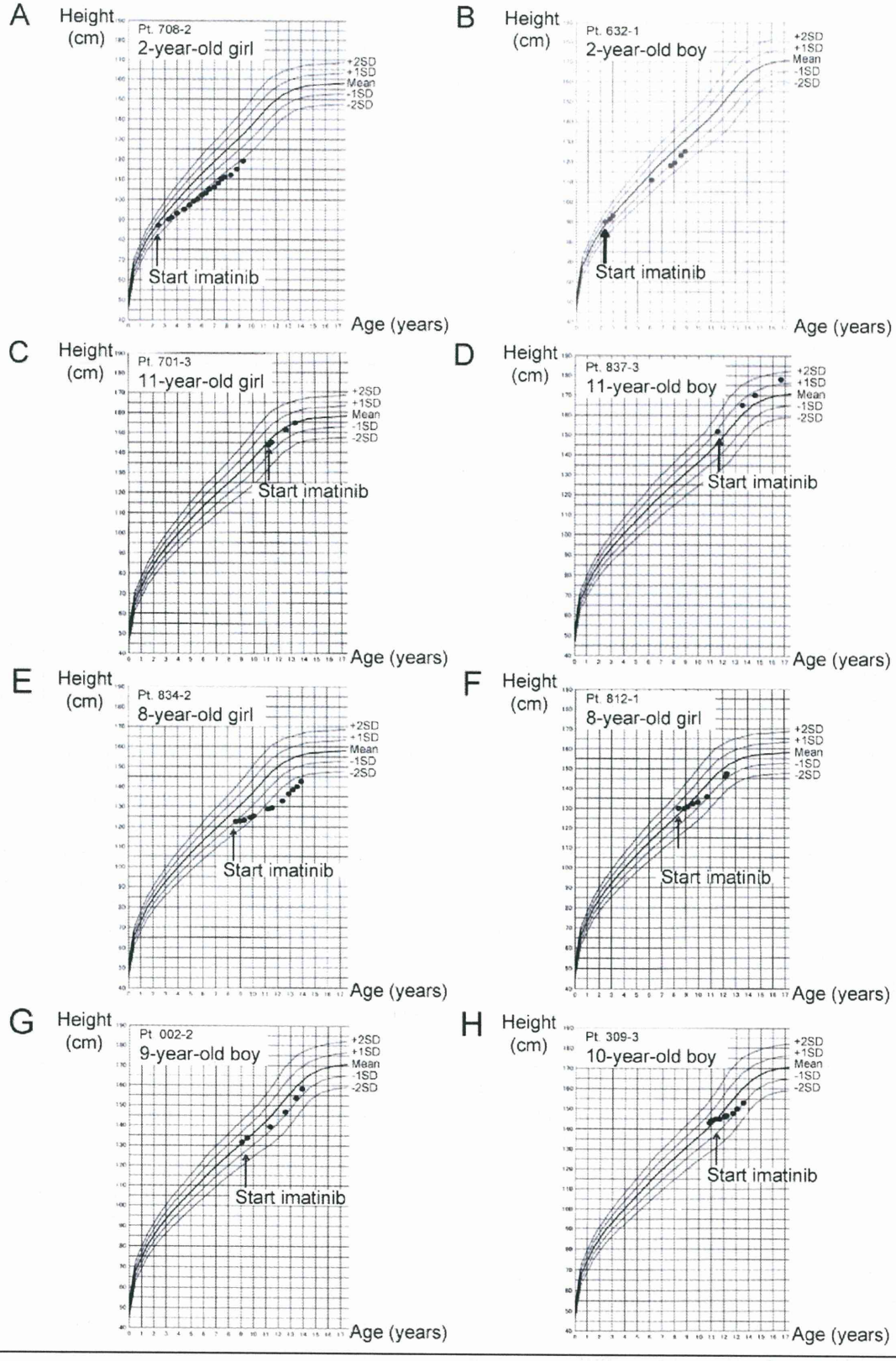

Figure 2. **A** and **B,** Representative height growth chart at the start of imatinib treatment of prepubertal children, and **C** and **D,** pubertal children. Growth impairment was observed in children at prepubertal age, but imatinib had little affect on growth in children at pubertal age. Impaired growth before puberty recovered as children reached pubertal age even during imatinib treatment. Catch-up growth was observed at **E** and **F,** approximately 11 years for girls, and **G** and **H,** 13 years for boys.

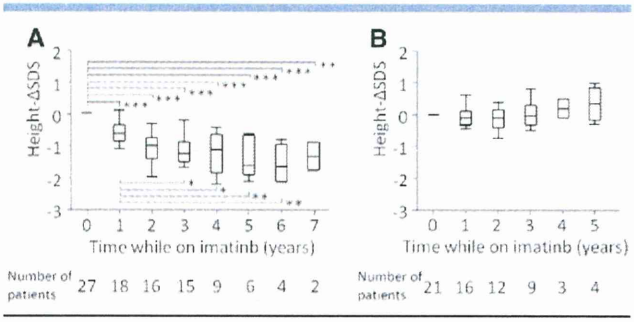

Figure 3. Height-ΔSDS during imatinib treatment of **A,** prepubertal (girls < 9 years, boys < 11 years) or **B,** pubertal children (girls ≥ 9 years, boys ≥ 11 years) in relation to age at the start of treatment. Annual height-ΔSDS is determined by subtracting height-SDS at each annual time point closest to each full-year point within ±6 months from the start of imatinib treatment. *$P < .05$; **$P < .01$; ***$P < .001$, Tukey-Kramer highly significant difference test.

Two previous reports demonstrated a recovery in growth velocity, one after discontinuation of imatinib treatment[3] and another at the onset of puberty even during imatinib treatment.[2] In our study, among 27 children who started imatinib at prepubertal age, 8 children were followed up over the pubertal age range; catch-up growth occurred in 4 children as they reached pubertal age, even during imatinib treatment (Figure 2, E-H). Human growth is described by the infancy-childhood-puberty growth model, and growth in puberty is dependent on the synergism between sex hormones and growth hormone (GH).[18] In these 4 children, noticeable catch-up growth was observed at approximately 11 years in girls (Figure 2, E and F) and 13 years in boys (Figure 2, G and H), consistent with the age at onset of the pubertal growth spurt.[18] These data support the hypothesis that imatinib has little effect on growth of children at pubertal age. Although more follow-up is needed to determine whether this catch-up is complete or incomplete, at least incomplete catch-up growth may be expected in the remaining 4 boys, who were only 13 years or younger at the last follow-up. Our study was performed based on generally agreed-upon prepubertal and pubertal ages, and more detailed studies are needed to determine the relationship between pubertal development and growth impairment.

Vandyke et al[19] recently reported that the rapid acceleration of growth plate closure resulting from the inhibition of PDGF-β receptor signaling by imatinib caused rapid acceleration of growth plate closure. However, bone age detected by wrist and hand X-rays showed no acceleration in other studies,[2,3] and the mechanism associated with the growth inhibitory effect of imatinib remains uncertain. A recent juvenile mouse model study indicated that long-term imatinib treatment impaired the length growth of tubular bone predominantly in prepubertal animals.[20] Consistent with this mouse model, growth impairment due to imatinib may be mild during the age period when height growth is dependent on sex hormones. Thus, imatinib may have a negative effect on GH or its functions. Indeed, Hobernicht et al[21] recently reported a case demonstrating iatrogenically induced GH deficiency due to tyrosine kinase inhibitor therapy for CML. However, performing a GH provocative test in all cohorts proved to be challenging, and moreover, the follow-up period was not of sufficient length for the majority of our cohort to allow determination of later effects on growth. To clarify the potential growth impairment mechanism of long-term imatinib treatment, further study with an extended follow-up period is needed to evaluate the growth recovery that likely would occur concomitantly with pubertal maturation. Because impaired bone remodeling and GH deficiency are caused by inhibition of tyrosine kinase, which is not specific to imatinib,[1,21] careful monitoring of growth velocity, as well as bone metabolic markers and serum insulin-like growth factor 1, is recommended for children treated with tyrosine kinase inhibitors. ■

We thank all of the participating institutions in Japanese Pediatric Leukemia/Lymphoma Study Group and all members of the Chronic Myeloid Leukemia Committee for their contributions to exact follow-up and data collection in each case.

Submitted for publication Nov 8, 2010; last revision received Feb 17, 2011; accepted Mar 22, 2011.

Reprint requests: Hiroyuki Shimada, MD, PhD, Department of Pediatrics, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. E-mail: hshimada@a5.keio.jp

References

1. Suttorp M, Millot F. Treatment of pediatric chronic myeloid leukemia in the year 2010: use of tyrosine kinase inhibitors and stem cell transplantation. Hematology Am Soc Hematol Educ Progr 2010;368-76.
2. Mariani S, Giona F, Basciani S, Brama M, Gnessi L. Low bone density and decreased inhibin-B/FSH ratio in a boy treated with imatinib during puberty. Lancet 2008;372:111-2.
3. Kimoto T, Inoue M, Kawa K. Growth deceleration in a girl treated with imatinib. Int J Hematol 2009;89:251-2.
4. Schmid H, Jaeger BA, Lohse J, Suttorp M. Longitudinal growth retardation in a prepubertal girl with chronic myeloid leukemia on long-term treatment with imatinib. Haematologica 2009;94:1171-9.
5. Millot F, Baruchel A, Guilhot J, Petit A, Leblanc T, Bertrandet Y, et al. Imatinib is efficient but has a negative impact on growth in children with previously untreated chronic myelogenous leukaemia (CML) in early chronic phase (CP): results of the French National Phase IV Trial (ASH annual meeting abstract). Blood 2009;110:863.
6. Suttorp M. Innovative approaches of targeted therapy for CML of childhood in combination with paediatric haematopoietic SCT. Bone Marrow Transplant 2008;42:S40-6.
7. Barr RD. Imatinib mesylate in children and adolescents with cancer. Pediatr Blood Cancer 2010;55:18-25.
8. Millot F, Guilhot J, Nelken B, Leblanc T, De Bont ES, Békassy AN, et al. Imatinib mesylate is effective in children with chronic myelogenous leukemia in late chronic and advanced phase and in relapse after stem cell transplantation. Leukemia 2006;20:187-92.
9. Champagne MA, Capdeville R, Krailo M, Qu W, Peng B, Rosamilia M, et al. Imatinib mesylate (STI 571) for treatment of children with Philadelphia chromosome–positive leukemia: results from a Children's Oncology Group phase 1 study. Blood 2004;104:2655-60.
10. Druker BJ. Imatinib as a paradigm of targeted therapies. Adv Cancer Res 2004;91:1-30.

11. Fitter S, Dewar AL, Kostakis P, To LB, Hughes TP, Roberts MM, et al. Long-term imatinib therapy promotes bone formation in CML patients. Blood 2008;111:2538-47.
12. Berman E, Nicolaides M, Maki RG, Fleisher M, Chanel S, Scheu K, et al. Altered bone and mineral metabolism in patients receiving imatinib mesylate. N Engl J Med 2006;354:2006-13.
13. O'Sullivan S, Naot D, Callon K, Porteous F, Horne A, Wattie D, et al. Imatinib promotes osteoblast differentiation by inhibiting PDGFR signaling and inhibits osteoclastogenesis by both direct and stromal cell–dependent mechanisms. J Bone Miner Res 2007;22:1679-89.
14. El Hajj Dib I, Gallet M, Mentaverri R, Sévenet N, Brazier M, Kamel S. Imatinib mesylate (Gleevec) enhances mature osteoclast apoptosis and suppresses osteoclast bone resorbing activity. Eur J Pharmacol 2006;551:27-33.
15. Vandyke K, Fitter S, Dewar AL, Hughes TP, Zannettino AC. Dysregulation of bone remodelling by imatinib mesylate. Blood 2010;115:766-74.
16. Dewar AL, Farrugia AN, Condina MR, Bik To L, Hughes TP, Vernon-Roberts B, et al. Imatinib as a potential antiresorptive therapy for bone disease. Blood 2006;107:4334-7.
17. Ando W, Hashimoto J, Nampei A, Tsuboi H, Tateishi K, Ono T, et al. Imatinib mesylate inhibits osteoclastogenesis and joint destruction in rats with collagen-induced arthritis (CIA). J Bone Miner Metab 2006; 24:274-82.
18. Tse WY, Hindmarsh PC, Brook CG. The infancy-childhood-puberty model of growth: clinical aspects. Acta Paediatr Scand Suppl 1989;356: 38-43.
19. Vandyke K, Dewar AL, Fitter S, Menicanin D, To LB, Hughes TP, et al. Imatinib mesylate causes growth plate closure in vivo. Leukemia 2009; 23:2155-9.
20. Suttorp M, Boehme J, Vaitl J, Mosch B, Pursche S, Jung R, et al. Side effects on the heart and skeleton of growing mice attributed to chronic imatinib exposure (ASH annual meeting abstract). Blood 2008;112:402.
21. Hobernicht SL, Schweiger B, Zeitler P, Wang M, Hunger SP. Acquired growth hormone deficiency in a girl with chronic myelogenous leukemia treated with tyrosine kinase inhibitor therapy. Pediatr Blood Cancer 2011;56:671-3.

Pediatr Blood Cancer 2011;57:36–40

Continuous and High-Dose Cytarabine Combined Chemotherapy in Children with Down Syndrome and Acute Myeloid leukemia: Report from the Japanese Children's Cancer and Leukemia Study Group (JCCLSG) AML 9805 Down Study

Takashi Taga, MD,[1]* Yasuto Shimomura, MD,[2] Yasuo Horikoshi, MD,[3] Atsushi Ogawa, MD,[4] Masaki Itoh, MD,[5] Masahiko Okada, MD,[6] Junichi Ueyama, MD,[7] Takeshi Higa, MD,[8] Arata Watanabe, MD,[9] Hirokazu Kanegane, MD,[10] Asayuki Iwai, MD,[11] Yutaka Saiwakawa, MD,[12] Kazuhiro Kogawa, MD,[13] Junko Yamanaka, MD,[14] and Masahito Tsurusawa[2,15]

Background. The aim of the JCCLSG AML 9805 Down study was to evaluate the effect of continuous and high-dose cytarabine combined chemotherapy on the survival outcome of acute myeloid leukemia (AML) with Down syndrome (DS). ***Procedure.*** From May 1998 to December 2006, DS patients with newly diagnosed AML were enrolled. Remission induction therapy consisted of two courses of pirarubicin, vincristine, and continuous-dose cytarabine (AVC1). The patients who achieved complete remission (CR) after two courses of AVC1 were subsequently treated with mitoxantrone and continuous-dose cytarabine (MC), etoposide and high-dose cytarabine (EC) and pirarubicin, vincristine, and continuous-dose cytarabine (AVC2). ***Results.*** Twenty-four patients were enrolled. All patients were younger than 4 years and diagnosed as having acute megakaryoblastic leukemia. Twenty-one patients achieved CR. Three patients died during remission induction therapy due to serious infection. No toxic deaths were observed during remission. All but one patient maintained CR without serious complications. The 5-year overall and event-free survivals were 87.5% ± 6.8% and 83.1% ± 7.7%, respectively. ***Conclusions.*** Continuous and high-dose cytarabine combined chemotherapy with reduced intensity would be effective in DS children with AML. Pediatr Blood Cancer 2011;57:36–40. © 2010 Wiley-Liss, Inc.

Key words: AML; Clinical trials; Down syndrome

INTRODUCTION

Down syndrome (DS) is one of the most common chromosomal abnormalities and is associated with an increased risk of leukemia [1]. The clinical and biological features of acute myeloid leukemia (AML) in DS children are quite different from those in children without DS: younger age, lower white blood cell count, and high incidence of acute megakaryoblastic leukemia [2,3]. Before the 1990s, most patients with AML with DS (AML-DS) received suboptimal therapy, resulting in poor outcomes. In 1992, high rates of event-free survival (EFS) with intensive AML treatment were reported from the pediatric oncology group (POG) [4]. After recognition of the favorable outcome of AML-DS patients treated with the AML protocol, recruitment to collaborative studies for AML-DS patients increased, but it became apparent that treatment-related toxicity was high in most series [5–7]. Since then, several collaborative groups have adapted their AML protocols for AML-DS by reducing the dosage of chemotherapeutic agents [6].

We report herein the results of the Japanese Children's Cancer and Leukemia Study Group AML 9805 Down study, which evaluated the feasibility, efficacy, and safety of continuous and high-dose cytarabine combined chemotherapy, which was adapted for DS patients by reducing dose intensity.

PATIENTS AND METHODS

Patients

Between May 1998 and December 2006, 24 AML patients with DS entered the Japanese Children's Cancer and Leukemia Study Group AML 9805 Down study after informed consent was obtained. Neonates with transient myeloproliferative disorder (TMD), defined as appearance of myeloid blasts within the first months of life, and those with spontaneous remission were not included. All children and adolescents less than 18 years of age with no prior treatment were eligible. The initial diagnosis of AML and its subtypes was determined according to the FAB classification by institution pathologists, with central review for most cases.

DOI 10.1002/pbc.22943
Published online 30 December 2010 in Wiley Online Library (wileyonlinelibrary.com).

Therapy

The scheme of treatment for the JCCLSG AML 9805 Down study is shown in Table I. Remission induction therapy consisted of two courses of AVC1 (cytarabine (Ara-C) 100 mg/m^2/day continuous infusion on days 1–7, pirarubicin 25 mg/m^2 by 60 min infusion on days 2, and 4, and vincristine (VCR) 0.7 mg/m^2 on day 7).

Patients who achieved complete remission (CR) after two courses of AVC1 were subsequently treated with MC (Ara-C 100 mg/m^2/day continuous infusion on days 1–5 and mitoxantrone (MIT) 3.5 mg/m^2 by 60 min infusion days 2–4). EC (high-dose Ara-C 1 g/m^2 every 12 hr on days 1–5, and etoposide 66 mg/m^2 by 2 h infusion on days 2–4) and AVC2 (Ara-C 100 mg/m^2/day continuous infusion on days 1–5, pirarubicin 35 mg/m^2 by 60 min infusion on day 2, and VCR 0.7 mg/m^2 on day 5).

[1]Department of Pediatrics, Shiga University of Medical Science, Japan; [2]Department of Pediatrics, Aichi Medical College, Japan; [3]Department of Hematology and Oncology, Shizuoka Children's Hospital, Japan; [4]Department of Pediatrics, Niigata Cancer Center, Japan; [5]Department of Pediatrics, Fukushima Medical College, Japan; [6]Department of Pediatrics, Nagasaki University, Japan; [7]Department of Pediatrics, Tottori University, Japan; [8]Department of Pediatrics, Ryukyu University, Japan; [9]Department of Pediatrics, Nakadori General Hospital, Japan; [10]Department of Pediatrics, Toyama University, Japan; [11]Department of Hematology and Oncology, Kagawa Children's Hospital, Japan; [12]Department of Pediatrics, Kanazawa Medical College, Japan; [13]Department of Pediatrics, National Defense Medical College, Japan; [14]Department of Pediatrics, International Medical Center of Japan, Japan; [15]Chairperson of JCCLSG, Japan

Conflict of Interest: Nothing to report.

*Correspondence to: Takashi Taga, MD, Department of Pediatrics, Shiga University of Medical Science, Tsukinowa-cho, Seta, Ohtsu, Shiga 520-2192. E-mail: ttaga@belle.shiga-med.ac.jp

Received 2 September 2010; Accepted 5 November 2010

TABLE I. Treatment Regimen of the JCCLSG AML9805 Down Study

	Regimen	Administration	Daily dose	Days
Induction				
AVC1	Cytarabine	IV (24 h)	100 mg/m^2	1–7
	Pirarubicin	IV (1 h)	25 mg/m^2	2–4
	Vincristine	IV	0.7 mg/m^2	7
	Methotrexate	IT	Age-adjusted[a]	1
	Cytarabine	IT	Age-adjusted[a]	1, (5, 10)[b]
	Hydrocortisone	IT	Age-adjusted[a]	1, (5, 10)[b]
Consolidation				
MC	Cytarabine	IV (24 h)	100 mg/m^2	1–5
	Mitoxantrone	IV (1 h)	3.5 mg/m^2	2–4
EC	Cytarabine	IV (2 h)	1 g × 2 /m^2	1–5
	Etoposide	IV (2 h)	66 mg/m^2	2–4
AVC2	Cytarabine	IV (24 h)	100 mg/m^2	1–5
	Pirarubicin	IV (1 h)	35 mg/m^2	2
	Vincristine	IV	0.7 mg/m^2	5
	Methotrexate	IT	Age-adjusted[a]	1
	Cytarabine	IT	Age-adjusted[a]	1
	Hydrocortisone	IT	Age-adjusted[a]	1

Recommended interval of each cycle was 4 weeks. [a]The doses were adjusted according to patient's age as follows: younger than 1 year, methotrexate (MTX) 5 mg, cytarabine (Ara-C) 10 mg, hydrocortisone (HDC) 10 mg; younger than 2 years, MTX 8 mg, Ara-C 20 mg, HDC 15 mg; younger than 3 years, MTX 10 mg, Ara-C 30 mg, HDC 20 mg; 3 years and older, MTX 12 mg, Ara-C 40 mg, HDC 25 mg. [b]For CNS-positive patients. The doses were adjusted according to patient's age as follows: younger than 1 year, cytarabine (Ara-C) 20 mg, hydrocortisone (HDC) 10 mg; younger than 2 years, Ara-C 30 mg, HDC 15 mg; younger than 3 years old, Ara-C 50 mg, HDC 20 mg; 3 years and older, Ara-C 70 mg, HDC 25 mg.

Prophylactic treatment for central nervous system (CNS) leukemia was performed by intrathecal injection of Ara-C, methotrexate, and hydrocortisone on the first day of AVC1 and AVC2. An absolute neutrophil count of more than 1,500/μL and a platelet count of more than 75,000/μL were the criteria for starting the first course of consolidation therapy, and an absolute neutrophil count of more than 1,500/μL and a platelet count of more than 100,000/μL were the criteria for starting the second course.

Definitions and Statistics

Evaluation of each treatment was performed on the 28th day. Treatment response was defined as follows: CR, less than 5% blasts in the bone marrow; partial remission (PR), less than 15% blasts; and no response (NR), more than 15% blasts or progressive disease at other sites.

CNS involvement was diagnosed if more than 5 leukocytes/μL were identified in the cerebrospinal fluid (CSF) in combination with detectable leukemic cells in the cytospin and/or with neurological symptoms (e.g., cranial nerve palsy).

EFS was calculated from the date of the first day of chemotherapy to last follow-up or to the first event (early death, resistant leukemia, relapse, or death from any cause). The EFS time of patients with an induction failure was calculated as zero. Toxicity was graded according to the Common Terminology Criteria for Adverse Events version 3.

Univariate comparisons of the survival data were performed using the log-rank test. The Statistical Analysis Software (SAS) computer program was used for the analysis. Follow-up data were actualized as of July 31, 2009.

RESULTS

Patient Characteristics

The relevant initial clinical and hematological data of the 24 patients in this study are shown in Table II. Males predominated, and all patients were younger than 4 years (median age, 17 months). The median white blood cell count was 6,500/μL (range 500–70,900/μL). All patients showed FAB M7 morphologically. No patients had CNS involvement. One patient had an extramedullary mass (skin) at initial diagnosis. Cytogenetic analysis of leukemic blasts was available for 22 patients. Favorable cytogenetics, such as inv (16) and t (8; 21), were not observed. Six patients had normal karyotypes with constitutional trisomy 21 only. The remainder had complex karyotypes with aneuploidy and translocation. GATA1 mutation was confirmed only in one patient.

Seven patients had a history of TMD. No patients of them received cytarabine therapy. Nine patients had documented congenital heart disease. Most patients had either surgically repaired defects or asymptomatic atrial septal defect or ventricular septal defect with normal function.

Overall Outcome

Overall, 21 (87.5%) of 24 patients achieved first remission. One patient relapsed with an isolated extramedullary mass after cessation of chemotherapy. The patient has been in third remission after chemotherapy, electron beam irradiation and cord blood cell transplantation following reduced intensity conditioning. The other 20 patients remain in first CR. Estimated 5-year OS and EFS were 87.5% ± 6.8% and 82.6% ± 7.9%, respectively (Fig. 1). No patients with secondary malignancy and severe cardiotoxicity were observed. Median follow-up period for all patients was 75 (range, 0–131) months.

Treatment-Related Mortality

Three deaths occurred that were not related to leukemia during induction therapy. Two of them occurred during the initial induction therapy, and the other occurred during second induction therapy.

TABLE II. Patients' Characteristic in the JCCLSG AML 9805 Down Study (N = 24)

Characteristic	No	%
Age, months		
Median	17	—
0–12	4	17
12–24	12	46
24–36	4	17
36–48	4	17
Sex		
Male	19	79
Female	5	21
History of TMD		
Yes	7	29
No	13	54
Unknown	4	17
Hepatomegaly		
Yes	10	42
No	12	50
Unknown	2	8
Splenomegaly		
Yes	10	42
No	12	50
Unknown	2	8
WBC, $\times 10^9$/L		
Median	6.5	—
Range	2.8–70.9	—
Hb, g/dL		
Median	8.1	—
Range	3.2–11.8	—
Plt, $\times 10^9$/L		
Median	26	—
Range	3–139	—
Cytogenetics		
Trisomy 8	5	21
Monosomy 7	4	17
Additional 21	2	8

Toxic Events

The incidence of grade 3 or 4 toxicity during induction and each intensification phase of therapy is shown in Table III. Three patients died during remission-induction therapy. One death was attributable to intracranial hemorrhage with disseminated intravascular coagulation, and the others were due to sepsis. The rate of induction death was 12.5%. No toxic deaths were observed during remission.

Prognostic Factors

Extramedullary invasion at initial diagnosis was a significant prognostic factor for 5-year EFS on univariate analysis ($P = 0.046$). Other factors, including sex, initial age, initial WBC, history of TMD, and chromosomal abnormality, were not significant.

DISCUSSION

The results of the JCCSLG AML 9805 Down study, which was conducted to evaluate the efficacy and safety of continuous and high-dose cytarabine combined chemotherapy with reduced intensity for AML-DS patients were presented. All patients enrolled in our study were younger than 4 years and had a phenotype of acute megakaryocytic leukemia (AMKL), which was consistent with previous reports for AML-DS. The number of patients was limited, but this regimen appears to be highly effective because there were no non-responders, and only one patient relapsed.

Contemporary clinical trials for AML-DS children are summarized in Table IV [5–11]. Treatment strategies for AML-DS are based on reduced intensity for AML non-DS, such as BFM and our study, or on a specifically designed strategy, such as the AT/DS study and the AML99 Down study in Japan [8,9]. The EFS of these studies, including the present study, has been between 80% and 90%.

The key drugs for the treatment of AML-DS are anthracyclines, cytarabine, and etoposide; it was also confirmed by in vitro studies that AMKL-DS blasts were significantly more sensitive to these drugs than non-DS AML cells [12]. AMKL-DS blasts are especially sensitive to cytarabine, possibly to the effect of the GATA1 mutations and trisomy 21 on the levels of cytarabine-metabolizing enzymes [13].

In the BFM 98 DS study, with a 3-year EFS of 89%, high-dose cytarabine (3 g/m^2) was used as intensification [6]. The authors reported that a high cure rate could be achieved in DS patients with therapy protocols including high-dose cytarabine. However, they also mentioned that it should be confirmed whether a dosage of 3 g/m^2 of cytarabine is necessary because of its toxicity. In

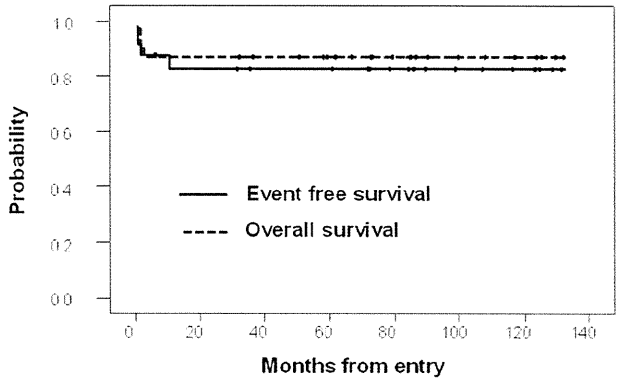

Fig. 1. Actuarial survival rate for the JCCLSG AML 9805 Down study. Of the 24 patients, 22 achieved CR. One patient relapsed. Two patients died during induction therapy. One patient died as a result of sepsis during the first CR. The 5-year overall survival (OS) was 87.5%, and the 5-year EFS was 82.6%.

TABLE III. Severe Adverse Events in the JCCLSG AML9805 Down Study (Grade III–IV)

Adverse events	AVC1-1 no.	n = 24 (%)	AVC1-2 no.	n = 22 (%)	MC no.	n = 21 (%)	EC no.	n = 21 (%)	AVC2 no.	n = 21 (%)
ALT/AST	5	23	2	9	0	0	1	5	0	0
Gastrointestinal	9	41	5	23	5	24	2	10	2	10
Renal	0	0	0	0	0	0	0	0	0	0
Cardiac	0	0	0	0	0	0	0	0	0	0
Pulmonary	1	5	0	0	0	0	0	0	0	0
Neurology	0	0	0	0	0	0	0	0	0	0
Pain	1	5	1	5	0	0	0	0	1	5
Fever/infection	14 (2)	64	9 (1)	41	11	52	15	71	7	33
Others	0	0	0	0	0	0	0	0	0	0

Number of patients who died.

our JCCLSG AML9805 Down study. 1 g/m^2 of cytarabine with etoposide was used for intensification. Serious non-hematological adverse effects, including infection, were not more frequent in this phase than in the other phase of this study (Table III). The dosage of 1 g/m^2 used in the present study may be sufficient for the treatment of AML-DS.

In the Japanese trial AML 99 Down study, the 4-year EFS was 83%, and treatment-related mortality was only 1.4%, which is much lower than that of recent reports for AML-DS [9]. However, relapse and induction failure were more frequent than in other reports with an intensive regimen. The regimen consisted of simple repeating of intermediate doses of pirarubicin and etoposide, so it is possible to reduce the rate of relapse and resistant disease using continuous and high-dose cytarabine combined chemotherapy, as in the JCCLSG AML9805 Down study.

As for other types of leukemia, risk-oriented therapy is proposed if any prognostic factors are identified in AML-DS. In the CCG 2891 study, patients with AML-DS who were older than 2 years had an increased risk of relapse [5]. However, in the BFM 98 DS study and in the Japanese AML 99 Down study, there was no difference in outcome between those 2 years or younger and those older than 2 years [6,9]. The present study also did not identify age older than 2 years as a risk factor, because all 7 patients older than 2 years survived without relapse after completing this protocol.

For cytogenetic factors, monosomy 7 is known to be a risk factor in children with AML [14,15]. In AML-DS, the presence of monosomy 7 adversely affected the outcome in the previous two Japanese trials, but not in the CCG 2891 study [5,8,9]. In the present study, four patients were found to have monosomy 7, and they all maintained remission. Continuous and high-dose cytarabine combined chemotherapy might affect intensification, which negates risk factors such as age and monosomy 7.

It is important to note that only one patient relapsed in the present study. Moreover, the cumulative doses of anthracycline and etoposide in this JCCLSG AML9805 Down study were lower than in other recent reports with intensive regimens for AML-DS. No patients had developed secondary cancer or cardiac insufficiency at the time of this analysis. The survival of DS patients has become longer, and it would be more important to decrease the late toxicity by reducing the cumulative doses of antileukemic drugs for AML-DS patients.

On the contrary, treatment-related mortality occurred in 3 of 24 patients (12.5%), which is more frequent than in other recent reports with intensive regimens for AML-DS. All three patients died from infection during the initial and second courses of this protocol. We could not identify any risk factors for toxicity in these patients, such as age or cardiac disease, compared with the patients who were successfully treated by this protocol. Serious non-hematological adverse effects, including infection, were more frequent during the remission induction phase than during the intensification phase. Induction therapy with combined continuous cytarabine might be toxic for AML-DS patients, although the induction rate is high. On the other hand, toxicity during the intensification phase including high-dose cytarabine was tolerable.

On the basis of the results of the previous Japanese trials and the present study, we have designed a risk-oriented therapy protocol for our next trial with AML-DS. Patients with M2, M3 marrow after induction therapy by pirarubicin, intermediate-dose cytarabine, and etoposide classified into a high-risk group will receive the continuous and high-dose cytarabine combined regimen of this JCCSLG AML9805 Down study.

TABLE IV. Comparison of Recent Clinical Trials for AML-DS

Study	Registry (year)	N	Daunorubicin (mg/m^2)	Ara-C (mg/m^2)	Etoposide (mg/m^2)	TRM (%)	OS (%)	EFS (%)
BFM98 for DS	1998–2003	67	220–240	23–29,000	950	5	91	89 (3y)
BFM93	NA	51	220–400	23,000	950	4	70	68 (3y)
NOPHO AML93	1988–2002	41	300	48,600	1,600	5	NA	85 (8y)
MRC AML10/12	1988–2002	46	670	10,600	NA	15	74	74 (5y)
CCG 2861/2891	1989–1999	160	320	15,800	1,600	4	79	77 (6y)
POG 9421	1995–1999	57	100	20,700	—	0	NA	79 (3y)
AT/Down	1987–1997	33	100–400	4,200	2,700	9	NA	80 (8y)
AML99 DS	2000–2004	72	250	3,500	2,250	1	84	83 (4y)
JCCLSG 9805DS	1998–2006	24	190	12,600	200	12.5	88	83 (5y)

TRM, treatment-related mortality; OS, overall survival; EFS, event-free survival; NA, not evaluated.

ACKNOWLEDGMENT

We are grateful to all participating institutions in the JCCLSG and all members of the JCCLSG AML committee for their contributions to the thorough follow-up and data collection in each case.

REFERENCES

1. Hasle H, Clemmensen IH, Mikkelsen M. Risks of leukaemia and solid tumours in individuals with Down's syndrome. Lancet 2000;355:165–169.
2. Kojima S, Matsuyama T, Sato T, et al. Down's syndrome and acute leukemia in children: an analysis of phenotype by use of monoclonal antibodies and electron microscopic platelet peroxidase reaction. Blood 1990;76:2348–2353.
3. Zipursky A, Thorner P, De Harven E, et al. Myelodysplasia and acute megakaryoblastic leukemia in Down's syndrome. Leukemia Res 1994;18:163–171.
4. Ravindranath Y, Abella E, Krischer JP, et al. Acute myeloid leukemia in Down's syndrome in highly responsive to chemotherapy: experience on Pediatric Oncology Group AML study 8498. Blood 1992;80:2210–2214.
5. Gamis AS, Woods WG, Alonzo TA, et al. Increased age at diagnosis has a significantly negative effect on outcome in children with Down syndrome and acute myeloid leukemia: a report from the Children's Cancer Group Study 2891. J Clin Oncol 2003; 21:3415–3422.
6. Creutzig U, Reinhardt D, Diekamp S, et al. AML patients with Down syndrome have a high cure rate with AML-BFM therapy with reduced dose intensity. Leukemia 2005;19:1355–1360.
7. Rao A, Hills RK, Stiller C, et al. Treatment for myeloid leukaemia of down syndrome: population-based experience in the UK and results from the Medical Research Council AML 10 and AML 12 trials. Br J Haematol 2006;132:576–583.
8. Kojima S, Sako M, Kato K, et al. An effective chemotherapy regimen for acute myeloid leukemia and myelodysplastic syndrome with Down's syndrome. Leukemia 2000;14:786–791.
9. Kudo K, Kojima S, Tabuchi K, et al. Prospective study of a pirarubicin, intermediate-dose cytarabine, and etoposide regimen in children with Down syndrome and acute myeloid leukemia: the Japanese Childhood AML Cooperative Study Group. J Clin Oncol 2007;25:5442–5447.
10. Abildgaard L, Ellebaek E, Gustafsson G, et al. Optimal treatment intensity in children with Down syndrome eloid leukaemia: data from 56 children treated on NOPHO-AML protocols and review of the literature. Ann Haematol 2006;85:275–280.
11. Stevens RF, Hann IM, Wheatley K, et al. Marked improvements in outcome with chemotherapy alone in paedriac acute myeloid leukemia: results of the United Kingdom medical research council's 10th AML trial. MRC childhood leukaemia working party. Br J Haematol 1998;101:130–140.
12. Zwaan CM, Kaspers GJ, Pieters R, et al. Different drug sensitivity profiles of acute myeloid and lymphoblastic leukemia and normal peripheral blood mononuclear cells in children with and without Down syndrome. Blood 2002;99:245–251.
13. Ge Y, Stout ML, Tatman DA, et al. GATA1, cytidine deaminase, and the high cure rate of Down syndrome children with acute megakaryocytic leumemia. J Natl Cancer Inst 2005;97:226–231.
14. Raimondi SC, Chang MN, Ravindranath Y, et al. Chromosomal abnormalities in 478 children with acute myeloid leukemia: clinical characteristics and treatment outcome in a cooperative Pediatric Oncology Group study-POG8821. Blood 1999;94:3707–3716.
15. Wells RJ, Arthur DC, Srivastava A, et al. Prognostic variables in newly diagnosed children and adolescents with acute myeloid leukemia: Children's Cancer Group Study 213. Leukemia 2002; 16:601–607.

Pediatr Blood Cancer 2011;57:461–466

Assessment of Late Cardiotoxicity of Pirarubicin (THP) in Children With Acute Lymphoblastic Leukemia

Yasuto Shimomura, MD, Reizo Baba, MD, Arata Watanabe, MD, Yasuo Horikoshi, MD, Keiko Asami, MD, Nobuyuki Hyakuna, MD, Asayuki Iwai, MD, Takeshi Matsushita, MD, Kazutaka Yamaji, MD, Toshinori Hori, and Masahito Tsurusawa, MD* for The Japanese Childhood Cancer and Leukemia Study Group (JCCLSG)

Background. Pirarubicin (tetrahydropyranyl-adriamycin: THP) is a derivative of doxorubicin with reportedly less cardiotoxicity in adults. However no studies of cardiotoxicity in children treated with THP have been reported. This study was performed to assess the THP-induced cardiotoxicity for children with acute lymphoblastic leukemia (ALL). ***Patients and Methods.*** This study comprised 61 asymptomatic patients aged from 7.6 to 25.7 years old. Median follow-up time after completion of anthracycline treatment was 8.1 years (range: 1.7–12.5). The cumulative dose of THP ranged from 120 to 740 mg/m^2 with a median of 180 mg/m^2. Patients underwent electrocardiogram (ECG), echocardiography, the 6-min walk test (6MWT), and measurements of serum brain natriuretic peptide (BNP) before and after exercise. ***Results.*** All subjects showed normal left ventricular function assessed by echocardiography. Ventricular premature contraction in Holter ECG and reduced exercise tolerance in the 6MWT were detected in 2/46 (3.3%) and 5/41(12.2%), respectively. Abnormal BNP levels were detected in 6/60 (10%) both before and after exercise. The cumulative dose of THP was significantly correlated with BNP levels after exercise ($r = 0.27$, $P = 0.03$), but not with any other cardiac measurements. Further analysis revealed that subjects with a high cumulative dose $\geqq$300 mg/m^2 had significantly higher BNP levels after exercise compared with subjects with a low cumulative dose <300 mg/m^2 ($P = 0.04$). ***Conclusions.*** No significant cardiac dysfunction was detected in long-term survivors who received THP treatment. The use of post-exercise BNP level to indicate high cardiotoxicity risk should be verified by further study. Pediatr Blood Cancer 2011; 57:461–466. © 2011 Wiley-Liss, Inc.

Key words: BNP; cardiotoxicity; childhood ALL; pirarubicin

INTRODUCTION

During the past 30 years, the use of anthracyclines (AC) for the treatment of childhood cancers has significantly improved survival outcomes [1,2]. However, the therapeutic potential of these agents is limited by their cardiotoxicity: acute cardiotoxicity occurs immediately after treatment, early-onset chronic cardiotoxicity presents within 1 year after treatment, and late-onset chronic cardiotoxicity appears after a prolonged asymptomatic period with a latency of one or more years following AC therapy [3–5].

In children, late-onset cardiotoxicity is more common than acute or early-onset toxicity [6–11]. In an effort to reduce overall cardiotoxicity, various AC derivatives have been studied [5]. Pirarubicin (tetrahydropyranyl-adriamycin: THP) is a derivative of doxorubicin (DOX) with reportedly low cardiotoxicity in adult patients [12–20]. However, these reports were limited to acute cardiotoxicity immediately after THP treatment, and there are no available data of late-onset cardiotoxicity in both adult and childhood patients [21,22]. Since the 1990s, the Japanese Childhood Cancer and Leukemia Study Group (JCCLSG) has employed THP in the treatment of acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphomas, and recently, it reported long-term patient outcomes, finding a very low incidence of congestive heart failure among survivors [23–25]. This finding led to assessment of the incidence of subclinical cardiac abnormalities among these survivors, because many previous studies had shown a considerable proportion of asymptomatic childhood cancer survivors who had received AC therapy with possible abnormalities of cardiac function or myocardial biomarkers [26–31]. That is, the importance of longer follow up has become apparent with the increasing numbers of asymptomatic cancer survivors at risk of cardiac dysfunction late in life.

In this study, THP-induced late cardiotoxicity was evaluated for asymptomatic children who received THP therapy in three consecutive JCCLSG studies (ALL911/ALL941/ALL2000). The results showed that THP-induced late cardiac dysfunction was not detected in any subjects, but careful observation may be necessary for subjects who show elevated biomarker levels following the exercise test.

DOI 10.1002/pbc.23012
Published online 4 February 2011 in Wiley Online Library (wileyonlinelibrary.com).

PATIENTS AND METHODS

Study Population

The 33 member institutes of the JCCLSG participated in three consecutive ALL trials, and the total number of long-term survivors was 825 (161 in ALL911, 381 in ALL941, and 283 in ALL2000). This study was performed on subjects from the 7 of these hospitals which had follow-up systems for long-term survivors with the collaboration of cardiologists. In each institute, survivors who had clinical heart failure, as defined by the New York Heart Association classification (NYHA, class III-IV) [32] or cardiovascular disease were excluded. Prior written informed consent was obtained from patients or legal guardians. Finally, 61 patients (9 in ALL911, 48 in ALL941, 4 in ALL2000) were enrolled in this study (Table I). Since many survivors from ALL911 (1991–1993) are now adults with no time to participate the study, and those from ALL2000 (2000–2003) have had a very short follow-up duration, 80% of patients consisted of survivors

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

Department of Pediatrics, Aichi Medical University, Aichi-gun, Aichi-ken, Japan

Conflict of interest: Nothing to declare.

*Correspondence to: Masahito Tsurusawa, MD, Department of Pediatrics, Aichi Medical University, Aichi-gun, Aichi-ken 480-1195, Japan. E-mail: mtsuru@aichi-med-u.ac.jp

Received 24 August 2010; Accepted 9 December 2010

TABLE I. Characteristics of Patients

Sex—male:female		30:31
Age at onset (years old)		5.7 ± 3.5
Age at evaluation (years old)		14.7 ± 3.5
Follow-up period (years)		7.2 ± 2.8
Treatment protocol		
ALL 911	Total	9
	LR	2
	IR	3
	HR	4
ALL 941	Total	48
	LR	7
	IR	21
	HR	17
	HHR	3
ALL 2000	Total	4
	IR	1
	HR	3
Total dose of THP (mg/m^2)		299 ± 192 (120–740; 180)[a]
Total dose of anthracyclins converted to THP (mg/m^2)		346 ± 206 (135–812; 207)[a]

Data are expressed as mean ± SD. HHR, high-high-risk; HR, high-risk; IR, intermediate -risk; LR, low-risk. [a]The number of parenthesis shows the range and median value.

from the ALL941 (1994–1999) study. Ages ranged from 7.6 to 25.7 years old with a median of 14.7, and the median follow-up time after completion of AC therapy ranged from 1.7 to 12.5 years with a median of 8.1. Ten age-matched healthy controls were also recruited (6 males and 4 females; mean age 13.8 ± 2.4 years old). They had normal cardiac function and had not received any treatment affecting the heart, kidneys, or fluid balance before the study.

Intralaboratory Exercise Testing

Master two-step intralaboratory testing with triple exercise loads was performed on every subject. The electrocardiogram (ECG) tracing was recorded before, immediately following, and 1 min after exercise. An abnormal ECG response was defined as a horizontal or downsloping ST segment depression of 0.10 mV (1 mm) for 80 msec [33].

Natriuretic Peptide

Blood samples for measuring brain natriuretic peptide (BNP) before intralaboratory exercise testing were obtained during fasting in the morning, and further samples were obtained after the exercise test. 1.5 ml of blood was drawn into ice-chilled tubes containing ethylene-diamine-tetraacetic acid while the subjects were in a supine position. The blood was centrifuged at 4°C to separate plasma, and stored below −20°C until analysis. Plasma BNP concentrations were measured using chemiluminescent enzyme immunoassay kits (Shionogi BNP; Shionogi & Co., Ltd., Osaka, Japan) [34].

Heart Rate Variability

Holter ambulatory ECG was recorded for every subject to evaluate heart rate variability (HRV). The measurements of heart rate adopted in the present study were standard deviation of NN intervals (SDNN) and co-variance of NN intervals (CVNN).

Heart periods with arrhythmia were excluded from the HRV analyses.

Echocardiography

Echocardiograms were recorded for each subject from the parasternal and apical windows. Two-dimensionally guided M-mode echocardiography was performed, and the measurements were expressed as indices [35]. Variables of systolic functions included: left ventricular diastolic dimension (LVDd), left ventricular end-systolic dimension (LVDs), ejection fraction (EF) defined as (LVDd3 − LVDs3)/LVDd3, and fractional shortening (FS) defined as (LVDd − LVDs)/LVDd. FS < 28% and EF < 54% were considered abnormal [36]. The end-diastolic and end-systolic phases were defined as the beginning of the QRS wave of the ECG tracing and the point at which the second heart sound was recorded by the phonocardiogram, respectively. The variable of diastolic function was the ratio between early (E) and late or atrial (A) ventricular filling velocity (the E/A ratio) [37,38] by a pulsed Doppler measurement. The sample volume was placed between the mitral anulus and the leaflet tips where the greatest velocities were found. Cardiac dysfunction was defined by abnormal FS, and abnormalities of the other determinations were used as confirmatory evidence.

The 6-Minute Walk Test

The 6-min walk test (6MWT) was used to evaluate the functional capacity of the subjects. The field test was performed on a running track to measure the furthest distance a subject can walk. Normal values according to age and sex were defined by Geiger et al. [39].

Statistical Analyses

Regression analyses were used to study the correlation between cumulative THP dose on one side and cardiac function and biomarkers. The unpaired Student's *t*-test was used for the comparison of mean values. SPSS statistical analysis software (SPSS 12.0 J, SPSS Japan Inc., Tokyo, Japan) was used for all computations.

RESULTS

Cumulative dose of THP ranged from 120 to 740 mg/m^2 with a median of 180 mg/m^2. In addition to THP, subjects in ALL941 and ALL2000 received DOX. Thus, total cumulative doses of AC (THP + DOX) ranged from 135 to 812 mg/m^2 with a median of 207 mg/m^2 (Table I). To calculate this, the DOX/THP ratio used was 1:1.08 based on the molecular weight ratio.

The measurements of cardiac functions and the number of abnormal subjects are listed in Table II. ECG at rest was normal in all subjects. However, abnormal ST elevation on ECG was found after laboratory exercise testing in one subject (1.6%). The Holter recording was performed on 59 subjects, and abnormal findings with supra-ventricular premature contraction were detected in 2 (3.3%). These two did not show any other cardiac abnormal measurements. Heart rate variability was normal in all