abdominal DSCT was recognized as a small cell tumor that usually occurs in the intra-abdominal region of children or young adults. DSCT is characterized by small cell proliferation, prominent stromal desmoplasia, and intra-abdominal serosal involvement. A distinct molecular property of this tumor is the presence of an in-frame fusion of the *EWS* gene and the *WT1* gene.[7–11]

Intra-abdominal DSCT has been described in the retroperitoneum, pancreatic region, gastric wall, and the pelvic, mesenteric, and omental regions.[7,9,12] Light microcopy has revealed a divergent differentiation pattern (small cell proliferation in an intermingled pattern) because the majority of cases exhibit a trabecular, basalioid, or glandular architecture associated with abundant interstitial fibrous proliferation, and, in some cases, solid cellular areas.[7] The small cells are typically positive for cytokeratin, EMA, vimentin, desmin, or NSE immunohistochemical staining.[7] Immunoreactivity for S100, Leu7, or LeuM1 has been observed in some cases.[6,13–20]

Cytogenetic investigation of intra-abdominal DSCT has revealed a consistent chromosomal translocation: t(11;22)(p13;q12). This translocation causes an in-frame fusion between the *EWS* gene and the *WT1* gene, producing a chimeric transcript. In most cases of intra-abdominal DSCT, the first seven exons of *EWS* are fused to the last three exons of *WT1*. *EWS-ERG* or *EWS-FLI1* fusion transcripts have also been described in some DSCT cases.[10–12,21]

The soft-tissue tumor in the present case was similarly characterized by divergent immunoreactivity pattern of EMA, cytokeratin, desmin, and vimentin in the solid and trabecular areas of the tumor. Electron microscopy showed the tumor cells to be arrayed with distinct desmosome-type intercellular junctions[6] and with intracytoplasmic intermediate fibrils in a few cells. These characteristics were morphologically and immunohistochemically consistent with a diagnosis of DSCT, despite the extra-abdominal location of the tumor. Molecular analysis of the first and second recurrent tumors showed the same in-frame fusion transcript, even though the tumor tissues had different morphological phenotypes.

Extra-abdominal DSCT is extremely rare, although ovarian or paratesticular involvement has been described.[13–15] DSCT of the soft tissue and bone of the hand, intracranial DSCT, and pleural cavity DSCT have also been reported.[16–19] To the best of our knowledge, no extra-abdominal DSCT confirmed by RT-PCR to exhibit an *EWS-WT1* chimeric fusion have been reported in Japanese studies. The morphological, immunohistochemical, ultrastructural, and molecular features of soft-tissue DSCT are similar to those of intra-abdominal DSCT. Gerald *et al.* reviewed and summarized 109 cases of DSCT, of which 103 were located in the abdominal cavity, four were in the thoracic region, one was in the cranial fossa, and one was in the hand.[20] Swanson *et al.* described 12 cases of polyphenotypic small cell tumors in children; two of these tumors arose from bone, six arose from soft tissue or the axial skeleton, two arose from the CNS, and two arose from the retroperitoneum.[1] All of the tumors exhibited primitive round cell features or neuroectodermal characteristics with or without myogenic, epithelial, or combined differentiation.

The present patient exhibited an uncommon form of the *EWS-WT1* fusion transcript: a fusion of *EWS* exon 9 and *WT1* exon 8, instead of the more common fusion of *EWS* exon 7 and *WT1* exon 8 seen in intra-abdominal DSCT.[8,22] Interestingly, two cases of DSCT of soft tissue and bone and one case of DSCT of the kidney exhibited the same type of fusion transcript.[6,19,23] This molecular aberration might be a variant of the in-frame *EWS-WT1* fusion transcript, because the *EWS-WT1* fusion gene has been reported to exhibit molecular heterogeneity, such as the fusion of *EWS* exon 10 and *WT1* exon 8[24] or the fusion of *EWS* exon 7 and *WT1* exon 9.[12] In contrast, intra-abdominal DSCT with a fusion of *EWS* exon 9 and *WT1* exon 8 has not been previously described. Furthermore, in spite of the cytological heterogeneity of the tumor phenotypes in the present case, the same in-frame *EWS-WT1* fusion was noted in the solid area and the desmoplastic epithelioid region. The genetic findings in the present case suggest that fusion gene heterogeneity may be related to the tissue-specific phenotypes of DSCT, although further investigation of other cases of extra-abdominal DSCT is needed to confirm this speculation.

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Association of 11q Loss, Trisomy 12, and Possible 16q Loss with Loss of Imprinting of Insulin-Like Growth Factor–II in Wilms Tumor

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We evaluated the *WT1* and *IGF2* status and performed chromosome and/or comparative genomic hybridization analysis in 43 tumor samples from patients with Wilms tumor. On this basis, we classified them into 4 groups: *WT1* abnormality, loss of heterozygosity (LOH) of *IGF2*, loss of imprinting (LOI) of *IGF2*, and retention of imprinting (ROI) of *IGF2*, which were seen in 12%, 30%, 16%, and 42% of the tumors, respectively. Patients in the LOI group were older than those in other groups ($P < 0.01$), and tumors in the *WT1* group had fewer cytogenetic changes than did those in the other groups ($P < 0.01$). It was found that 11q− and +12 were more frequent in the LOI group than in the *WT1*+LOH+ROI group ($P < 0.01$ and $P < 0.01$). There was no difference in the incidence of 16q− between the LOI group and the other groups; however, when we excluded 16 tumors with LOH on 11p15, 16q− tended to be more frequent in the LOI group than in the *WT1*+ROI group ($P = 0.06$). The association of 11q− or +12 with LOI of *IGF2* found in the present study suggests that many tumors with no *WT1* abnormalities need overexpression of *IGF2* together with biallelic inactivation of the tumor-suppressor gene on 11q and/or overexpression of growth-promoting genes on chromosome 12. The 11q gene may code for one of the proteins that constitute a CTCF insulator complex, and its mutation, deletion, or haploinsufficiency may cause insulator abnormalities that might lead to LOI of *IGF2*.

INTRODUCTION

Wilms tumor is the most common kidney tumor in childhood. A tumor-suppressor gene, *WT1*, was isolated in the 11p13 chromosomal region, but deletion or mutation has been found in only 15%–20% of Wilms tumors (Huff, 1998; Nakadate et al., 2001). Loss of imprinting (LOI) of insulin-like growth factor–II (*IGF2*), a paternally expressed gene at 11p15.5, has been reported to occur in 40%–70% of tumors (Ogawa et al., 1993; Rainier et al., 1993), and it was associated with a pathological subtype that occurs in a later stage of renal development (Ravenel et al., 2001). Several studies found the type of loss of heterozygosity (LOH) on 11p that is always caused by loss of the maternal chromosome in 30%–40% of tumors investigated (Schroeder et al., 1987; Grundy et al., 1994; Nakadate et al., 2001). LOI or LOH of *IGF2* may cause overexpression of a gene that gives tumor cells a growth advantage or modifies their differentiation stage (Sakatani et al., 2005), and *IGF2* is the primary candidate for being the *WT2* gene. Cytogenetic, comparative genomic hybridization (CGH), and LOH analyses of Wilms tumors showed gain or loss of specific chromosomes or chromosomal regions, indicating that *WT1*-wild-type tumors had more genomic alterations than *WT1*-mutant-type tumors (Nakadate et al., 1999; Hing et al., 2001; Ruteshouser et al., 2005). Furthermore, association of the long arm loss of chromosome 16 (16q−) with LOI of *IGF2* in Wilms tumor was recently reported (Mummert et al., 2005). However, 16q− was found in only a small portion of the tumors with LOI investigated, and no other cytogenetic abnormalities are known to be associated with LOI in the tumors. These studies indicate that Wilms tumor is a genetically heterogeneous disease, and further

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studies are needed to clarify the genetic/epigenetic and cytogenetic background of the tumor.

We evaluated the *WT1* and *IGF2* status and performed chromosome and/or CGH analysis of 43 Wilms tumors, on the basis of which we classified them into 4 genetic/epigenetic groups: *WT1* abnormality, LOH of *IGF2*, LOI of *IGF2*, and retention of imprinting (ROI) of *IGF2*. We analyzed the relationship between cytogenetic and genetic/epigenetic changes and found an association of LOI of *IGF2* with 11q– and +12 and possibly also with 16q–.

MATERIALS AND METHODS

Patient Samples

Tumor samples were available from 68 Japanese infants or children ranging in age from 2 months to 8 years who underwent surgery or biopsy between August 1984 and February 2003. These samples were selected on the basis of tissue availability and were not gathered consecutively. Of the 68 patients, 21 were registered in the Japan Wilms Tumor Group Study (JWITS). Samples of normal tissue were obtained from either the peripheral blood or normal renal tissue adjacent to the tumor from the same patients. Informed consent was obtained from the parents, and the study design was approved by the ethics committee of Saitama Cancer Center. The tumors were staged according to the National Wilms' Tumor Study group (NWTS) staging system, and most patients were treated according to NWTS protocols (d'Angio et al., 1989). None of the 68 patients had a family history of Wilms tumor. One patient (275) had Drash syndrome, and another patient (953) had bilateral tumors; the remaining patients had sporadic and unilateral tumors (Table 1).

Histological Examination

In all tumors, the diagnosis of Wilms tumor was made with routine hematoxylin- and eosin-stained pathology slides by local pathologists from each institution according to the classification proposed by the Japanese Pathological Society and/or the NWTS pathology panel (Beckwith et al., 1978; Japanese Pathological Society, 1988). Twenty-one cases that were registered at the JWITS were also reviewed by the pathology panel.

Cytogenetic, Fluorescence In Situ Hybridization, and CGH Studies

Chromosomes from tumor cells were studied by methods reported previously (Nakadate et al., 1999), and karyotypes were described according to the International System of Human Cytogenetic Nomenclature (ISCN, 1995). Fluorescence in situ hybridization (FISH) using Vysis probes [CEP 3 (chromosome 3 centromere), CEP 12 (chromosome 12 centromere), CBFB (16q22), and MLL (11q23); Downers Grove, IL) were carried out as described previously (Watanabe et al., 2002). CEP 12 was used to detect trisomy 12 and CEP 3 was used as a control because chromosome and CGH analyses detected 2 copies of chromosome 3 in almost all Wilms tumors, and the CBFB and MLL probes were used to detect 16q– and 11q–, respectively. Karyotypes of 11 of the 43 tumors described in Table 1 were reported previously (Nakadate et al., 1999).

CGH analysis was performed as described previously (Kumon et al., 2000). A chromosomal region was considered overrepresented or underrepresented if the average ratio profile was above 1.25 or below 0.75, respectively.

Analyses of WT1 Abnormalities and Allelic Loss on 11p and 11q

DNA preparation and digestion and Southern blot analysis using a *WT1* cDNA probe (WT33; Call et al., 1990), PCR–single-strand conformation polymorphism (SSCP) and subsequent direct-sequencing analysis, and allelic loss analysis on 11p and 11q were performed as described previously (Nakadate et al., 2001). Whether there was allelic loss on 11p and 11q was determined by PCR using microsatellite markers of D11S922, *TH*, *IGF2*, D11S932, *PAX6*, D11S903, D11S4100, *NCAM*, D11S1885, D11S29, and D11S1364 and using the restriction fragment length polymorphism (RFLP) sites of *WT1* (Tadokoro et al., 1993). The primer sequences used for PCR were obtained from the Genome Database (http://www.gdb.org). The results of the allelic loss analysis on 11p and 11q for 21 of the 43 tumors described in Tables 1 and 2 were reported previously (Nakadate et al., 2001).

The results of the study of promoter hypermethylation of *WT1* were reported previously (Satoh et al., 2003).

Analysis of *IGF2* Allelic Expression and Loss

The *Apa*I/*Ava*II polymorphism site in exon 9 was used to evaluate allelic expression of *IGF2*. PCR with genomic DNA from normal tissue and identification of heterozygous specimens after *Ava*II and *Hin*fI digestion were performed as described previously (Watanabe et al., 2002). RT-PCR products from the tumor RNA also were

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TABLE 1. Clinical, Genetic, Karyotypic and CGH findings in 43 Wilms Tumors

Patients number	Age/Sex	Stage of disease	*WT1* Abnormality	Karyotype	CGH	CEP 12/CEP 3	CBFB
Tumors with *WT1* abnormalities and LOH or ROI of *IGF2* (n = 5)							
275*	1 y 0 m/F	I	Mutation in exon 8	48,XX,+3,+6	ND		
832*	9 m/F	II	Mutation in exon 2	45,XX,del(3)(p12p14),−7	ND		
949*	1 y 3 m/F	II	Promoter methylation	44,X,-X,dic r(1;11)(p3?q3?;q25?p1?), inv(9)(p11q12)c	ND		
2375	1 y 9 m/M	IV	Homozygous deletion	46,XY	N		
M289	5 y 4 m/F	II	Mutation in exon 7	ND	enh(18),dim(11p13–11q12, 19,22)		
Tumors with LOH of *IGF2* and no *WT1* abnormalities (n = 13)							
325*	1 y 6 m/M	I	None	47,XY,+8,del(14)(q22)	ND		
528*	4 y 1 m/F	II	None	56,XX,+5,+7,+7,+9,+10,+12,+13,+18,+19,+22	enh(1q,4p,7,8,9,10,12,13,18)	3/2	2
575	3 y 11 m/M	II	None	46,XY	enh(1q)		
871	1 y 4 m/F	I	None	NM	N	2/2	2
918*	4 y 6 m/M	III	None	45,X,-Y	ND		
1075	2 y 4 m//M	IV	None	NM	N		
1390	4 y 0 m/M	I	None	NM	enh(Yq)		
1570*	11 m/F	II	None	51,XX,+7,+8,+10,+12,+13,?del(16)(q22)	enh(7,12,13),dim(16q22–qter)	3/2	1
1658*	2 y 8 m/M	III	None	46,XY,der(16)t(1;16)(q21;q12)	ND		
1752	1 y 0 m/F	III	None	46,XX	N		
2488	3 m/F	I	None	46,XX	N		
M134	10 m/F	II	None	ND	enh(6q),dim(7p)		
M204	3 y 9 m/F	IV	None	ND	enh(8,9,20),dim(Y)		
Tumors with LOI of *IGF2* and no *WT1* abnormalities (n = 7)							
548	3 y 1 m/M	II	None	NM	enh(6,8,9,12)		
1206*	3 y 10 m/F	II	None	50,XX,+12,inc/11q- detected by FISH (MLL)	ND	3/2	2
1207	4 y 4 m/F	III	None	76–87 complex changes	enh(12),dim(9,10p,11q,16q,18p)		
1435*	6 y 1 m/F	III	None	53,XX,+12,inc	ND	3/2	2
1535*	3 y 8 m/F	II	None	46,XX,dup(1)(q21q25),der(11)t(1;11)(q21;q22),del(16)(q22)	enh(1q,4p15–pter),dim(11q13–qter,16q)		
M269	4 y 6 m/F	IV	None	ND	enh(7q,14q21–qter),dim(7p,X)		
M291	8 y 0 m/F	I	None	ND	enh(1q,6,9p,12,13,18q),dim(1p,11q,19)		

(Continued)

TABLE 1. Clinical, Genetic, Karyotypic and CGH findings in 43 Wilms Tumors (Continued)

Patients number	Age/Sex	Stage of disease	*WT1* Abnormality	Karyotype	CGH	CEP 12/CEP 3	CBFB
Tumors with ROI of *IGF2* and no *WT1* abnormalities (n = 18)							
884	2 m/M	III	None	46,XY	N	2/2	2
953	1 y 1 m/F	V	None	47,XX,add(2)(p25), del(7)(q11q22),+8	ND		
1371	5 m/F	IV	None	NM	N		
1420	6 m/F	Unknown	None	46,XX	N		
1879	7 m/M	I	None	46,XY	N		
2011	2 y 7 m/M	II	None	55,XY,+2,+6,+7,+8, +10,+del(12)(q23) +del(12)(q23),+13,+14	enh(1q,2,6,7q21–qter,8,10, 12pter–q23,13,15), dim(1p,18p)		
2385	1 y 4 m/F	IV	None	46,XX	N		
2677	4 y 4 m/F	II	None	46,XX	enh(2)		
2749	5 y 2 m/M	II	None	46,XY	dim(22)		
M126	2 y 5 m/F	III	None	ND	enh(2p14–pter,3q,6,7,8, 12,13,17)		
M175	1 y 9 m/F	I	None	ND	N		
M188	1 y 0 m/M	I	None	ND	N		
M196	1 y 5 m/F	III	None	ND	N		
M232	1 y 2 m/F	II	None	ND	N		
M233	5 y 3 m/F	IV	None	ND	enh(6,8)		
M238	6 m/F	I	None	ND	enh(7,8,10,12,13,17,18)		
M258	4 m/M	I	None	ND	N		
M290	2 y 1 m/M	II	None	ND	enh(1q,6,7,9,12),dim(18p,Y)		

*Karyotypes of these tumors were reported previously (Nakadate et al., 1999).

Abbreviations: NM, no mitotic cells; ND, not done; N, normal; 3/2, 3 copies of CEP 12 and 2 copies of CEP 3 detected by FISH; 2, 2 copies of CBFB detected by FISH.

TABLE 2. Allelic Status of 11p and 11q and *IGF2* Imprinting Status in *WT1*, LOH of *IGF2*, and LOI of *IGF2* Wilms Tumor Groups

	p15					p13		p11	q21–22	11q23					
	S922	*IGF2*	*IGF2-LOI*	*TH01*	S932	*PAX6*	*WT1*	S903	S4100	*NCAM*	S1855	S29	S1364	*WT1* abnormality[a]	11q-detected by CGH/ cytogenetics
Tumors with *WT1* abnormalities and LOH, LOI, or ROI of *IGF2* ($n = 5$)															
275	—	●	—	●	●	—	●	—	—	○	—	○	—	Mutation in exon 8	Not detected
832	—	—	—	●	●	●	●	—	○	○	—	—	○	Mutation in exon 2	Not detected
949	—	—	—	●	—	—	●	●	●	—	—	—	●	Promoter methylation	Not detected
M289	—	○	□	○	○	○	●	●	—	—	○	○	○	Mutation in exon 7	Not detected
2375	○	○	□	○	○	—	▲	—	○	○	—	—	○	Homozygous deletion	Not detected
Tumors with LOH of *IGF* and no *WT1* abnormalities ($n = 13$)															
325	●	—	—	—	—	—	●	—	○	○	—	○	○	None	Not detected
528	—	●	—	●	●	●	—	—	●	—	●	—	●	None	Not detected
575	●	●	—	●	●	—	○	—	○	○	—	—	○	None	Not detected
871	●	—	—	●	—	●	—	—	—	○	○	○	○	None	Not detected
918	●	—	—	—	—	○	○	○	—	—	○	○	○	None	Not detected
1075	—	—	—	●	●	—	○	○	○	○	○	—	○	None	Not detected
1390	—	—	—	●	—	—	●	○	○	○	○	—	—	None	Not detected
1570	—	●	—	—	●	●	—	—	—	●	●	●	—	None	Not detected
1658	—	—	—	●	—	—	—	—	—	—	○	○	—	None	Not detected
1752	●	—	—	●	●	—	●	●	—	—	—	○	○	None	Not detected
2488	●	—	—	—	●	●	●	—	○	—	○	○	○	None	Not detected
M134	—	●	—	—	●	●	●	●	○	○	○	○	—	None	Not detected
M204	—	—	—	—	●	●	●	—	—	●	—	—	○	None	Not detected
Tumors with LOI of *IGF2* and no *WT1* abnormalities ($n = 7$)															
548	—	○	■	○	○	—	○	—	○	—	—	○	○	None	Not detected
1206	—	○	■	○	○	○	○	○	○	—	—	●	●	None	Detected
1207	—	○	■	○	—	—	—	○	—	—	—	—	●	None	Detected
1435	—	○	■	—	—	○	○	—	○	○	—	○	○	None	Not detected
1535	○	○	■	○	—	—	○	—	—	●	●	●	—	None	Detected
M269	○	○	■	○	○	○	○	○	○	—	—	○	○	None	Not detected
M291	○	—	■	○	—	○	○	—	●	—	●	●	●	None	Detected

[a]Details of *WT1* abnormality are described in the text.
● Loss of heterozygosity; ○ Retention of heterozygosity; — Not informative; ■ Loss of *IGF2* imprinting; □ Retention of *IGF2* imprinting; ▲ Homozygous *WT1* deletion.

digested with *Ava*II and *Hinf*I, and allelic expression of *IGF2* was determined.

Statistical Analysis

The significance of differences in various clinical and cytogenetic aspects of the disease among the 4 genetic/epigenetic groups of tumors was determined by the chi-square or Fisher's exact tests. Differences in the mean age of the patients and in the average number of chromosome changes between any 2 of the 4 groups were examined with Welch's *t* test.

RESULTS

Allelic Loss on 11p and 11q

Allelic loss on 11p and 11q was analyzed in Wilms tumor samples from 68 patients. Informative 11p15 loci were found in normal tissue from 64 of the patients; the 11p15 loci in the tissue from the other 4 patients were uninformative. Of the 64 informative tumors, 16 showed LOH. Of the 48 tumors without LOH, 27 were informative for the *Apa*I/*Ava*II polymorphism site of the *IGF2* gene. Thus, 43 tumor samples were the subject of the present study.

Three tumors (949, 528, and 1570) showed LOH for the entire chromosome 11; 1 tumor (M204) showed LOH on 11p15–11q23, retaining heterozygosity in the more distal 11q locus; 3 tumors (575, 918, and 1075) showed LOH limited to the 11p15 region; and 9 (275, 832, 325, 871, 1390, 1658, 1752, 2488, and M134) showed LOH limited to the 11p15–11p13 region (Table 2). Of the 27 tumors without LOH on 11p15, 1 (M289) showed

LOH limited to 11p13–11p11, and 4 (C1206, C1207, C1535, and M291) showed LOH on 11q (Table 2).

WT1 Abnormalities

Of the 9 tumors with LOH limited to the 11p15–11p13 region, 2 showed a *WT1* mutation; one (275) had a missense mutation in exon 8 (G to A conversion in nucleotide 1064; Haber et al., 1991), and the other (832) had a nonsense mutation (C to T conversion in nucleotide 550) in exon 2 (Table 2). Another tumor (C949) was found to have *WT1* promoter methylation, which was examined in 21 of the 43 tumors, of which only 1 showed the methylation (Satoh et al., 2003). This tumor had a ring chromosome containing chromosomes 1 and 11. Because the incidence of promoter methylation was quite low, and no other tumors showed a ring chromosome containing chromosome 11 and LOH for the entire chromosome 11, the other 22 tumors whose *WT1* promoter methylation status was not examined were assumed to be unmethylated.

Of 27 tumors without LOH on 11p15, 1 (M289) with LOH limited to the 11p13–11p11 region had a missense mutation in exon 7 (G to T conversion in nucleotide 895), and another (C2375) with retention of heterozygosity (ROH) for the entire chromosome 11 had homozygous deletion of the 6.6-kb fragment of *WT1*, detected by Southern blotting with a *WT1* cDNA probe and *Eco*RI digestion (Call et al., 1990; Table 2).

LOI of *IGF2*

Of the 27 tumors with ROH in 11p15 and the informative *Apa*I/*Ava*II polymorphism site of *IGF2*, 7 showed LOI of *IGF2* (Tables 1 and 2, Fig. 1). Of the 20 ROI tumors, 2 (M289 and C2375) showed *WT1* abnormalities as described before.

Four Groups of Tumors Classified by *WT1* and *IGF2* Status

We classified 43 Wilms tumors into 4 groups on the basis of major genetic abnormalities: *WT1* abnormality, LOH of *IGF2*, LOI of *IGF2*, and tumors without *WT1* or *IGF2* abnormalities. Three tumors with a *WT1* abnormality and LOH on 11p15–11p13 were included in the *WT1* group because *WT1* abnormalities are believed to have a stronger impact on tumorigenicity than LOH of *IGF2*. Thus, of the 43 tumors, 5 were classified into the *WT1* group, 13 into the LOH group, 7 into the LOI group, and 18 into the ROI group (Table 1).

CGH patterns and/or karyotypes were available for all 43 tumors (Table 1). Four tumors (528, 1206, 1435, and 1570) with a hyperdiploid karyotype (≥50 chromosomes) and trisomy 12 with or without other changes were also studied by FISH using the CEP 3, CEP 12, and CBFB probes. All 4 tumors were shown to have trisomy 12, and 1 was shown to have 16q–. One tumor (1206) was shown to have 11q– using FISH with the *MLL* probe.

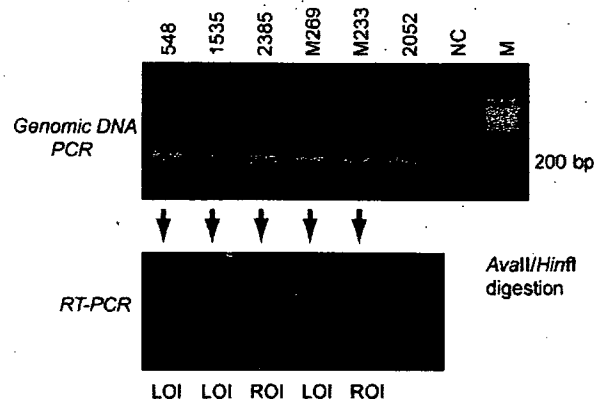

Figure 1. Electrophoretic patterns of products of genomic DNA PCR or reverse-transcription PCR after *Ava*II and *Hin*fI digestion. Normal tissue from samples 548, 1535, 2385, M269, and M233 was observed to have heterozygous *IGF2* alleles, and normal tissue from sample 2052 was observed to have homozygous *IGF2* alleles, in upper lanes; loss of imprinting was found in tumor tissue from samples 548, 1535, and M269 and retention of imprinting in tumor tissue from samples 2385 and M233, in lower lanes [NC, negative control (H_2O); M, size marker].

Clinical Characteristics of Patients in Each Tumor Group

The mean age of the patients was higher in the LOI group than in the *WT1* ($P = 0.03$), the LOH ($P = 0.01$), the ROI ($P < 0.01$), or the *WT1* + LOH + ROI ($P < 0.01$) groups (Table 3). There were no differences in stage distribution among the 4 groups. The tumors of 42 patients were classified as having a favorable histology, and the tumor of 1 patient (1390) was classified as having unfavorable histology (the diffuse anaplasia type). Of the 43 patients, 41 were alive with no evidence of disease at the last follow-up (November 30, 2004). Two patients had died: the patient who had the diffuse anaplasia–type tumor died of the disease, and the patient in the *WT1* group who had Drash syndrome (275) died of renal failure.

Association of Chromosome Abnormalities with *IGF2* and *WT1* Status

Ten chromosome/CGH abnormalities were seen in 4 or more tumors (Table 3). Loss limited to 11q was more frequent in the LOI group than in the *WT1* ($P = 0.08$), the LOH ($P < 0.01$), the ROI ($P < 0.01$), or the *WT1* + LOH + ROI ($P < 0.01$)

TABLE 3. Relationship between Cytogenetic Abnormalities with 4 Wilms Tumor Groups Classified by *WT1* or *IGF2* Status

Tumors classified by *WT1* and *IFG2* status	Number of tumors	Mean age of patients in months (range)	Mean number of cytogenetic changes	+1q	+6	+7/+7q	7p-	+8	+10	11q-[a]	+12[b]	+13	16q-[c]
A. Tumors with *WT1* abnormalities and LOH or ROI of *IGF2*	5	24.2 (9–64)	0.4	0	1	0	1	0	0	0	0	0	0
B. Tumors with LOH of *IGF2* and no *WT1* abnormalities	13	28.7 (3–54)	1.5	3	1	2	1	4	2	0	2	2	2
C. Tumors with LOI of *IGF2* and no *WT1* abnormalities	7	57.4 (37–96)	2.7	2	2	1	1	1	0	4	5	1	2
D. Tumors with ROI of *IGF2* and no *WT1* abnormalities	18	23.2 (2–63)	1.3	2	4	4	0	6	2	0	4	3	0

Mean age: C versus A, $P = 0.03$; C versus B, $P = 0.01$; C versus D, $P < 0.01$; C versus A+B+D, $P < 0.01$.
Mean number of cytogenetic changes: A versus B, $P = 0.12$; A versus C, $P < 0.01$; A versus D, $P = 0.13$; A versus B+C+D, $P < 0.01$.
[a]11q-: C versus A, $P = 0.08$; C versus B, $P < 0.01$; C versus D, $P < 0.01$; C versus A+B+D, $P < 0.01$; C versus A (2 tumors with ROI)+D, $P < 0.01$.
[b]+12: C versus A, $P = 0.03$; C versus B, $P = 0.02$; C versus D, $P = 0.06$; C versus A+B+D, $P < 0.01$; C versus A (2 tumors with ROI)+D, $P = 0.02$.
[c]16q-: C versus A, $P = 0.46$; C versus B, $P = 0.6$; C versus D, $P = 0.06$; C versus A+B+D, $P = 0.12$; C versus A (2 tumors with ROI)+D, $P = 0.06$.

groups. When we added 4 tumors with LOH in the entire chromosome 11 or in the 11p15–11q23 region to the 11q− category, 11q− was still more frequent in the LOI group than in the *WT1*+LOH+ROI group ($P = 0.02$).

Trisomy 12 was more frequent in the LOI group than in the *WT1* ($P = 0.03$), LOH ($P = 0.02$), ROI ($P = 0.06$), or LOH+ROI+*WT1* ($P < 0.01$) groups. Loss of 16q was found only in the LOI or the LOH group, but there was no significant difference among the 4 groups, or between the LOI and the *WT1*+LOH+ROI groups ($P = 0.12$). Mummert et al. (2005) excluded tumors with LOH on 11p15 in a correlation analysis of 16q− and LOI of *IGF2* because LOI of the maternal *IGF2* allele prior to its deletion could not be ascertained. When we excluded 16 tumors with LOH on 11p15, 16q− tended to be more frequent in the LOI group than in the *WT1* group with the ROI of *IGF2* + ROI group ($P = 0.06$). No other associations between chromosome abnormalities with any of the 4 groups were found (Fig. 2).

For the 10 chromosome/CGH abnormalities observed in 4 or more tumors, the mean number per tumor was lower in the *WT1* group (0.4/tumor) than in the LOH (1.5/tumor; $P = 0.12$), LOI (2.7/tumor; $P < 0.01$), ROI (1.3/tumor; $P = 0.13$), or LOH+LOI+ROI (1.7/tumor; $P < 0.01$) groups (Table 3).

DISCUSSION

Wilms tumor is a heterogeneous disease showing various genetic/epigenetic abnormalities, including mutations/deletions of the *WT1* gene, LOH or LOI of the *IGF2* gene, and *CTNNB1* mutations frequently associated with *WT1* abnormalities (Ogawa et al., 1993; Rainier et al., 1993; Koesters et al., 1999; Mati et al., 2000; Ravenel et al., 2001). In addition, we previously reported that hyperdiploid tumors, usually including trisomy 12, might be a unique subgroup of tumors with no *WT1* abnormalities (Nakadate et al., 1999). Cytogenetic, CGH, and LOH studies have found recurrent abnormalities, including gains of 1q, 2, 6, 7, 8, 10, 12, 13, and 18 and losses of 1p, 7p, 9q, 11p, 11q, 16q, and 22q (Nakadate et al., 1999; Hing et al., 2001; Ruteshouser et al., 2005). None of the previous studies simultaneously examined the status of *WT1*, LOH or LOI of *IGF2*, LOH on 11p and 11q, and all chromosome/CGH patterns. The present study showed *WT1* abnormalities, LOH of *IGF2*, LOI of *IGF2*, and ROI of *IGF2* in 12%, 30%, 16%, and 42%, respectively, of 43 Wilms tumors.

Recently, Mummert et al. (2005) reported that Wilms tumors with 16q− had expression of *CTCF* half that of expression in tumors with normal chromosomes 16 and that LOI of *IGF2* was associated with loss of 16q. The *CTCF* gene, at 16q22, codes for an insulator protein. According to Mummert et al. (2005), when less CTCF was available to bind the differentially methylated region (DMR) upstream of *H19*, access of maternal *IGF2* to an enhancer downstream of *H19* might occur. The present study confirmed that tumors with 16q− showed either LOI or LOH of *IGF2* (Yeh et al., 2002; Mummert et al., 2005) and provided support for the association of 16q− with LOI of *IGF2*. Furthermore, the present study disclosed that 11q− and +12 were more frequent in tumors with LOI than in those with LOH, ROI, or *WT1* abnormalities.

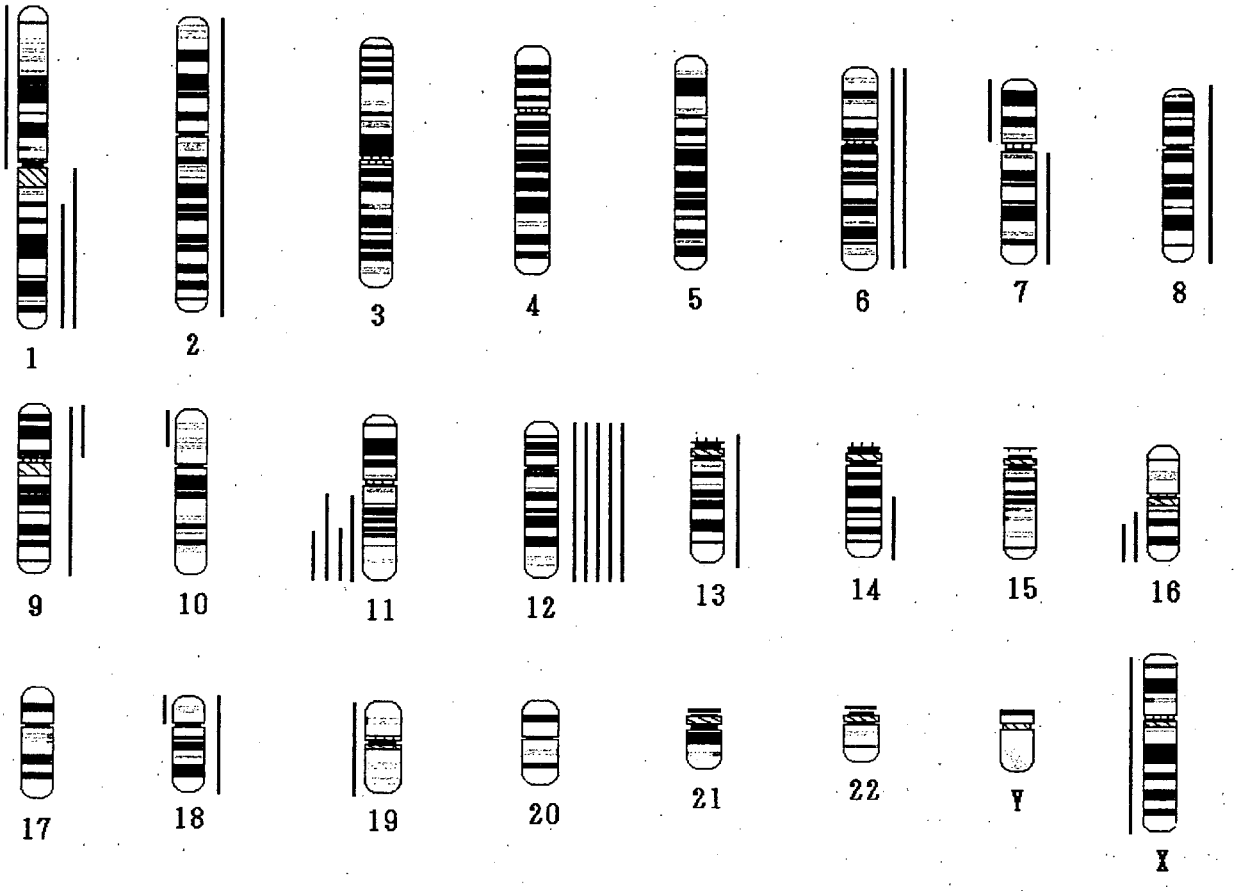

Figure 2. Summary of chromosome changes in the LOI group detected by CGH, chromosome, FISH, and/or LOH analyses. Gains and losses are shown on the right and left sides, respectively.

Overexpression of *IGF2* can be caused by LOI or by duplication of the paternal chromosome 11 with loss of the maternal chromosome 11 (LOH). LOI or LOH of *IGF2* has been detected in various embryonal tumors, including Wilms tumor, rhabdomyosarcoma, and hepatoblastoma (Ogawa et al., 1993; Rainier et al., 1993, 1995; Zhan et al., 1994). More recently, microdeletion of the maternal *H19* DMR was reported in a large family of people with Beckwith–Wiedemann syndrome (Prawitt et al., 2005). Although LOI of *IGF2* was found in fibroblasts from all 4 individuals with the microdeletion, 3 with a second genetic lesion (duplication of the microdeleted maternal *IGF2* locus), but not the one without it, developed Beckwith–Wiedemann syndrome and Wilms tumor. These findings suggest that LOI of *IGF2* or duplication of the paternal *IGF2* may be one of several genetic and epigenetic events that promote tumor cell proliferation.

The present study found an association of 11q– with LOI of *IGF2*. Very recently, Yuan et al. (2005) studied LOI of *IGF2* by assessing DNA methylation of the *H19* DMR and LOH by single-nucleotide polymorphism (SNP) chips in 58 sporadic Wilms tumors, 22 of which showed LOI. Partial loss of 11q and loss of whole chromosome 11 were found in 6 and 0, respectively, of the 22 LOI tumors, and in 1 and 13, respectively, of the 36 non-LOI tumors. They stated that 11q– was not associated with LOI. When we added 4 tumors with LOH for the entire chromosome 11 or the 11p and 11q regions into the 11q– category in the present series, 11q– was still more frequent in the LOI group than in the *WT1* + LOH + ROI group. Whole loss of chromosome 11 may play a role in loss of the wild-type *WT1* allele or in loss of the maternal *IGF2* allele, and 11q– may be a bystander in tumors with whole loss of chromosome 11 and *WT1* mutation or duplication of the paternal *IGF2* (LOH). When the 13 tumors with loss of the entire chromosome 11 from the series reported by Yuan et al. (2005) were excluded, partial loss of 11q was more frequent in the LOI tumors (6 of 22 tumors) than in the non-LOI tumors (1 of 23 tumors), $P < 0.01$, Fisher's exact

test. Thus, the present study and that of Yuan et al. (2005) lead to the same conclusion: chromosomal loss limited to 11q is associated with LOI of *IGF2* in Wilms tumor.

It has been hypothesized that 11q harbors a tumor-suppressor gene involved in the development of Wilms tumor (Radice et al., 1995; Nakadate et al., 2001). The association between 11q− and LOI of *IGF2* found in the present study suggests that Wilms tumors with overexpression of *IGF2* require deletion/mutation of the putative 11q gene in order to develop to full-blown tumors. As we have shown (Tables 1 and 2, Fig. 2), the present CGH and cytogenetic study detected physical loss of 11q DNA, rather than mitotic recombination, in the 4 tumors with LOI and 11q LOH. The gene on 11q may code for one of the proteins that constitute a CTCF insulator complex, and mutation, deletion, or haploinsufficiency of the gene may cause insulator abnormalities that might lead to LOI of *IGF2* (Ohlsson et al., 2001).

The present study also found an association between trisomy 12 and LOI of *IGF2*. We previously proposed that hyperdiploid tumors (≥50 chromosomes) make up a unique subgroup of Wilms tumors characterized by the absence of *WT1* abnormalities and nonrandom gains of chromosomes, usually including trisomy 12 (Nakadate et al., 1999). The present study added another characteristic, namely, the tendency to show LOI of *IGF2*, to the list of characteristics of hyperdiploid tumors. *CCND2* and *CDK4*, which are growth-promoting genes on chromosome 12, are overexpressed in Wilms tumors (Faussillon et al., 2005), and it is speculated that tumors with LOI of *IGF2* also need trisomy 12 in order to proliferate in an accelerated manner.

Ravenel et al. (2001) reported that patients who had Wilms tumors with LOI of *IGF2* were older than those who had tumors with normal imprinting and that the tumors with LOI were more likely to be of a pathological subtype associated with a later stage of renal development. The present study confirmed that patients with tumors with LOI were older than those who had tumors of other subtypes. Chromosome changes were most frequent in the LOI group and least frequent in the *WT1* group (Table 3). We suggest from the findings described above that tumors with LOI need far more genetic events to develop into full-blown tumors than do those with certain genetic types of tumors; it will take time to accumulate the genetic and epigenetic events that might explain why patients with LOI of *IGF2* are older.

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Treatment of lung damage

Retrospective analysis of steroid therapy for radiation-induced lung injury in lung cancer patients

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Abstract

***Purpose*: To disclose characteristics of lung cancer patients developing radiation-induced lung injury treated with or without corticosteroid therapy.**

***Methods and materials*: Radiographic changes, symptoms, history of corticosteroid prescription, and clinical course after 50–70 Gy of thoracic radiotherapy were retrospectively evaluated in 385 lung cancer patients.**

***Results*: Radiation-induced lung injury was stable without corticosteroid in 307 patients (Group 1), stable with corticosteroid in 64 patients (Group 2), and progressive to death despite corticosteroid in 14 patients (Group 3). Fever and dyspnea were noted in 11%, 50% and 86% ($p < 0.001$), and in 13%, 44% and 57% ($p < 0.001$) patients in Groups 1–3, respectively. Median weeks between the end of radiotherapy and the first radiographic change were 9.9, 6.7 and 2.4 for Groups 1–3, respectively ($p < 0.001$). The initial prednisolone equivalent dose was 30–40 mg daily in 52 (67%) patients. A total of 16 (4.2%) patients died of radiation pneumonitis or steroid complication with a median survival of 45 (range, 8–107) days.**

***Conclusion*: Development of fever and dyspnea, and short interval between the end of radiotherapy and the first radiographic change were associated with fatal radiation-induced lung injury. Prednisolone 30–40 mg daily was selected for the treatment in many patients.**

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Keywords: Radiation pneumonitis; Radiotherapy; Lung cancer; Corticosteroid

Thoracic radiotherapy is widely used for the curative and palliative treatment of lung cancer. Radiation-induced lung injury was first described as early as 1922 [1,2], and two types of lung injury, radiation pneumonitis and radiation fibrosis, were recognized in 1925 [3]. Radiation pneumonitis occurs in 5–15% of patients who have received radiation therapy for lung cancer. Its clinical symptoms are characterized by cough, dyspnea and fever developing between 1 and 3 months after the end of radiotherapy. Distinctive radiographic changes of radiation pneumonitis are a ground-glass opacification or diffuse haziness in early phase, and then alveolar infiltrates or dense consolidation in late phase in the region corresponding to the irradiated area [4–7]. Radiation pneumonitis may persist for a month or more and subside gradually. In severe cases, however, pneumonitis progresses to death due to respiratory failure within few weeks [4].

Use of adrenocorticotropic hormone (ACTH) and cortisone for radiation pneumonitis in a case was first reported in 1951 [8], and 9 cases of radiation pneumonitis treated with cortisone therapy in the literature were reviewed in 1968 [9]. Although no case series or clinical trials of corticosteroid therapy have been reported since that time, prednisolone has been given in patients with severe pneumonitis in clinical practice. The initial dose of prednisolone, approximately 30–100 mg daily, and very slow tapering schedule are in agreement among experts [4–6,10], because early withdrawal results in aggravation of pneumonitis [11–13]. There is no consensus, however, about criteria to define when steroids are required for radiation-induced lung injury. The objective of this study is to disclose general characteristics of lung cancer patients developing radiation-induced lung injury treated with or without corticosteroid therapy, to obtain data on the initiation criteria, dose, and taper schedule of corticosteroid therapy for further prospective trials.

Patients and methods

Consecutive lung cancer patients treated with thoracic radiotherapy at a total dose of 50–70 Gy in National Cancer

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Center Hospital between January 1998 and December 2003 were subjects of this study. We retrospectively reviewed all chest X-ray films taken during 6 month period from the end of thoracic radiation to identify the first radiographic change and its progress. History of corticosteroid prescription, symptoms at the time of and one-month period after the first radiographic change in a chest X-ray film, and clinical course of radiation-induced lung injury were obtained from medical charts. The diagnosis of radiation-induced lung injury was defined as radiographic changes including opacification, diffuse haziness, infiltrates or consolidation conforming to the outline of the sharply demarcated irradiated area in a chest X-ray film. During clinical course, scarring (fibrosis) was developed within the irradiated area leading to a reduction in lung volume. In contrast, pulmonary infection spreads through anatomical structure of the lung, and the boundary of infiltrates corresponds to anatomical boundary of the lung. For patients with fever, the radiographical response to antibiotics was also evaluated. Observed differences in the proportions of patients in various patient subgroups were evaluated using Chi-square test. Differences between continuous variables were compared using Mann–Whitney tests. The Dr. SPSS II 11.0 for Windows software package (SPSS Japan Inc., Tokyo, Japan) was used for all statistical analyses.

Results

Of 544 lung cancer patients receiving thoracic radiotherapy at a total dose of 50–70 Gy, 111 patients were excluded from this study because they were not evaluable: loss of follow-up in 88 patients, early lung cancer progression in 18 patients, chemotherapy-induced neutropenic fever and pneumonia in three patients, death of bleeding from the esophageal stent in one patient, and no chest X-ray films available in one patient. In addition, 48 patients (11% of 433 evaluable patients) were also excluded because no radiation-induced lung injury was noted. Thus, the subject of this study was 385 patients.

Of the 385 patients, 78 (20%) received corticosteroid therapy for radiation-induced lung injury, and 307 did not. Radiation-induced lung injury was stable without corticosteroid in the 307 (80%) patients (Group 1), stable or in remission with corticosteroid in 64 (17%) patients (Group 2), and progressive to death despite corticosteroid in 14 (4%) patients (Group 3). No difference in sex, total dose, intent of radiotherapy, and combination chemotherapy was noted among three Groups, but median age of patients was higher in Group 3 (Table 1). Fever was developed in 50% of patients in Group 3 at the initial radiographic change, and in 86% of them during subsequent clinical course, while it was developed in only 11–12% of patients in Group 1 through their clinical course (Table 2). Dyspnea was developed in 57% of patients in Group 3 and in 44% of patients in Group 2 during clinical course, while it was developed in only 14% of patients in Group 1 (Table 2). A total of 88 patients developed fever at the initial change in chest X-ray and/or during subsequent clinical course. Of these, 43 patients received antibiotics, but no radiographical response was obtained in these patients. Five (2%) and seven (2%) patients in Group 1 developed bloody sputum and chest pain, respectively, but none in Group 2 or 3 developed these symptoms. The average interval of chest X-rays taken between the start of radiotherapy and the first appearance of radiographic change was 1.7 weeks for group 1, 1.3 weeks for group 2, and 0.9 weeks for group 3 ($P < 0.001$, Table 3). Interval between the end of radiotherapy and the first change in a chest X-ray was shorter in Group 3 than in Group 2 or Group 1 (Table 3). Of 57 patients in whom the first radiographic change was noted within three weeks, 9 (16%) died of pneumonitis, while radiation-induced lung injury that occurred 10 weeks or later after the end of radiation was easily managed with or without steroid therapy (Table 3). Oxygen content in the blood at the start of steroid therapy was examined in 70 patients of Groups 2 and 3. Oxygen content

Table 1
Patient demographics and radiotherapy performance

Characteristics	Total *N* (%)	Group 1	Group 2	Group 3	*p*-value
		N (%)	*N* (%)	*N* (%)	
Total	385 (100)	307 (80)	64 (17)	14 (4)	
Sex					
Male	300 (78)	240 (78)	47 (73)	13 (93)	0.28
Female	85 (22)	67 (22)	17 (27)	1 (7)	
Age median (range)	65 (28–87)	63 (28–87)	65 (37–83)	71 (65–84)	0.008
Total dose (Gy)					
Median (range)	60 (50–70)	60 (50–70)	60 (50–61)	60 (50–60)	0.50
Intent of radiotherapy					
Curative	298 (77)	232 (76)	52 (81)	14 (100)	0.074
Palliative	87 (23)	75 (24)	12 (19)	0 (0)	
Chemotherapy					
None	121 (31)	101 (33)	15 (23)	5 (36)	0.48
Sequential	121 (31)	93 (30)	25 (39)	3 (21)	
Concurrent	143 (37)	113 (37)	24 (38)	6 (43)	

Table 2
Symptoms through clinical courses

Symptom	At the initial change in chest X-ray				During subsequent clinical course			
	Group 1	Group 2	Group 3	p	Group 1[a]	Group 2[b]	Group3[b]	p
Cough	96 (31)	35 (56)	5 (36)	0.001	85 (28)	38 (59)	5 (36)	<0.001
Sputum	32 (10)	11 (18)	4 (29)	0.049	30 (10)	11 (17)	3 (21)	0.12
Hemosputum	5 (2)	0 (0)	0 (0)	0.53	4 (1)	0 (0)	0 (0)	0.60
Chest pain	7 (2)	0 (0)	0 (0)	0.40	2 (0.6)	0 (0)	0 (0)	0.78
Fever								
None	269 (88)	35 (56)	7 (50)	<0.001	272 (89)	32 (50)	2 (14)	<0.001
37.0–37.9 °C	18 (6)	11 (18)	2 (14)	24 (8)	16 (25)	5 (35)		
38 °C⩽	13 (4)	14 (22)	5 (36)	8 (3)	13 (20)	7 (50)		
Not specified	7 (2)	3 (4)	0 (0)	3 (1)	3 (4)	0 (0)		
Dyspnea	43 (14)	14 (22)	6 (43)	0.007	40 (13)	28 (44)	8 (57)	<0.001
Fever or dyspnea	75 (24)	37 (58)	10 (71)	<0.001	65 (21)	49 (77)	14 (100)	<0.001
Any	150 (49)	51 (81)	13 (93)	<0.001	118 (38)	60 (94)	14 (100)	<0.001

[a] During one month period following the initial change in the chest X-ray.
[b] At the start of steroid therapy.

Table 3
The chest X-ray intervals and first radiographic change

Weeks	Group 1	Group 2	Group 3	p-value
The average interval of chest X-rays (weeks)[a]				
Median (range)	1.7 (0.7 to 6.0)	1.3 (0.5 to 4.4)	0.9 (0.5 to 3.8)	<0.001
Duration between the end of radiotherapy and the first radiographic change (weeks)				
Median (range)	9.9 (−2.9 to 45.1)	6.7 (0 to 24.9)	2.4 (0.4 to 10.1)	<0.001
<6	82 (27)	26 (41)	11 (79)	<0.001
6–11.9	116 (38)	29 (45)	3 (21)	
12–17.9	71 (23)	7 (11)	0 (0)	
18⩽	38 (12)	2 (3)	0 (0)	

[a] Calculated as follows: the average interval of chest X-rays = (the first radiographic change − the start of radiotherapy)/the number of chest X-rays taken during this period/7).

was slightly decreased (PaO2 = 70–74.9 Torr) in 12 (19%) patients of Group 2 and one (7%) patient of Group 3, and moderately to severely decreased (PaO2 ⩽ 69.9 Torr or SpO2 ⩽ 92%) in 21 (33%) patients of Group 2 and 7 (50%) patients of Group 3 ($p = 0.38$).

Prednisolone was administered as the initial therapy in 69 (88%) patients of Groups 2 and 3. The initial prednisolone equivalent dose of steroid was 30–40 mg daily in 52 (67%), and 60 mg of higher only in 8 (10%) patients (Table 4). The median duration of the initial dose was 10 (range, 2–64) days, and the dose was reduced within 14 days in 57 (77%) patients. The median duration of steroid therapy was 10 (range, 2–28) weeks (Table 4). Steroid pulse therapy (methylprednisolone 1000 mg daily for three days) was administered as the initial therapy in one patient, and as salvage therapy in six patients at the time of pneumonitis aggravation. Among the seven patients, six died of respiratory failure due to progressive radiation pneumonitis.

Outcome of steroid therapy was evaluated in 76 patients (Fig. 1). Symptomatic relief was obtained and the steroid dose was reduced in 71 (93%) of the 76 patients, while no effect was noted in the remaining five patients, who all died of radiation pneumonitis despite escalated steroid administration. Of the 71 patients, 15 (21%) developed recurrent symptoms at the median daily prednisolone dose of 20 mg (range, 10–40 mg) within median 33 days (range, 21–42 days) from the start of the steroid therapy, and required steroids to be escalated. Of the 15 patients, nine died of radiation pneumonitis and one died of complication of steroid therapy. A total of 54 (71%) patients were in remission from pneumonitis and steroid therapy was terminated. The remainder 22 patients died during steroid therapy, 14 of radiation pneumonitis, two of infectious complication (bacterial pneumonia in one, and lung aspergillosis in another patient), five of lung cancer progression, and one of hemoptysis. Thus, 16 patients, who accounted for 4.2% of 385 patients receiving 50–70 Gy of thoracic radiotherapy, and who accounted for 21% of 78 patients treated with steroid therapy, died of radiation pneumonitis or complication associated with steroid therapy. Median survival from the start of steroid therapy in these patients was 45 (range, 8–107) days.

Discussion

Patients with radiation-induced lung injury have been managed in compliance with the expert opinions, because there has been no case series or clinical trial report on clinical course and corticosteroid use for this lung injury. This

Table 4
Corticosteroid, dose and duration of steroid therapy

	N (%)
Corticosteroid	
Prednisolone	69 (88)
Dexamethasone	4 (5)
Betamethasone	4 (5)
Methylprednisolone	1 (1)
Initial dose, mg/body daily (prednisolone equivalent)	
Pulse therapy	1 (1)
60	7 (9)
50	1 (1)
40	10 (13)
30	42 (54)
10–25	17 (22)
Duration of the initial dose, days	
Median (range)	10 (2–64)
⩽14	57 (77)
15–28	9 (12)
29⩽	8 (11)
Not evaluable	4
Total duration of steroid therapy, weeks	
Median (range)	10 (2–28)
⩽6	16 (30)
6.1–12	19 (35)
12.1–18	14 (26)
18.1⩽	5 (9)
Not evaluable	24

study is the first systemic review of these patients both who received corticosteroid therapy and who did not. Comparison between the expert opinions and the results of this study is given below. First, radiation-induced lung injury is severer when a radiographic change appears earlier [5]. In this study, the initial change in a chest X-ray film was observed in 9.9 (range, −3 to 45) weeks in Group 1, in 6.7 (range, 0–25) weeks in Group 2, and 2.4 (range, 0–10) weeks in Group 3 after the end of thoracic radiotherapy. If patients present with symptoms, presumably they receive a chest X-ray. Thus, the patients with symptoms may have radiographic findings seen sooner, since they receive an X-ray when they complain of symptoms. The average interval of chest X-rays taken between the start of radiotherapy and the first appearance of radiographic change was longer in Group 1 than that in groups 2 and 3. The difference, however, was negligibly small when compared with the difference in duration between the end of radiotherapy and the first radiographic change. Second, steroid administration is determined generally based on the severity of symptoms [5]. In this study steroid was used when patients developed dyspnea or fever. Dyspnea has been thought to be the cardinal symptom of radiation pneumonitis but fever to be unusual [5,10]. In this study, however, fever was highly associated with fatal radiation pneumonitis; fever was noted in 12% patients of Group 1, in 58% patients of Group 2, and 86% patients of Group 3. This study failed to show utility of blood gas analysis. An oxygen content in the blood was decreased moderately to severely in only 28 (36%) patients in Groups 2 and 3, and did not differ between the two groups. The oxygen content in Group 1 was measured in only small number of patients, and therefore it was not evaluable in this study. Third, 30–100 mg/day of prednisolone has been recommended as the initial dose [4–6,10]. In our practice, a dose of 30–40 mg was the most frequently used. We selected this relatively low dose of steroid mostly because steroid therapy was started in out patient clinic. Forth, duration of the initial dose was within two weeks in 73% of patients, which is consistent to most expert opinions [6,10]. In contrast, tapering schedules varied between a pa-

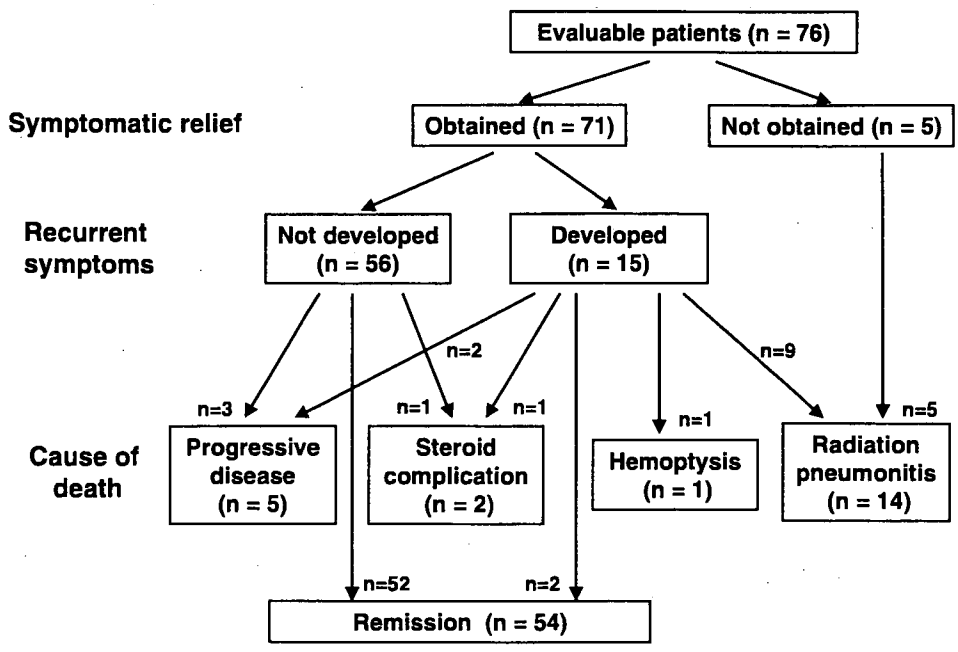

Fig. 1. Outcome of patients who received steroid therapy. Two patients were excluded because of loss of follow-up. Of 76 evaluable patients, 71 (93%) experienced symptomatic relief by steroid therapy.

tient and another in this study. This may be partly due to the diversity in clinical course of radiation pneumonitis, but mostly due to lacking in available recommendation for tapering schedules. In this study, median total duration of steroid therapy was 10 weeks, which may be a tentative guide. A guideline of taper schedule appeared in the latest textbook: the dose should be tapered by 10 mg every two weeks, and be terminated in 12 weeks [10].

Although our clinical practice mostly followed the expert opinions on the management of radiation-induced lung injury as mentioned above, there is little evidence that our steroid use, dose and duration for radiation-induced lung injury were correct. In this study, 21% of patients received steroid therapy and 4% of patients died of radiation pneumonitis among lung cancer patients treated with thoracic radiotherapy at a total dose of 50 Gy or higher. These figures are comparable to the incidence of grade 3 pneumonitis, 3–20%, and that of fatal pneumonitis, 1–4%, in other reports [10].

In conclusion, development of fever and dyspnea, and short interval between the end of radiotherapy and the first radiographic change were associated with fatal radiation-induced lung injury. Prednisolone 30–40 mg daily for two weeks followed by slow taper was selected for the treatment in many patients.

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Docetaxel Consolidation Therapy Following Cisplatin, Vinorelbine, and Concurrent Thoracic Radiotherapy in Patients with Unresectable Stage III Non-small Cell Lung Cancer

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Background: To evaluate the feasibility and efficacy of docetaxel consolidation therapy after concurrent chemoradiotherapy for unresectable stage III non–small cell lung cancer (NSCLC).

Patients and Methods: The eligibility criteria included unresectable stage III NSCLC, no previous treatment, age between 20 and 74 years, and performance status 0 or 1. Treatment consisted of cisplatin (80 mg/m^2 on days 1, 29, and 57), vinorelbine (20 mg/m^2 on days 1, 8, 29, 36, 57, and 64), and thoracic radiotherapy (TRT) (60 Gy/30 fractions over 6 weeks starting on day 2), followed by consolidation docetaxel (60 mg/m^2 every 3 to 4 weeks for three cycles).

Results: Of 97 patients who were enrolled in this study between 2001 and 2003, 93 (76 males and 17 females with a median age of 60) could be evaluated. Chemoradiotherapy was well tolerated; three cycles of chemotherapy and 60 Gy of TRT were administered in 80 (86%) and 87 (94%) patients, respectively. Grade 3 or 4 neutropenia, esophagitis, and pneumonitis developed in 62, 11, and 3 patients, respectively. Docetaxel consolidation was administered in 59 (63%) patients, but three cycles were completed in only 34 (37%) patients. The most common reason for discontinuation was pneumonitis, which developed in 14 (24%) of the 59 patients. During consolidation therapy, grade 3 or 4 neutropenia, esophagitis, and pneumonitis developed in 51, 2, and 4 patients, respectively. A total of four patients died of pneumonitis. We calculated a V_{20} (the percent volume of the normal lung receiving 20 Gy or more) on a dose–volume histogram in 25 patients. Of these, five patients developed grade 3 or more severe radiation pneumonitis. A median V_{20} for these five patients was 35% (range, 26–40%), whereas the median V_{20} for the remaining 20 patients was 30% (range, 17–35%) (p = 0.035 by a Mann–Whitney test). The response rate was 81.7% (95% confidence interval [CI], 72.7–88.0%), with 5 complete and 71 partial responses. The median progression-free survival was 12.8 (CI, 10.2–15.4) months, and median survival was 30.4 (CI, 24.5–36.3) months. The 1-, 2-, and 3-year survival rates were 80.7, 60.2, and 42.6%, respectively.

Conclusion: This regimen produced promising overall survival in patients with stage III NSCLC, but the vast majority of patients could not continue with the docetaxel consolidation because of toxicity.

Key Words: Non-small cell lung cancer, Chemoradiotherapy, Consolidation, Docetaxel.

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Locally advanced unresectable non–small cell lung cancer (NSCLC), stage IIIA with bulky N2 and stage IIIB disease without pleural effusion, is characterized by large primary lesions and/or involvement of the mediastinal or supraclavicular lymph nodes and occult systemic micrometastases. A combination of thoracic radiotherapy and chemotherapy is the standard medical treatment for this disease, but the optimal combination has not been established.[1] Although the available data are insufficient to accurately define the size of a potential benefit,[2] concurrent chemoradiotherapy using a platinum doublet has been shown to be superior to the sequential approach in phase III trials of this disease.[3–5] However, third-generation cytotoxic agents, which have provided better patient survival with extrathoracic spread than the old-generation agents, must be reduced when administered concurrently with thoracic radiotherapy.[6] Thus, it has been hypothesized that the addition of systemic dose chemotherapy with a new cytotoxic agent to concurrent chemoradiotherapy, either as induction or as consolidation chemotherapy, might further improve patient survival.[1]

The consolidation chemotherapy with docetaxel was based on the observation that this drug was highly active in the primary treatment of metastatic NSCLC, producing a response rate (RR) as high as 20% after platinum-based chemotherapy failed.[7–9] Highly encouraging results of a me-

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dian survival time (MST) of more than 2 years and a 3-year survival rate of nearly 40% were obtained in a phase II trial of docetaxel consolidation after chemoradiotherapy with cisplatin and etoposide in patients with stage IIIB NSCLC (SWOG study S9504).[10]

We have developed a combination chemotherapy schedule with cisplatin and vinorelbine concurrently administered with thoracic radiotherapy at a total dose of 60 Gy in 30 fractions in patients with unresectable stage III NSCLC. The results of a phase I study in 18 patients were very promising, with a RR of 83%, a MST of 30 months, and a 3-year survival rate of 50%.[6] Thus, addition of docetaxel consolidation to this regimen is a particularly interesting therapeutic strategy. The objectives of the current study were to evaluate the feasibility of docetaxel consolidation therapy after concurrent chemoradiotherapy with cisplatin and vinorelbine and to evaluate the efficacy and safety of the whole treatment regimen including both the chemoradiotherapy and consolidation therapy in patients with unresectable stage IIIA and IIIB NSCLC.

PATIENTS AND METHODS

Patient Selection

The eligibility criteria were histologically or cytologically proven NSCLC; unresectable stage IIIA or IIIB disease; no previous treatment; measurable disease; tumor within an estimated irradiation field no larger than half the hemithorax; age between 20 and 74 years; Eastern Cooperative Oncology Group performance status (PS) of 0 or 1; adequate bone marrow function (12.0×10^9/liter ≥ white blood cell [WBC] count ≥4.0×10^9/liter, neutrophil count ≥2.0×10^9/liter, hemoglobin ≥10.0 g/dl, and platelet count ≥100×10^9/liter), liver function (total bilirubin ≤1.5 mg/dl and transaminase no more than twice the upper limit of the normal value), and renal function (serum creatinine ≤1.5 mg/dl and creatinine clearance ≥60 ml per minute); and a PaO_2 of 70 torr or more under room air conditions. Patients were excluded if they had malignant pleural or pericardial effusion, active double cancer, a concomitant serious illness such as uncontrolled angina pectoris, myocardial infarction in the previous 3 months, heart failure, uncontrolled diabetes mellitus, uncontrolled hypertension, interstitial pneumonia or lung fibrosis identified by a chest x-ray, chronic obstructive lung disease, infection or other diseases contraindicating chemotherapy or radiotherapy, pregnancy, or if they were breast feeding. All patients gave their written informed consent.

Pretreatment Evaluation

The pretreatment assessment included a complete blood cell count and differential count, routine chemistry determinations, creatinine clearance, blood gas analysis, electrocardiogram, lung function testing, chest x-rays, chest computed tomographic (CT) scan, brain CT scan or magnetic resonance imaging, abdominal CT scan or ultrasonography, and radionuclide bone scan.

Treatment Schedule

Treatment consisted of a chemoradiotherapy phase with three cycles of cisplatin and vinorelbine followed by a consolidation phase with three cycles of docetaxel (Figure 1). Cisplatin 80 mg/m^2 was administered on days 1, 29, and 57 by intravenous infusion for 60 minutes with 2500 to 3000 ml of fluid for hydration. Vinorelbine diluted in 50 ml of normal saline was administered intravenously on days 1, 8, 29, 36, 57, and 64. All patients received prophylactic antiemetic therapy consisting of a $5HT_3$-antagonist and a steroid.

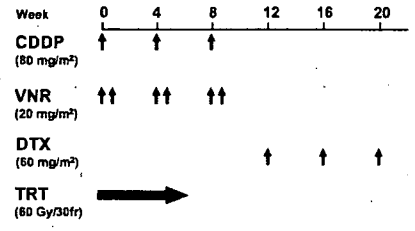

FIGURE 1. Treatment schema. CDDP, cisplatin; DTX, docetaxel; TRT, thoracic radiotherapy; VNR, vinorelbine.

Radiation therapy was delivered with megavoltage equipment (≥6 MV) using anterior/posterior opposed fields up to 40 Gy in 20 fractions including the primary tumor, the metastatic lymph nodes, and the regional nodes. A booster dose of 20 Gy in 10 fractions was given to the primary tumor and the metastatic lymph nodes for a total dose of 60 Gy using bilateral oblique fields. A CT scan–based treatment planning was used in all patients. The clinical target volume (CTV) for the primary tumor was defined as the gross tumor volume (GTV) plus 1 cm taking account of subclinical extension. CTV and GTV for the metastatic nodes (>1 cm in shortest dimension) were the same. Regional nodes, excluding the contralateral hilar and supraclavicular nodes, were included in the CTV, but the lower mediastinal nodes were included only if the primary tumor was located in the lower lobe of the lung. The planning target volumes for the primary tumor, the metastatic lymph nodes, and regional nodes were determined as CTVs plus 0.5- to 1.0-cm margins laterally and 1.0- to 2.0-cm margins craniocaudally, taking account of setup variations and internal organ motion. Lung heterogeneity corrections were not used.

The criteria for starting consolidation chemotherapy were completion of three cycles of cisplatin and vinorelbine and a full dose of thoracic radiotherapy, the absence of progressive disease, adequate general condition within 6 weeks of the start of the third cycle of cisplatin and vinorelbine (PS 0 or 1, WBC count ≥3.0×10^9/liter, neutrophil count ≥1.5×10^9/liter, hemoglobin ≥9.0 g/dl and platelet count ≥100×10^9/liter, total bilirubin ≤1.5 mg/dl and transaminase no more than twice the upper limit of the normal value, and a PaO_2 of 70 torr or more at room air). Docetaxel (60 mg/m^2) was administered intravenously for 1 hour every 3 to 4 weeks for three cycles.

Toxicity Assessment and Treatment Modification

Complete blood cell counts and differential counts, routine chemistry determinations, and a chest x-ray were performed once a week during the course of treatment. Acute toxicity was graded according to the NCI Common Toxicity Criteria, and late toxicity associated with thoracic radiother-

apy was graded according to the Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer late radiation morbidity scoring scheme. Vinorelbine administration on day 8 was omitted if any of the following were noted: WBC count $<3.0 \times 10^9$/liter, neutrophil count $<1.5 \times 10^9$/liter, platelet count $<100 \times 10^9$/liter, elevated hepatic transaminase level or total serum bilirubin of at least grade 2, fever ≥38°C, or PS ≥2. Subsequent cycles of cisplatin and vinorelbine chemotherapy were delayed if any of the following toxicities were noted on day 1: WBC count $<3.0 \times 10^9$/liter, neutrophil count $<1.5 \times 10^9$/liter, platelet count $<100 \times 10^9$/liter, serum creatinine level ≥1.6 mg/dl, elevated hepatic transaminase level or total serum bilirubin of at least grade 2, fever ≥38°C, or PS ≥2. The dose of cisplatin was reduced by 25% in all subsequent cycles if the serum creatinine level rose to 2.0 mg/dl or higher. The dose of vinorelbine or docetaxel was reduced by 25% in all subsequent cycles if any of the following toxicities were noted: WBC count $<1.0 \times 10^9$/liter, platelet count $<10 \times 10^9$/liter, or grade 3 or 4 infection or liver dysfunction. Thoracic radiotherapy was suspended if any of the following were noted: fever ≥38°C, grade 3 esophagitis, PS of 3, or PaO_2 <70 torr. Thoracic radiotherapy was terminated if any of the following were noted: grade 4 esophagitis, grade 3 or 4 pneumonitis, PS of 4, or duration of radiotherapy of over 60 days. The use of granulocyte colony-stimulating factor during radiotherapy was not permitted unless radiotherapy was on hold. The criteria for termination of docetaxel consolidation were not defined in the protocol.

Response Evaluation

Objective tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumor.[11] Local recurrence was defined as tumor progression in the primary site and in the hilar, mediastinal, and supraclavicular lymph nodes after a partial or complete response; regional recurrence as the development of malignant pleural and pericardial effusions; and distant recurrence as the appearance of a distant metastasis.

Study Design, Data Management, and Statistical Considerations

This study was conducted at three institutions: the National Cancer Center Hospital, National Cancer Center Hospital East, and Tochigi Cancer Center. The protocol and consent form were approved by the institutional review board of each institution. Registration was conducted at the registration center. Data management, periodic monitoring, and the final analysis were performed by the study coordinator.

The primary objective of the current study was to evaluate the feasibility of docetaxel consolidation therapy. The secondary endpoints were toxicity observed during chemoradiotherapy and consolidation therapy, the best response, and overall survival in all patients eligible to participate in this study. Because no standard method to evaluate consolidation chemotherapy after chemoradiotherapy has been established, we arbitrarily defined the primary endpoint of this study as a ratio (R) of the number of patients receiving docetaxel without grade 4 nonhematological toxicity or treatment-related death to the total number of patients receiving docetaxel. The sample size was initially estimated to be 34 patients with a power of 0.80 at a significance level of 0.05, under the assumption that a R of 0.95 would indicate potential usefulness, whereas a R of 0.8 would be the lower limit of interest, and that 85% of patients would move into the consolidation phase. An analysis of the first 13 patients, however, showed that only 8 (61%) patients advanced into the consolidation phase. The reasons for not receiving docetaxel were disease progression in one, delay in completion of chemoradiotherapy in two, grade 3 esophagitis in one, and death due to hemoptysis in one patient. Considering that the SWOG trial S9504 included 83 patients, we decided to revise the number of patients in the current study. According to Simon's two-stage minimax design, the required number of patients was calculated to be 59 with a power of 0.80 at a significance level of 0.05, under the assumption that a R of 0.85 would indicate potential usefulness, whereas a R of 0.7 would be the lower limit of interest.[12] Assuming that 61% of registered patients would move into the consolidation phase, the sample size was determined to be 97 patients.

Overall survival time and progression-free survival time were estimated by the Kaplan–Meier method, and confidence intervals (CI) were based on Greenwood's formula.[13] Overall survival time was measured from the date of registration to the date of death (from any cause) or to the last follow-up. Progression-free survival time was measured from the date of registration to the date of disease progression, death (from any cause), or the last follow-up. Patients who were lost to follow-up without event were censored at the date of their last known follow-up. A CI for RR was calculated using methods for exact binomial CIs. The Dr. SPSS II 11.0 for Windows software package (SPSS Japan Inc., Tokyo, Japan) was used for statistical analyses.

RESULTS

Registration and Characteristics of the Patients

A total of 97 patients were enrolled in this study between April 2001 and June 2003. Four patients were excluded from this study before the treatment was started because the radiation treatment planning disclosed that their tumors were too advanced for curative thoracic radiotherapy. Thus, 93 patients who received the protocol-defined treatment were the subjects of this analysis (Figure 2). There were 76 males and 17 females, with a median age of 60 (range 31–74). Body weight loss was less than 5% in 77 patients; adenocarcinoma histology was noted in 57 patients, and stage IIIA disease was noted in 41 patients (Table 1).

Treatment Delivery

Treatment delivery was generally well maintained in the chemoradiotherapy phase (Table 2). Full cycles of cisplatin and vinorelbine and the full dose of thoracic radiotherapy were administered in 80 (86%) and 87 (94%) patients, respectively. Delay in radiotherapy was less than 5 days in 61 (66%) patients. In contrast, the delivery of docetaxel was poor (Table 2). A total of 59 (63%) patients could enter the consolidation phase, and only 34 (37%) patients completed three cycles of docetaxel chemotherapy. The reasons for not