Table 3. Results of postoperative radiation therapy for stage IB-IIA cervical cancer according to pelvic nodal status

Author (year)	Pelvic node metastases	Number of patients	5 year overall survival
Gonzalez (1989)[30]	Negative	43	85%
	Positive	89	60%
Frigerio (1994)[31]	Negative	98	88%
	Positive	39	44%
Garipagaoglu (1999)[32]	Negative	67	95%
	Positive	33	65%
Yeh (1999)[33]	Negative	113	81%
	Positive	66	53%
Tsai (1999)[34]	Negative	150	87%*
	Positive	72	71%*

*Disease-free survival.

Table 4. Clinical significance and intrinsic problems in the intergroup study

Significance	
	Prospectively demonstrated that difference in the postoperative treatment strategy significantly influences overall survival in patients with cervical cancer
	Prospectively demonstrated that patients with positive nodes benefit from chemotherapy
Problems	
	Unknown role of PORT in both groups
	Suboptimal fractionation schedule in PORT
	Lower photon energy in PORT was accepted
	Eligibility also included risk factors other than positive pelvic node
	Patients with positive surgical margin were included

PORT, postoperative radiation therapy.

affects overall survival in patients with cervical cancer. The results of the study surely influenced subsequent clinical practices. Several pitfalls, however, exist in this study. First, both the control arm and the experimental arm included PORT — the role of which in patients with positive pelvic nodes is still unknown. Second, prolonged overall treatment time with PORT utilized a suboptimal dose/fractionation schedule (1.7 Gy per day for pelvic irradiation, 1.5 Gy per day for pelvic and paraaortic irradiation). Third, treatment with inappropriately lower photon energy (4 MV) was accepted. Fourth, 15% of patients had negative pelvic nodes, which are not included in the criteria for high-risk patients described by Thomas and Dembo. This inclusion resulted in a group of relatively heterogenic study subjects. Finally, eligibility included patients with positive surgical margins, despite the study's intention to examine the role of "adjuvant" treatment. Thus, from this context, it is questionable whether concurrent chemoradiation was necessary for all study subjects in the intergroup trial; for some patients, concurrent chemoradiation might have been overtreatment. Patients with parametric invasion without pelvic lymph node metastases could have achieved a good clinical course with PORT alone.[36] A separate analysis with a longer follow-up of the intergroup study performed by Monk *et al.* revealed that patients with only one positive pelvic node did not benefit from concurrent chemotherapy.[37] It is not surprising that the most important prognostic factor determined by multivariate analyses in the intergroup study was not positive pelvic nodes but tumor diameter. The clinical significance and intrinsic problems in interpreting the intergroup phase III clinical trial results are summarized in Table 4.

Because reports of clinical trials tend to insufficiently publish late adverse effects of radiation therapy, long-term follow-up with careful monitoring of each participant is crucial, especially for those receiving concurrent chemoradiation. In the experimental arm of the ongoing European Organization for Research and Treatment of Cancer clinical trial for IB2, large IIA, or IIB cervical cancer, neoadjuvant chemotherapy followed by radical

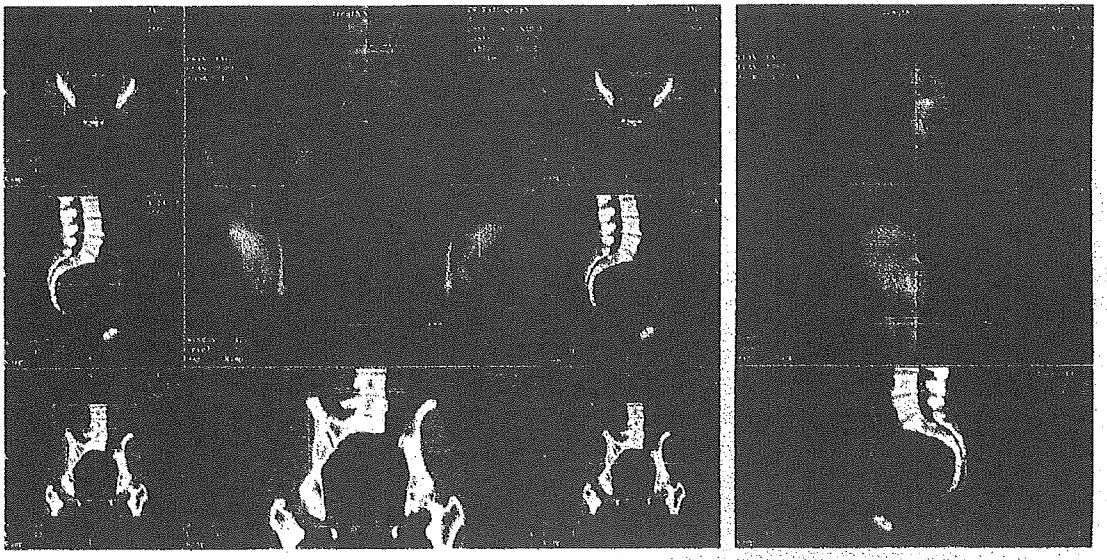

Fig. 1. A four-field technique consisting of the anterior-posterior (A) and two opposed lateral fields (B) determined three-dimensionally by using CT-simulator.

A | B

hysterectomy with tailored PORT is being compared with a standard definitive chemoradiation. Initial radical hysterectomy followed by PORT with concurrent chemotherapy is now not a treatment of choice in this setting. This practice underlines that, to reduce toxicities, surgery and PORT should not be combined if possible.

Technical Concerns of PORT

Once indicated, PORT should be performed using the proper radiation therapy technique. A conventional opposed anterior-posterior field technique using bony landmarks is inadequate and now out of date. To reduce the dose to normal tissues such as the small intestine, pelvic irradiation should be given with the four-field technique consisting of the anterior, posterior, and two opposed lateral fields using 10 MV or higher photon energies (Fig. 1). Treatment machines should be equipped with a multi-leaf collimator to shape each port automatically. By using the four-field technique, the incidence of late complications can be reduced.[38] In general, a fractional daily dose of 1.8-2.0 Gy, 5 times per week, up to a total dose of 45-50 Gy, is prescribed at the beam intersection point. Because bony landmarks are suboptimal to cover clinical target volumes such as tumor bed and lymph node area, care must be taken to cover them adequately when lateral fields are added.[39,40] A modern three-dimensional treatment plan using a CT-simulator is required to decrease the potential geographic miss when PORT is given using the four-field technique.

Conclusion

Efficacy of PORT is mainly supported by retrospective studies. There is no conclusive evidence that PORT eventually improves patient survival. Based on the results of large surgical series, patients are now divided into three risk-groups by combining risk factors: low-risk patients for whom no adjuvant therapy is necessary, intermediate-risk patients for whom PORT increases pelvic control rate and therefore may improve patient survival, and high-risk patients for whom systemic chemotherapy should be incorporated. Recent prospective clinical studies have demonstrated the efficacy of PORT for the intermediate-risk group as well as survival benefit of PORT with concurrent chemotherapy for the high-risk group. However, care should be taken in interpreting these results to evaluate the role of PORT. Difference in the concept of PORT in Europe/USA and Japan should also be of concern.

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Germline polymorphism of *p53* codon 72 in gynecological cancer

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Abstract

Objective. To investigate the biological significance of single nucleotide polymorphism at codon 72 of the *p53* gene in the development of gynecological cancer.

Methods. *p53* codon 72 polymorphism was examined in a total of 354 blood samples from 95 normal, 83 cervical, 108 endometrial and 68 ovarian cancer cases using polymerase chain reaction and restriction fragment length polymorphism techniques.

Results. When *p53* codon 72 genotype was classified into two subgroups of Arg/Arg and Arg/Pro+Pro/Pro, the Arg/Arg genotype was associated with an increased risk for the development of endometrial cancer (OR = 1.86, 95% CI = 1.06 to 3.26) compared with the Arg/Pro+Pro/Pro genotype ($P = 0.0301$). The Arg allele also increased the risk of endometrial cancer (OR = 1.42, 95% CI = 0.93 to 1.52) compared with the Pro allele, but no statistical difference was found ($P = 0.1031$). There was no significant difference in the genotype or allele prevalence between control subjects and cervical or ovarian cancer patients.

Conclusion. Homozygous Arg at codon 72 of the *p53* gene may be a risk factor for developing endometrial cancer in a Japanese population.

Keywords: *p53*; Polymorphism; Gynecological cancer

Introduction

p53 is a tumor suppressor gene involved in multiple pathways including apoptosis, cellular transcriptional control, and cell cycle regulation [1,2]. A large number of human tumors, including smoke-induced lung cancer, show mutations and deletions of the *p53* gene that result in loss of tumor suppression function and cell cycle deregulation [3]. The *p53* gene shows a polymorphism at codon 72 with a single-base change that causes an amino acid replacement in the transactivation domain of the protein of Arg (CGC) by Pro (CCC) [4]. Single nucleotide polymorphism (SNP) at codon 72 of the *p53* gene has been associated in the last decade with the risk of developing various neoplasms such as lung [5–7], esophageal [8], and cervical cancer [9]. An influence of this polymorphism on endometrial and ovarian cancer has been recently suggested [10–13]. However, the correlation between this SNP and gynecological cancer susceptibility has not been extensively studied, and previous experimental results are controversial. In the present study, we investigated germline polymorphism at codon 72 of the *p53* gene in human cervical, endometrial, and ovarian cancer patients and reevaluated the role of this SNP in the development of these gynecological malignancies in a Japanese population.

Materials and methods

Sample collection

We conducted genotype analysis of *p53* codon 72 in a total of 354 blood samples from normal healthy women and gynecological cancer patients. They consist of 95 normal controls and 83 cervical, 108 endometrial and 68 ovarian cancer patients with invasive diseases. All subjects were Japanese women who visited Osaka Medical College in the past 3 years. The non-cancer controls had no history of gynecological disease and for whom there was no present evidence of gynecological cancer. Women with any malignant disease or other systemic problems such as chronic liver diseases were excluded from the control group. Final histologic diagnosis was confirmed by biopsy or surgical specimens from each cancer patient. Table 1 shows the clinical characteristics of cancer patients examined in this study. For 108 endometrial cancer patients, putative risk factors, such as body mass index (BMI), a history of hypertension or diabetes, and family history, were also obtained by reviewing the medical

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Table 1
Clinical characteristics of gynecological cancer patients

Variable	Cervical cancer (n = 83)	Endometrial cancer (n = 108)	Ovarian cancer (n = 68)
Age distribution (years)			
≤30	4 (4.8%)	3 (2.8%)	3 (4.4%)
31–50	39 (47.0%)	26 (24.1%)	20 (29.4%)
51–70	35 (42.2%)	68 (62.9%)	41 (60.3%)
>70	5 (6.0%)	11 (10.2%)	4 (5.9%)
Menstrual status			
Premenopause	45 (54.2%)	34 (31.5%)	25 (36.8%)
Postmenopause	38 (45.8%)	74 (68.5%)	43 (63.2%)
Stage (FIGO)			
I	37 (44.6%)	84 (77.8%)	26 (38.2%)
II	31 (37.3%)	4 (3.7%)	7 (10.3%)
III	15 (18.1%)	19 (17.6%)	32 (47.1%)
IV	0 (0%)	1 (0.9%)	3 (4.4%)
Histologic type	Squamous	Endometrioid	Serous
	59 (71.1%)	97 (89.8%)	34 (50.0%)
	Non-squamous	Non-endometrioid	Non-serous
	24 (28.9%)	11 (10.2%)	34 (50.0%)

records. No statistically significant differences were found between control subjects and cancer patients in each group in terms of age distribution, smoking, and menstrual status. The protocol of this study was approved by our institutional review board, and all samples were obtained with informed consent. Genomic DNA was extracted from peripheral blood lymphocytes using the standard method of proteinase K treatment and phenol/chloroform extraction.

Genotyping of p53 codon 72

Polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis of codon 72 of the *p53* gene, modified from a technique described by Ara et al. [4], was conducted to identify p53 genotypes with the primers, 5′-TTGCCGTCCCAAGCAATG-GATGA-3′ and 5′-TCTGGGAAGGGACAGAAGATGAC-3′. 100 ng of the DNA template from each sample was amplified by PCR in a final volume of 50 μl reaction containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.01% (w/v) gelatin, 200 μM dNTP, 0.5 μM each primer, and 1.25 units Taq polymerase (Applied Biosystems, Branchburg, NJ) as previously described [14]. After an initial denaturation at 96°C for 3 min, 40 cycles of denaturation (94°C for 1 min), annealing (60°C for 1 min), and extension (72°C for 2 min) were carried out on a Perkin-Elmer GeneAmp PCR System 9700. The final extension was performed at 72°C for 10 min. After confirmation of an amplified fragment of the expected size (199 bp) on a 1.5% agarose gel, 17 μl of each PCR product was digested with 10 units of restriction enzyme *Bst*UI (New England Biolabs, ME) at 60°C for 3 h. DNA fragments were visualized on a 3.0% agarose gel with ethidium bromide. As shown in Fig. 1, the Arg allele is cleaved by *Bst*UI and yields two small fragments (113 and 86 bp). The Pro allele is not cleaved by *Bst*UI and has a single 199 bp band. The heterozygote contains three bands (199, 113, and 86 bp). We have previously demonstrated that these experimental procedures are quite useful to minimize the misclassification of *p53* codon 72 genotype [15,16].

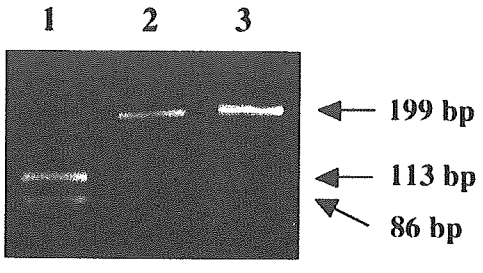

Fig. 1. Genotyping of *p53* codon 72 by PCR-RFLP. Lane 1, Arg/Arg homozygote. Lane 2, Arg/Pro heterozygote. Lane 3, Pro/Pro homozygote. The fragment of 199 bp is the nondigested PCR product from the Pro allele. Fragments of 113 and 86 bp result from *Bst*UI digestion of the Arg allele.

Statistical analysis

To compare the polymorphic features of *p53* codon 72 between control subjects and cancer patients, Pearson's chi-square test was used. A level of $P < 0.05$ was accepted as statistically significant.

Results

Table 2 shows the genotype and allele frequencies of *p53* codon 72 in 354 samples examined. There was no significant difference in the genotype or allele prevalence between control subjects and cervical, endometrial, or ovarian cancer patients. However, when *p53* codon 72 genotype was classified into two subgroups of Arg/Arg and Arg/Pro+Pro/Pro, the Arg/Arg genotype was associated with an increased risk for the development of endometrial cancer (OR = 1.86, 95% CI = 1.06 to 3.26) compared with the Arg/Pro+Pro/Pro genotype (P = 0.0301) as shown in Table 3. The Arg allele also increased the risk of endometrial cancer (OR = 1.42, 95% CI = 0.93 to 1.52) compared with the Pro allele, but there was no significant difference (P = 0.1031). Thus, homozygous Arg genotype of *p53* codon 72 was found to be a risk factor for endometrial cancer.

We further examined the correlation between genotypic and allelic frequencies of *p53* codon 72 and epidemiological factors including putative risk factors for endometrial cancer, such as BMI, a history of hypertension or diabetes, and family history. 108 endometrial cancer patients were also classified into two groups of type I and type II. Type I cancer is related to hyperestrogenism by association with endometrial hyperplasia, whereas type II cancer is unrelated to estrogen, associated with atrophic endometrium. Histologically, non-endometrioid carcinomas, such as serous and clear cell carcinomas, are considered

Table 2
Genotypic and allelic frequencies of *p53* codon 72 in control subjects and cancer patients

Samples	Genotype frequency			Allele frequency	
	Arg/Arg	Arg/Pro	Pro/Pro	Arg	Pro
Normal (n = 95)	34 (35.8%)	54 (56.8%)	7 (7.4%)	122 (64.2%)	68 (35.8%)
Cervical cancer (n = 83)	28 (33.7%)	46 (55.4%)	9 (10.8%)	102 (61.4%)	64 (38.6%)
Endometrial cancer (n = 108)	55 (50.9%)	45 (41.7%)	8 (7.4%)	155 (71.8%)	61 (28.2%)
Ovarian cancer (n = 68)	21 (30.9%)	41 (60.3%)	6 (8.8%)	83 (61.0%)	53 (39.0%)

as type II cancer. There was no significant difference in the genotype or allele prevalence among these epidemiological factors as shown in Table 4.

Discussion

There is an expanding body of literature suggesting that host factors, including genetic polymorphisms, may explain some of the individual differences in cancer occurrence. A large number of previous studies have been conducted on the correlation between germline polymorphisms of cancer susceptibility genes and the higher risk of human malignant tumors.

Codon 72 of exon 4 was the first polymorphism detected in the *p53* gene, and it was suggested that the two alleles of codon 72 might have different oncogenic properties [17]. Previous studies have failed to find the correlation between the genetic polymorphism of *p53* codon 72 and the risk of endometrial cancer [18,19]. In a recent study published in this journal, Roh et al. [10] reported that SNP at codon 72 of the *p53* gene is associated with the development of endometrial cancer in Korean population. They stated that the *p53* genotype containing the Pro allele statistically increased the risk of endometrial cancer compared with homozygous Arg genotype. In contrast, our present results on germline polymorphism of *p53* codon 72 demonstrated that homozygous Arg genotype increased the risk of endometrial cancer compared with Arg/Pro+Pro/Pro genotype. Very recently, Agorastos et al. [11] reported that there was no significant difference in the genotype prevalence of *p53* codon 72 between control subjects and endometrial cancer patients in Greek women. However, they also found a positive linear trend of Arg/Arg towards poor differentiation in endometrial malignancies. These discrepancies may be due to the differences of sample size and the ethnic variation of genotype frequency of *p53* codon 72 in different geographical regions. Allelic differences of *p53* polymorphisms were observed in various ethnic groups [20]. The frequency of Arg/Arg homozygote in normal controls of Japanese population was reported to be 30–40% [20–22], which was consistent with our present results. Roh et al. [10] reported that homozygous Arg was observed in 58.3% of normal healthy women. The higher Arg/Arg genotype prevalence in Korean population may affect our discrepant results.

Currently, two different pathways are distinguished for tumorigenesis of sporadic endometrial cancer, one estrogen-related and another unrelated to estrogen [23]. The majority of sporadic endometrial cancers (at least approximately 70–80%), designated as type I cancers, follow the estrogen-related pathway. About 10–20% of endometrial cancers, designated as type II cancers, follow the estrogen-unrelated pathway and arise in the background of atrophic endometrium. These two types of tumors may have different genetic pathways, especially regarding *p53* mutations. However, there was no significant difference in the genotype or allele prevalence of *p53* codon 72 between type I and type II cancers in our series. Moreover, this SNP was not correlated with putative risk factors for endometrial cancer. The associations of germline polymorphism of *p53* codon 72 with these epidemiological factors should be further elucidated in a larger sample size of Japanese population.

Several mechanisms have been proposed to explain the role of the Arg allele in cancer development. The Arg allele is found to be more susceptible to degradation by the human papillomavirus E6 protein than the Pro allele [24]. In addition, the Arg allele may enhance mutant *p53* binding to *p73*, thus neutralizing *p73*-induced apoptosis independently of human papillomavirus-related mechanisms [25,26]. Moreover, several studies have examined the role of the codon 72 polymorphism in mutation of the *p53* gene in cancer. Langerod et al. [27] identified *p53* mutations more commonly in breast cancer from Arg/Arg homozygotes (28.5%) than among Arg/Pro heterozygotes (21%) or Pro/Pro homozygotes (4%). They have also suggested that the codon 72 Arg allele is preferentially mutated and retained in Arg/Pro heterozygotes [27,28]. These authors indicated that the codon 72 Arg containing mutants may have a

Table 3
Risk of endometrial cancer associated with *p53* codon 72 genotypes and alleles

p53 codon 72 polymorphism	Control subjects	Cancer patients	OR (95% CI)	P value
Genotype				
Arg/Pro+Pro/Pro	61 (64.2%)	53 (49.1%)	1.00 (referent)	
Arg/Arg	34 (35.8%)	55 (50.9%)	1.86 (1.06 to 3.26)	0.0301
Allele				
Pro	68 (35.8%)	61 (28.2%)	1.00 (referent)	
Arg	122 (64.2%)	155 (71.8%)	1.42 (0.93 to 1.52)	0.1031

Table 4
Correlation between genotypic and allelic frequencies of *p53* codon 72 and epidemiological factors in endometrial cancer patients

Variable	Genotype frequency			Allele frequency	
	Arg/Arg	Arg/Pro	Pro/Pro	Arg	Pro
Menstrual status					
Premenopause	17 (50.0%)	13 (38.2%)	4 (11.8%)	47 (69.1%)	21 (30.9%)
Postmenopause	38 (51.4%)	32 (43.2%)	4 (5.4%)	108 (73.0%)	40 (27.0%)
Obesity (BMI)					
>25 kg/m^2	22 (44.0%)	25 (50.0%)	3 (6.0%)	69 (69.0%)	31 (31.0%)
≤25 kg/m^2	33 (56.9%)	20 (34.5%)	5 (8.6%)	86 (74.1%)	30 (25.9%)
Hypertension history					
Yes	8 (50.0%)	7 (43.7%)	1 (6.3%)	23 (71.9%)	9 (28.1%)
No	47 (51.1%)	38 (41.3%)	7 (7.6%)	132 (71.7%)	52 (28.3%)
Diabetes history					
Yes	7 (58.3%)	4 (33.3%)	1 (8.3%)	18 (75.0%)	6 (25.0%)
No	48 (50.0%)	41 (42.7%)	7 (7.3%)	137 (71.4%)	55 (28.6%)
Family history					
Yes	4 (57.1%)	3 (42.9%)	0 (0%)	11 (78.6%)	3 (21.4%)
No	51 (50.5%)	42 (41.6%)	8 (7.9%)	144 (71.3%)	58 (28.7%)
Histologic type					
Endometrioid	50 (51.5%)	39 (40.2%)	8 (8.2%)	139 (71.6%)	55 (28.4%)
Non-endometrioid	5 (45.5%)	6 (54.5%)	0 (0%)	16 (72.7%)	6 (27.3%)
Cancer type					
Type I	45 (53.6%)	33 (39.3%)	6 (7.1%)	123 (73.2%)	45 (26.8%)
Type II	10 (41.7%)	12 (50.0%)	2 (8.3%)	32 (66.7%)	16 (33.3%)

selective growth advantage influencing the ratio of Arg and Pro containing mutants in tumors. However, previous reports have demonstrated that the incidence of mutations of the *p53* gene in endometrial cancer ranges from 4.8 to 20%, which is relatively infrequent compared with other malignancies [29–31]. Further studies are needed to clarify the molecular interaction between *p53* codon 72 polymorphism and mutation of the *p53* gene involving the development of human endometrial malignancies.

Our present results revealed that the differences in the polymorphic frequency of *p53* Arg/Arg, Arg/Pro and Pro/Pro genotypes between control subjects and cervical cancer patients were statistically not significant. Some previous studies have reported no correlation between germline polymorphisms of the *p53* codon 72 and increased risk of cervical cancer [32–35]. The recent study reported by Nishikawa et al. [36] using cervical condyloma, dysplasia, and cancer tissue samples demonstrated that no statistically significant differences in the distribution of *p53* genotypes were found among the patients with these diseases, regardless of HPV status. We have also reported the similar results in a recent issue of this journal using cytologic materials from abnormal cervix [16]. These data suggest that *p53* codon 72 polymorphism does not correlate with the development of cervical neoplasms in a Japanese population.

In the present study, we found no association between *p53* codon 72 polymorphism and the risk of ovarian cancer. However, Agorastos et al. [11] and Pegoraro et al. [12] reported that women with ovarian neoplasias had the Arg/Arg genotype or Arg allele more often than healthy controls. In contrast, Wang et al. [13] demonstrated that analyses of this SNP in tumor DNA of ovarian cancer patients gave a higher frequency of homozygosity or heterozygosity for the Pro allele, which was closely associated with higher frequency of *p53* sequence variants and poorer prognosis. Resistance to chemotherapy remains a complex problem in ovarian cancer which is one of the reasons for its poor prognosis. *p53* functional status may be a critical determinant for the success of systemic chemotherapy with drug that can induce *p53*-dependent apoptosis [37]. Previous studies have reported that wild-type *p53* of the Arg allele may be more efficient in suppression of the multiple drug resistance gene MDR1 or induction of the proapoptotic oncogene BAX, while wild-type *p53* of the Pro allele is more efficient at promoting cell cycle arrest and DNA repair via its induction of *p21*-Waf and GADD45 [38,39]. Recently, ovarian cancers were also divided into two broad categories designated as type I and type II cancers, which correspond to two main pathways of tumorigenesis [40]. Type I cancers tend to be low-grade neoplasms that arise in stepwise manner from borderline tumors, whereas type II cancers are high-grade neoplasms for which a morphologically recognizable precursor lesion has not been identified, so-called de novo development. These two types of ovarian cancer may have different genetic alterations including *p53* mutations. Although these designations only refer to pathways of tumorigenesis and are not specific histopathological terms, it would be of interest

to examine the correlation between genotypic and allelic frequencies of *p53* codon 72 and ovarian cancer types. Further studies on the functional differences of two variants of *p53* codon 72 and their biological correlation to apoptosis, cell cycle regulation, chemosensitivity, and genetic backgrounds would contribute to the better understanding for pathogenesis and clinical management of ovarian cancer.

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Extranodal Natural Killer/T-Cell Lymphoma, Nasal Type

The Significance of Radiotherapeutic Parameters

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BACKGROUND. The objective of this study was to investigate the correlation between local recurrence and radiotherapeutic parameters, including dose and RT radiotherapy (RT) field.

METHODS. The current study included 35 patients who were diagnosed with immunohistochemically confirmed nasal natural killer (NK)/T-cell lymphoma between 1976 and 2004. There were 21 males and 14 females, and they ranged in age from 18 years to 76 years (median, 51 yrs). The primary tumor originated in the nasal cavity in 28 patients, and 32 patients had Stage I disease. Seventeen patients received treatment solely with RT, and the remaining 18 patients received a combination of chemotherapy and RT. The median tumor dose was 50 grays (Gy) (range, 22–60 Gy). Twenty-seven patients received RT to include all macroscopic lesions, all paranasal sinuses, the palate, and the nasopharynx. Eight patients received RT to all macroscopic lesions with generous margins.

RESULTS. A complete remission (CR) or a CR/unconfirmed was achieved in 28 patients (80%). The 5-year overall survival (OAS) rate, disease-free survival (DFS) rate, and local control probability (LCP) were 47.3%, 42.9%, and 65.2%, respectively. Patients who received RT only to macroscopic lesions fared less well in terms of LCP (LCP 5 years, 71.9% vs. 41.7%; $P = 0.007$). The difference in RT field also affected both the OAS rate and the DFS rate. Patients who received RT doses $\geq$ 50 Gy tended to achieve favorable local control.

CONCLUSIONS. In the management of nasal NK/T-cell lymphoma, the RT field affected treatment outcomes. RT doses $\geq$ 50 Gy resulted in favorable local control. *Cancer* **2006;106:609–15.** © *2005 American Cancer Society.*

KEYWORDS: extranodal natural killer/T-cell lymphoma, angiocentric lymphoma, radiotherapy field, radiotherapy dose, chemotherapy.

Extranodal natural killer (NK)/T-cell lymphoma, nasal type (formerly known as midline lethal granuloma, polymorphic reticulosis, or angiocentric immunoproliferative lesions) recently was recognized as a distinct entity of malignant lymphoma.[1–4] Because this type of lymphoma often shows an angiocentric and angiodestructive growth pattern, together with a broad cytologic spectrum of atypical cells and a zonal necrosis, it was categorized as angiocentric lymphoma in the revised European–American lymphoma classification.[5] These lymphomas are uncommon in the U.S. and Europe, but they are prevalent in East Asia and in certain parts of Central and South America. In a recent nationwide study of malignant lymphoma among Japanese, it was reported that NK/T-cell lymphoma accounts for approximately 2.6% of all malignant lymphomas in Japan.[6] Patients with this lymphoma present either with symptoms of nasal

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obstruction or epistaxis due to the presence of a mass lesion or with destruction of midfacial structures. The tumor also extends to adjacent tissues, such as the nasopharynx, paranasal sinuses, orbit, oral cavity, palate, and oropharynx.

The confusing nomenclature and its rarity have prevented investigators from establishing the optimal treatment for patients with extranodal NK/T-cell lymphoma. Furthermore, the finding that much published research not only included patients who did or did not undergo immunohistochemical confirmation of NK/T-cell lymphoma[7–22] but also included patients who had B-cell lymphoma served to complicate the interpretation of that research.[22–30] However, the response to radiotherapy (RT) generally is so rapid and dramatic that the delivery of RT has been accepted as the preferred treatment of choice for localized disease. Several investigators have advocated the combination of chemotherapy and RT[9,18,26,30]; however, whether the addition of chemotherapy to RT offers any survival benefits to patients is questionable in most series.[11,12,14–17,19–22,24,25,27–29] Patients with nasal NK/T-cell lymphoma experience recurrence at various anatomic sites, including the lymph nodes, skin, liver, spleen, and bone marrow; however, local failure remains the predominant pattern of recurrence. Thus, it is indispensable to clarify the role of RT in each clinical setting. The optimal dose required to achieve appropriate local control and the volume to be treated also require clarification.

In the current study, we analyzed the effects of various factors on local control with special reference to RT parameters, such as the dose to be delivered and the fields to be covered. The objectives of this study were to investigate the correlation between RT field and local recurrence and to establish the dose-response relation that influenced the probability of local control in patients with Stage I and II nasal NK/T-cell lymphoma who were treated at our institutions.

MATERIALS AND METHODS

Between July 1976 and May 2004, 38 patients with nasal NK/T-cell lymphomas received RT at Chiba University Hospital and Narita Red Cross Hospital. Of these 38 patients, 3 were excluded from the current analysis for the following reasons: palliative intent for advanced disease (n = 2 patients) and consultation at the time of recurrence (n = 1 patient). The median follow-up was 27 months (range, from 1.5 mos to 28.0 yrs), and the median follow-up of surviving patients was 11.1 years.

Histologic specimens were evaluated by an expert hematopathologist (J. T.). Immunohistochemical examination was undertaken in every patient to exclude B-cell lymphomas. Consequently, all tumors were positive for either CD56 and CD3ϵ or for T-cell markers, such as CD3, CD43, and CD45RO. For staging of their disease, patients underwent a physical examination; complete blood counts; screening blood tests of hepatic and renal function; chest radiograph; gallium scintigraphy; computed tomography (CT) scans of the head and neck, chest, abdomen, and pelvis; examination of the gastrointestinal tract; and bone marrow aspiration and/or biopsy. All patients were staged according to the Ann Arbor criteria.

TABLE 1
Patient Characteristics

Characteristics	No. of patients (%)
Age	
Range	18–76 yrs
Mean ± SD	52 ± 16 yrs
Median	51 yrs
Gender	
Male	21 (60)
Female	14 (40)
Primary site	
Nasal cavity	28 (80)
Paranasal sinus	4 (11)
Pharynx	3 (9)
B symptoms	
Yes	7 (20)
No	28 (80)
Lactate dehydrogenase elevation	
Yes	7 (20)
No	28 (80)
Disease stage	
Stage I	32 (91)
Stage II	3 (9)

SD: standard deviation.

Patient Characteristics

There were 21 males and 14 females, and the patients ranged in age from 18 years to 76 years (median, 51 yrs). The primary tumor originated in the nasal cavity in 28 patients and in the paranasal sinuses in 4 patients, including 2 patients with mesopharynx tumors and 1 patient with a nasopharyngeal tumor. Thirty-two patients had Stage I disease. Systemic B symptoms were present in seven patients, and lactate dehydrogenase (LDH) elevation was found in seven patients (Table 1). Informed consent was provided according to the Declaration of Helsinki.

Treatment

All patients received RT from a cobalt-60 unit or a linear accelerator with 4-megavolt (MV), 6-MV, or 10-MV photons to achieve dose homogeneity. An ap-

TABLE 2
Treatment Characteristics

Characteristic	No. of patients (%)
Treatment	
RT alone	17 (49)
RT and chemotherapy	18 (51)
Anthracycline	
Yes	15 (83)
No	3 (17)
Dose	
Range	22.0–60.0 Gy
Mean ± SD	37.9 ± 9.3 Gy
Median	50.0 Gy
Field	
Primary alone	8 (23)
Others	27 (77)

RT: radiotherapy; SD: standard deviation; Gy: grays.

propriate energy of electron field also was applied to treat the tumor behind the lens block in the photon field. Generally, the planning target volume included all macroscopic lesions, the paranasal sinuses, the nasopharynx, the upper gum, and the palate with adequate margins. Regardless of primary tumor localization, elective cervical lymph node irradiation was not delivered unless the neck was involved clinically. The most common field arrangement was two lateral opposing photon fields with supplementation between the medial canthus by appropriate energy of electron. All patients received RT with a conventional fractionation schedule at a median tumor dose of 50 grays (Gy) (range, 22–60 Gy). Seventeen patients received treatment treated solely with RT, and the remaining 18 patients received a combination of chemotherapy followed by RT. Anthracycline-containing combination chemotherapy was administered to 15 patients (Table 2).

Statistical Analysis

Tumor response was assessed by using standard criteria.[31] Overall survival (OAS), disease-free survival (DFS), and the local control probability (LCP) were calculated using the method of Kaplan and Meier.[32] The log-rank test was used to assess significance in univariate analysis, and the Cox proportional hazards model was used to assess significance in multivariate analysis.[33]

RESULTS

Overall Results

At the time of evaluation, 28 patients achieved complete remission (CR) or CR/unconfirmed (CRu), which resulted in an 80% CR rate (95% confidence interval [95% CI], 66–94%). Of the seven patients who failed to obtain CR or CRu, the primary tumor was controlled well in three patients. The 2-year OAS rate, DFS rate, and LCP were 57.6% (95% CI, 40.7–74.4%), 53.0% (95% CI, 36.2–69.8%), and 73.8% (95% CI, 57.9–89.6%), respectively. The corresponding values at 5 years were 47.3% (95% CI, 29.8–64.7%), 42.9% (95% CI, 25.8–60.0%), and 65.2% (95% CI, 47.3–83.2%), respectively (Fig. 1). The 5-year OAS rate, DFS rate, and LCP for the patients who received RT alone were 43.8% (95% CI, 19.4–68.1%), 43.8% (95% CI, 19.4–68.1%), and 60.2% (95% CI, 35.2–85.2%), respectively. The corresponding values for patients who received both chemotherapy and RT were 51.8% (95% CI, 27.5–76%), 43.2% (95% CI, 19.8–66.6%), and 71.9% (95% CI, 48.2–95.7%), respectively. The administration of chemotherapy did not appear to have an impact on all endpoints.

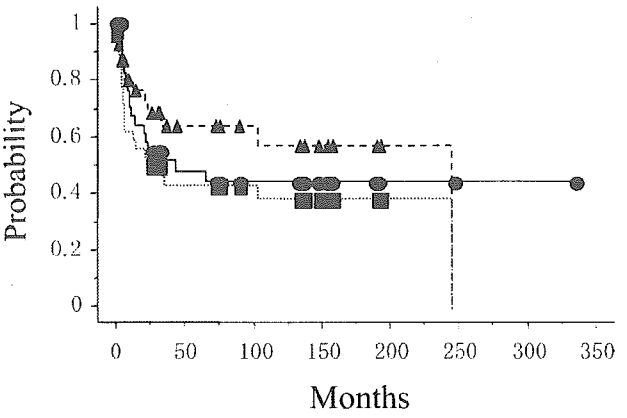

FIGURE 1. This chart illustrates the curves for overall survival (solid line), disease-free survival (dotted line), and local control probability (dashed line).

At the time of the current analysis, 16 patients were alive without evidence of disease, and 14 disease recurrences were observed. Of these 14 patients who developed disease recurrence, 5 patients had locally recurrent disease, 6 patients had distant metastasis, and the remaining 3 patients experienced both local and distant or regional failure. The median time to disease recurrence was 11.6 months. The metastatic sites included the liver, spleen, lymph nodes, subcutaneous soft tissue, skin, intestine, bone marrow, and brain. Two patients who experienced local recurrence were salvaged successfully by a second course of RT. Those 2 patients developed local disease recurrence at 8.5 years and 15.5 years after their initial course of RT. There were 18 deaths during the study period, all of which were due to progressive or recurrent tumors.

RT Field, RT Dose, and Local Control

We also assessed the relation between RT field and the LCP. During the study period, 27 patients received RT

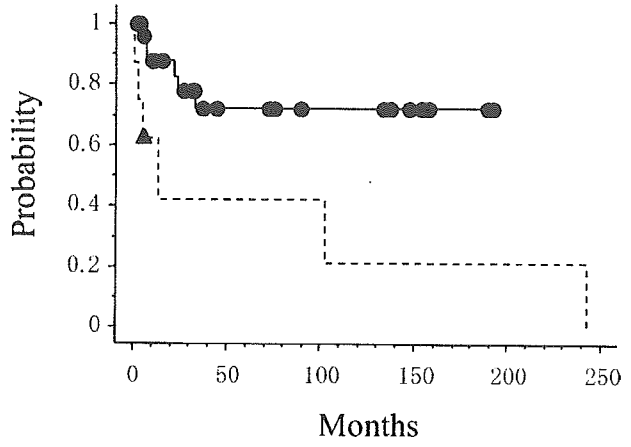

FIGURE 2. This graph illustrates the local control probability as a function of radiotherapy (RT) field. Solid line: an RT field that encompassed all sinuses, the nasopharynx, and macroscopic lesions; dashed line: an RT field that included macroscopic lesions with a margin.

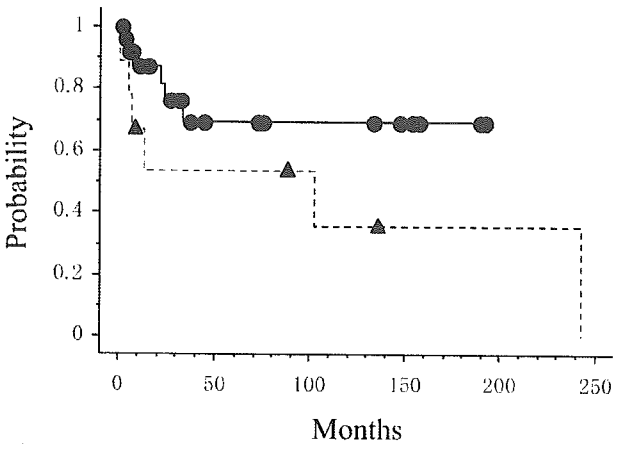

FIGURE 3. This graph illustrates the local control probability as a function of radiotherapy dose. Solid line: doses ≥ 50 grays (Gy); dashed line; doses < 50 Gy.

that included all macroscopic lesions and sites of potential contiguous spread (i.e., all paranasal sinuses, the palate, and the nasopharynx) with adequate margins. The RT field in the remaining eight patients encompassed all macroscopic lesions with generous margins. Although there were six local recurrences in the former group, all but two patients in the latter group experienced local disease recurrence. The LCP at 5 years was 71.9% versus 41.7%, respectively (P = 0.007) (Fig. 2).

Next, we assessed whether there was a dose-response relation for local control. Among the 26 patients who received ≥ 50 Gy of RT, 20 patients were able to achieve local control; however, only 3 of 9 patients in the low-dose group obtained local control (Fisher exact test; P = 0.038). Figure 3 illustrates the LCP as a function of RT dose. The 5-year LCP for patients who received ≥ 50 Gy versus patients who received < 50 Gy was 69.2% (95% CI, 47.9–90.5%) and 53.3% (95% CI, 19.4–87.3%), respectively (P = 0.13). The OAS and DFS rates for patients who received ≥ 50 Gy were 47.7% (95% CI, 28.6–68.6%) and 41.6% (95% CI, 21.3–61.9%), respectively, which did not differ significantly from patients who received < 50 Gy.

Prognostic Factors

The clinical and treatment factors that we assessed for potential prognostic impact included age, gender, primary site, B symptoms, LDH elevation, disease stage, dose of RT, and chemotherapy. However, none of those variables was identified as an independent prognostic factor for OAS and LCP. The only factor that was found to be associated with OAS, DFS, and LCP was the RT field (P = 0.027, P = 0.020, and P = 0.007, respectively). However, multivariate analysis failed to identify any prognostic factors for those three endpoints.

DISCUSSION

Extranodal NK/T-cell lymphoma, nasal type, which was recognized previously as angiocentric lymphoma, has a distinct position in the new World Health Organization classification system.[1] It is characterized by CD56 and cytoplasmic CD3 expression, a germline configuration of the T-cell receptor gene, and a strong association with Epstein–Barr virus (EBV). Many groups have reported treatment outcomes for patients with nasal non-Hodgkin lymphoma.[7–30] Some groups treated patients only with RT,[7,8,10,13,23] but others delivered both chemotherapy and RT.[9,11,12,14–22,24–30] Those studies demonstrated OAS and LCP rates at 5 years of 24–86%, and 31–84%, respectively. Although there are many suggestions in the literature regarding the management and natural history of nasal lymphoma, many of these reports included patients with B-cell lymphoma and patients who did not receive immunohistochemical examinations, which led to a great deal of confusion in relation to the roles of chemotherapy, failure patterns, and treatment outcomes.

There were eight studies, including our previous work,[14] that included only patients who had immunophenotypically confirmed nasal NK/T-cell lymphoma.[14,16–22] Furthermore, of those eight studies, five included only patients who had both CD3ε-positive and CD56-positive expression to exclude peripheral T-cell lymphoma.[17,18,20–22] A summary of those eight

TABLE 3
Summary of the Literature

Reference	Phenotype	Treatment	No.	LFR	5-yr OAS (%)
Itami et al., 1991[14]	NK or T-cell	CT → RT or RT	9	6/9	NR
Aviles et al., 2000[16]	NK or T-cell	RT → CT	108	NR	86 (8 yrs)
Kim et al., 2001[17]	NK cell	CT → RT	17	NR	59 (3 yrs)
Yamaguchi et al., 2001[18]	NK cell	RT → CT or CT → RT	12	7/12	39
Ribrag et al., 2001[19]	NK or T-cell	RT → CT or CT → RT or RT	20	NR	NR
Cheung et al., 2002[20]	NK cell	CT → RT	79	31.1%	37.1
Chim et al., 2004[21]	NK cell	CT → RT	67	35/67	42.5 (10 yrs)
You et al., 2004[22]	NK cell	CT → RT	46	NR	36.5
Current study	NK or T-cell	CT → RT or RT	35	34.8%	47.3

LFR: local failure rate; OAS: overall survival; NK: natural killer; CT: chemotherapy; RT: radiotherapy; NR: not reported.

studies is provided in Table 3. According to those reports, the OAS ranged from 36.5–86%. Although the Mexican group demonstrated very surprising results,[16] the remaining 7 groups reported that OAS was approximately 40%,[14,17–22] which was comparable to the results of the current study. All eight series administered chemotherapy and RT and concluded that conventional chemotherapy followed by RT appeared to be ineffective for the majority of patients and that innovative treatment modalities are needed to improve outcomes. However, Yamaguchi et al. observed that patients who received treatment with concurrent chemoradiotherapy or with RT followed by chemotherapy enjoyed favorable outcomes.[18] Therefore, those authors concluded that RT followed by, or combined with, chemotherapy was best as initial treatment, and they recommended nonanthracycline-containing chemotherapy (dexamethasone, etoposide, ifosphamide, and carboplatin) based on their previous observation that nasal NK/T-cell lymphomas express P-glycoprotein.[34] Since 1998, Cheung et al. also have employed concurrent chemoradiotherapy in an attempt to intensify local treatment.[20] Those authors selected cisplatin as the chemotherapeutic agent in this concurrent setting. Conversely, Kim et al. also administered anthracycline-containing chemotherapy concurrently with RT in two patients.[17] Furthermore, Ribrag et al. observed that two patients who were treated with alternated chemotherapy and RT achieved a CR.[19] The sequence of chemotherapy and RT that will most effectively achieve a satisfactory local control rate will be resolved by future studies; however, it can be concluded that chemotherapy followed by RT is disadvantageous.

In addition to controlling systemic disease, it is indispensable to achieve high LCP in patients with localized nasal NK/T-cell lymphoma. Local recurrence rates ranged from 31–67%, and these high local failure rates led to very poor outcomes.[14,16–22] Although it is evident that RT should play an essential role in achieving local disease control, the dose to be delivered and the field to be covered have not been resolved. With regard to treatment volume, Cheung et al. administered RT to the nasal cavity and nasopharynx,[20] two investigational groups encompassed all paranasal sinuses and the Waldeyer ring in addition to the nasal cavity,[21,22] and four investigational groups delivered RT to the tumor with an adequate margin.[14,17–19] The remaining Mexican investigators delivered RT with an extended field, but to our knowledge the details were not reported.[16] In the current study, we observed that the patients who received RT to macroscopic lesions with a margin achieved an inferior local control rate compared with patients who received with an RT field that encompassed all paranasal sinuses, the palate, and the nasopharynx in addition to the nasal cavity. In contrast, Cheung et al. recommended meticulous CT conformal planning with the aid of magnetic resonance imaging scans to deliver RT to the macroscopic tumor with an adequate margin.[20] Although the majority of reports in the literature do not mention the recommended RT field, three groups of investigators advocated that the RT field should encompass the paranasal sinuses in addition to macroscopic lesions,[7,23,24] a recommendation that is consistent with our current observations.

Many researchers have delivered 30–60 Gy to control macroscopic lesions. In the current study, we suggested that patients who received $\geq$ 50 Gy had a tendency to achieve superior local control rates compared with patients who received $<$ 50 Gy, which is well in accordance with the observation of Cheung et al.[20] You et al. administered higher RT doses (54–60 Gy) and achieved an 83.3% failure-free survival rate at 5 years.[22] Furthermore, although 50% of their patients did not have immunohistochemical confirmation of nasal NK/T-cell lymphoma, a Korean group demonstrated a clear dose-response relation within the range

of 20–54 Gy with a plateau at doses in excess of approximately 54 Gy.[13] Conversely, 2 other groups reported that 45 Gy appeared to be an effective dose for local control.[16,17] Those data indicated that it is necessary to deliver at least 45 Gy of RT to achieve a favorable local control rate; however, whether higher doses would achieve better local control rates remains unresolved.

We were able to identify that the RT field was a significant prognostic factor for LCP, DFS, and OAS. Several groups have advocated that disease stage,[15,17,20,22] performance status,[20] B symptoms,[17,20] LDH elevation,[22] and the International Prognostic Index (IPI)[21,22] are of prognostic importance. However, Aviles et al. reported that there was no evidence that the IPI was applicable in patients with nasal NK/T-cell lymphoma,[16] and there have been no widely accepted prognostic factors. With regard to molecular markers, Lin et al. examined 19 true NK-lineage nasal NK/T-cell lymphomas and demonstrated that CD94 expression was a favorable prognostic factor.[35] In addition, Au et al. demonstrated that the EBV DNA level at the time of presentation was correlated with disease stage and LDH, and high presentation EBV DNA levels were associated significantly with inferior DFS. Furthermore, patients with EBV DNA levels that increased further or that failed to become undetectable during treatment had significantly inferior survival. Those investigators concluded that, in patients with EBV-positive lymphomas, the plasma EBV DNA level is valuable as a tumor biomarker and for prognosis.[36] The significance of these new molecular markers will be elucidated in future clinical trials.

The rarity of this type of lymphoma limits large-scale, prospective, randomized trials. However, several of our findings have important implications in the management of nasal NK/T-cell lymphoma. The high efficacy of RT in achieving a CR with an RT field that encompasses all paranasal sinuses, the nasopharynx, and the palate, in addition to macroscopic lesions, and with RT doses $\geq$ 50 Gy suggests that it may be advantageous to incorporate adequate RT up-front in the treatment strategy. The occurrence of failures at distant sites implies that systemic chemotherapy also should be administered. Accordingly, we have launched a prospective study to evaluate the efficacy and toxicity of concurrent chemoradiotherapy for patients with nasal NK/T-cell lymphoma.

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