with a range from 35 to 80 years, and most of them were male patients (78%) and had stage IV disease (74%).

Presence of HPV DNA and p16 expression in OPSCC

Of the 104 tumor specimens, 40 (38%) were positive for HPV-16 or HPV-18 DNA by PCR analysis (Fig. 1). These 40 tumors included 37 positive for HPV-16 alone, two positive for HPV-18 alone, and one positive for both HPV-16 and HPV-18. HPV DNA was detected more frequently in the tonsils ($P = 0.002$) than in other regions (Table 1). Patients positive for HPV DNA presented significantly more often with lymph node metastasis (85 vs. 64%, $P = 0.021$) and included a higher proportion of never-smokers (55 vs. 30%, $P = 0.010$) compared with those negative for HPV. There was no significant association between HPV DNA status and gender, age, T classification, or disease stage.

Expression of p16 was detected by IHC in a total of 39 tumors (Fig. 2). Of the 40 cases positive for HPV DNA, 32 (80%) were positive for p16, whereas 57 (89%) of the 64 cases negative for HPV DNA were also negative for p16 (Fig. 1). There was thus good agreement between HPV DNA positivity and p16 positivity ($\kappa = 0.65$; 95% confidence interval [CI], from 0.50 to 0.80; $r = 0.631$; $P < 0.001$).

DNA methylation at the p16 gene promoter in OPSCC

To identify the underlying mechanism of p16 gene silencing in tumors positive for HPV DNA but negative for p16 expression, we examined the DNA methylation status of the p16 gene promoter region with the use of MS-PCR analysis. Among the eight such cases, DNA methylation at the p16 gene promoter was detected in six (cases 66, 69, 71, 82, 96, and 106) (Fig. 3).

Survival analysis

Oropharyngeal squamous cell carcinoma patients positive for HPV DNA showed a significantly better overall survival compared with those negative for HPV DNA [hazard ratio (HR), 0.214; 95% CI, from 0.074 to 0.614; $P = 0.002$] (Fig. 4A). For OPSCC of stages I to III, HPV-positive patients tended to have a better overall survival compared with their HPV-negative counterparts, but the difference was not statistically significant ($P = 0.129$), possibly because of the small sample size ($n = 27$) (Fig. S1A). On the other hand, for OPSCC of stage IV

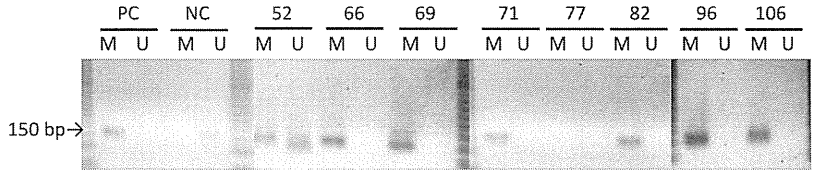

Figure 3. MS-PCR analysis of the p16 gene promoter in eight OPSCC tumors positive for HPV DNA but negative for p16 by IHC. The position of a 150-bp amplification product corresponding to the methylated promoter is indicated. PC, positive control; NC, negative control; M, methylated; U, unmethylated; MS-PCR, methylation-specific polymerase chain reaction; OPSCC, oropharyngeal squamous cell carcinoma; HPV, human papillomavirus; IHC, immunohistochemistry.

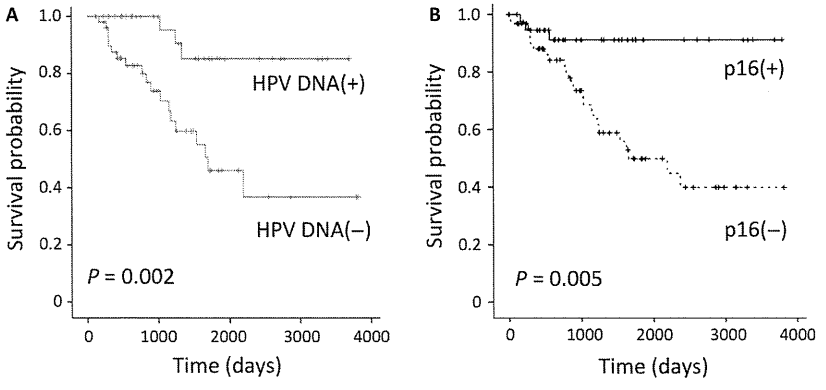

Figure 4. Kaplan–Meier curves for overall survival of OPSCC patients according to HPV DNA (A) or p16 (B) status. *P*-values were calculated by the log-rank test. OPSCC, oropharyngeal squamous cell carcinoma; HPV, human papillomavirus.

(n = 77), patients with HPV DNA showed a significantly better overall survival than did those without it (P = 0.002) (Fig. S1B). Stratification based on p16 expression also revealed a significantly better outcome for OPSCC patients positive for p16 than for those negative for this marker (HR, 0.245; 95% CI, from 0.085 to 0.705; P = 0.005) (Fig. 4B). To rule out potential confounding effects for the presence of HPV DNA and other factors, we performed multivariate analysis for overall survival (Table 2). The presence of HPV DNA was revealed to be an independent and significant prognostic factor for overall survival (HR, 0.248; 95% CI, from 0.080 to 0.766; P = 0.015) after taking into account gender, age, T and N classification, smoking history, tumor location, and radiation therapy.

Sensitivity of OPSCC cell lines with or without HPV DNA to radiation and cisplatin

We next investigated the biological impact of HPV DNA status with OPSCC cell lines positive (UPCI-SCC-090, -152, and -154) or negative (UPCI-SCC-003, -036, and -089) for HPV DNA. A clonogenic survival assay performed after exposure of the cells to various doses of radiation revealed no significant difference in survival between the cell lines positive or negative for HPV DNA (Fig. 5A). We also examined the effect of cisplatin on the growth of the cell lines, again detecting no difference in the IC_{50} value of cisplatin between those positive or negative for HPV DNA (Fig. 5B, Table 3).

Table 2. Multivariate analysis of overall survival in patients with OPSCC (n = 104).

	Overall survival		
Factor	HR	95% CI	P
HPV DNA (positive vs. negative)	0.248	0.080–0.766	0.015
Gender (female vs. male)	0.870	0.231–2.151	0.539
Age (≤63 vs. >64 years)	0.833	0.392–1.770	0.634
T classification (1–2 vs. 3–4)	0.718	0.315–1.640	0.432
N classification (0 vs. 1–3)	1.536	0.640–3.680	0.337
Smoking history (nonsmoker vs. smoker)	0.541	0.120–2.445	0.424
Tumor location (tonsil vs. other)	0.597	0.277–1.289	0.189
RT[1], RT(+) vs. RT(−)	2.233	0.390–13.89	0.355

OPSCC, oropharyngeal squamous cell carcinoma; HPV, human papillomavirus; HR, hazard ratio; CI, confidence interval.

[1]RT(+), treatment with radiation, including radiation therapy alone (n = 3), chemoradiotherapy alone (n = 13), or surgery followed by radiation therapy (n = 46) or by chemoradiotherapy (n = 23); RT(−), treatment without radiation, including surgery alone (n = 11), surgery followed by chemotherapy (n = 1), chemotherapy alone (n = 4), and best supportive care (n = 3).

Discussion

In this study, we applied PCR-based detection of viral DNA and IHC-based detection of p16 to tumor specimens from Japanese patients with OPSCC, given that this combination of approaches is the most reliable means to determine HPV status, with a sensitivity of 97% and specificity of 94% [13]. We found that 38% of the patients were positive for HPV DNA, consistent with recent studies that detected HPV DNA in 30–50% of OPSCC patients in Asian countries [14–16]. In the United States, the incidence of HPV-positive OPSCC increased by 225% from the late 1980s to the early 2000s [17], with 40–80% of OPSCCs now being caused by HPV [3]. This increase is thought to have resulted from the decrease in tobacco use and increased oral HPV exposure due to changes in sexual behavior among recent birth cohorts [3, 4]. As in other Asian countries, the prevalence of smoking in Japan is much higher than that in the United States, especially among men (32 vs. 17%) [18]. The lower proportion of OPSCC cases associated with HPV in Asian countries compared with Western countries might therefore be attributable, at least in part, to the difference in tobacco exposure. Given that the proportion of active smokers has recently been decreasing each year in Japan, the proportion of OPSCCs related to HPV in the Japanese population is likely to increase.

We found that overall survival for Japanese OPSCC patients positive for HPV DNA was significantly better than that for those negative for HPV DNA. The presence of HPV DNA was associated mostly with tumors of the palatine tonsils, lymph node metastasis, and nonsmoking. HPV-positive OPSCC was more frequent in younger individuals than was HPV-negative OPSCC, but the difference was not significant, possibly due to the relatively small sample size. These results are consistent with those for OPSCC in the United States and Europe [3, 4], suggesting similarity in the features of HPV-associated OPSCC between Japan and Western countries.

The reason for the more favorable prognosis of HPV-associated OPSCC remains unclear, although it may be related to a younger age at onset, minimal exposure to established risk factors such as cigarette smoking, or a better response to therapy [3, 19]. Indeed, recent studies have provided evidence that HPV-positive OPSCC shows a better response to chemotherapy [20, 21] or to radiotherapy either alone [22, 23] or in combination with chemotherapy [20, 21, 24, 25]. Although these findings are suggestive of an inherent radio- or chemosensitivity of HPV-positive OPSCC, we did not detect a difference in sensitivity to radiation or cisplatin in vitro between OPSCC cell lines positive or negative for HPV DNA. This apparent discrepancy between the in vitro and clinical

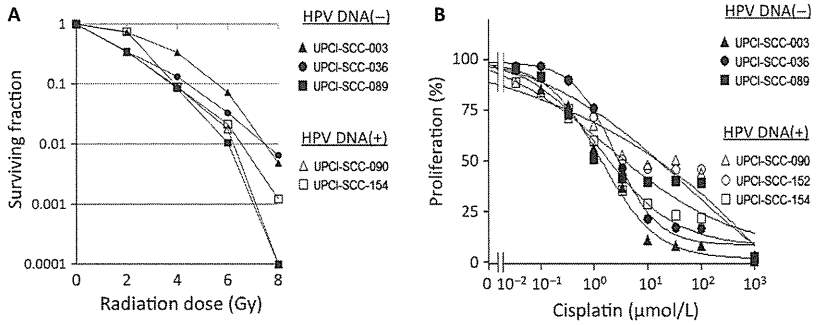

Figure 5. Sensitivity of OPSCC cell lines to radiation or cisplatin according to HPV DNA status. (A) Clonogenic assay for cells exposed to the indicated doses of radiation. This assay was not performed with UPCI-SCC-152 cells. (B) Effect of cisplatin concentration on cell growth. All data are means from three independent experiments. OPSCC, oropharyngeal squamous cell carcinoma; HPV, human papillomavirus.

Table 3. IC_{50} values of cisplatin for inhibition of OPSCC cell growth in vitro.

Cell line	HPV DNA	Cisplatin IC_{50} (μmol/L)
UPCI-SCC-003	(−)	1.7
UPCI-SCC-036	(−)	3.0
UPCI-SCC-089	(−)	1.2
UPCI-SCC-090	(+)	5.7
UPCI-SCC-152	(+)	4.6
UPCI-SCC-154	(+)	2.0

IC, inhibitory concentration; OPSCC, oropharyngeal squamous cell carcinoma; HPV, human papillomavirus.

data might be due to the limitations of in vitro assays, which do not accurately reflect the tumor microenvironment in vivo. Further study is thus needed to determine the molecular mechanism underlying the favorable outcome of patients with HPV-positive OPSCC, with the prospect that such knowledge might inform the development of therapeutic approaches to improve the poor prognosis of those with HPV-negative OPSCC.

In HPV-positive OPSCC, production of the viral oncoprotein E7 results in inactivation of the retinoblastoma (RB) protein and consequent upregulation of p16 expression [3, 26–28]. IHC positivity for p16 is thus associated with HPV-positive OPSCC, being regarded as a surrogate marker for HPV infection in such tumors [3, 6]. We also found a significant correlation between positivity for HPV DNA and IHC-based detection of p16 in Japanese patients with OPSCC, and the results of survival analysis based on p16 status as a stratification factor were similar to those of such analysis based on HPV DNA status.

Although most HPV-associated OPSCC tumors express p16, we found that 20% of HPV DNA-positive tumors (eight cases) were negative for p16 by IHC. A similar level of discordance was observed in previous studies based on the same approaches for detection of HPV DNA and p16 [7, 13, 29], although the underlying mechanism remains largely unknown. Given that DNA methylation at the p16 gene promoter has been identified as a key mechanism of p16 gene silencing in various types of primary tumor [30], we analyzed the methylation status of the p16 gene promoter in the eight tumors positive for HPV DNA but negative for p16 in this study with the use of MS-PCR analysis. We found a high frequency (6/8, 75%) of DNA methylation at the p16 gene promoter in these cases. As far as we are aware, this is the first demonstration of DNA methylation at the p16 gene promoter in OPSCC tumors positive for HPV DNA but negative for p16 by IHC. A recent meta-analysis showed that heavy cigarette consumption was associated with p16 gene methylation in patients with non-small cell lung cancer [12]. In this study, among the HPV DNA-positive subgroup, patients with tumors negative for p16 expression had a significantly more extensive smoking history than those with tumors positive for p16 ($P < 0.001$, Student's two-tailed t-test), suggesting that heavy smoking might be responsible, at least in part, for DNA methylation at the p16 gene promoter and a consequent loss of p16 expression. Consistent with the results of a previous study [7], we also found that the survival of patients with HPV DNA-positive, p16-negative tumors was not as good as that of those with HPV DNA-positive, p16-positive tumors (data not shown). These data thus suggest that IHC-based detection of p16 provides suboptimal prognostic information unless combined with PCR-based detection of HPV DNA.

Seven (11%) of the 64 HPV DNA-negative tumors in this study were positive for p16 by IHC. Given that the HPV DNA analysis was initially restricted to HPV types 16 and 18, we further investigated the possible presence of DNA for other high-risk types of HPV (types 31, 33, and 35), which, together with types 16 and 18, account for most cases of HPV-associated OPSCC [8, 13, 31]. However, none of the seven HPV DNA-negative,

p16-positive tumors was found to be positive for these other high-risk types of HPV (data not shown). Similar results have been obtained in previous studies based on detection of HPV by PCR or in situ hybridization [19], with a discordance rate of ~10–20%. Expression of p16 in such HPV DNA-negative tumors might reflect disturbances of the RB signaling pathway unrelated to HPV infection, as has been found to be the case in malignant lymphoma and small cell lung cancer [32]. The mechanism of p16 expression in the absence of detectable HPV DNA in OPSCC warrants further investigation.

Two prophylactic HPV vaccines against HPV types 6, 11, 16, and 18 (quadrivalent) or HPV types 16 and 18 (bivalent) have shown clinical efficacy for prevention of HPV-related cervical cancer [33] and anal cancer [34]. Both vaccines thus target HPV type 16, which accounts for >90% of HPV-associated OPSCCs [4]. Given the causal relation between HPV infection and OPSCC, clinical evaluation of the potential efficacy of HPV vaccines for reducing the incidence of HPV-associated OPSCC is warranted.

In conclusion, we found that 38% of Japanese patients with OPSCC are positive for HPV DNA, with such positivity being an independent prognostic factor for overall survival. Given that expression of p16 can be affected by genetic or epigenetic changes in addition to HPV infection, our results suggest that IHC-based detection of p16 provides suboptimal prognostic information if not combined with detection of HPV DNA. Further clinical studies are warranted to characterize the mechanism underlying the survival benefit conferred by HPV positivity in patients with OPSCC as well as to identify optimal treatments for this patient population.

Acknowledgment

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Conflict of Interest

None declared.

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Supporting Information

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

Figure S1. Kaplan–Meier curves for overall survival of patients with OPSCC of stages I to III (A) or of stage IV (B) according to HPV DNA status. *P*-values were calculated by the log-rank test.

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Alternating chemoradiotherapy in patients with nasopharyngeal cancer: prognostic factors and proposal for individualization of therapy

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The purpose of this study is to assess the efficacy of alternating chemoradiation in patients with nasopharyngeal cancer. From 1990–2006, 100 patients with nasopharyngeal cancer were treated with alternating chemoradiation at the Aichi Cancer Center. Of these, 4, 2, 23, 34, 13 and 23 patients were staged as I, IIA, IIB, III, IVA and IVB, respectively. The median radiation doses for primary tumors and metastatic lymph nodes were 66.6 Gy (range, 50.4–80.2 Gy) and 66 Gy (range, 40.4–82.2 Gy), respectively. A total of 82 patients received chemotherapy with both cisplatin and 5-fluorouracil (5-FU), while 14 patients received nedaplatin (CDGP) and 5-FU. With a median follow-up of 65.9 months, the 5-year rates of overall survival (OAS) and progression-free survival (PFS) were 78.1% and 68.3%, respectively. On multivariate analysis (MVA), elderly age, N3, and WHO type I histology proved to be significantly unfavorable prognostic factors of OAS. As for PFS, there were T4, N3, and WHO type I histology in MVA. Acute toxicities of hematologic and mucositis/dermatitis ≥ Grade 3 were relatively high (32%); however, they were well-managed. Late toxicities of ≥ Grade 3 were three (3%) mandibular osteomyelitis and one (1%) lethal mucosal bleeding. Results for alternating chemoradiation for nasopharyngeal carcinoma are promising. In order to improve outcomes, usage of intensity-modulated radiation therapy and application of active anticancer agents are hopeful treatments, especially for groups with poor prognosis factors with WHO type I histopathology, T4 and/or N3 disease.

Keywords: nasopharyngeal carcinoma; alternating chemoradiation; WHO type I histopathology

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a common disease among Southern Chinese, Southeast Asian, Northern African and Inuit populations. In Japan, the USA and Western European countries it is relatively rare. Because of anatomical characteristics, surgical treatment is very difficult. In addition, the majority of NPC patients revealed undifferentiated carcinoma, which is relatively sensitive to radiation therapy. Therefore, radiotherapy is widely accepted as the first choice of therapy for NPC. In recent years, by randomized-control trials, chemoradiotherapy has shown significant survival benefits over radiotherapy alone, improving both local and distant control [1–4]. In addition, meta-analysis of eight randomized trials showed significant benefits for OAS and event-free survival [5]. The pooled hazard ratio of death was 0.82 (95% confidence interval, 0.71–0.94; $P = 0.006$), corresponding to an absolute survival benefit of 6% at 5 y from the addition of chemotherapy. Thus, the standard treatment for locally advanced NPC is now believed to be concurrent chemoradiotherapy. However, several key factors need further clarification. Firstly, the chemotherapy used in the Intergroup 0099 study (IGS) consisted of three courses each of concurrent administration of cisplatin (CDDP) and adjuvant chemotherapy with both CDDP and 5-fluorouracil (5-FU). However, about two thirds (63%) of patients could receive concurrent chemotherapy, and about half (55%) could receive the full course of adjuvant chemotherapy. Secondly, a higher incidence of adverse events ≥ Grade 3 was observed in the chemoradiation group than in the radiation alone group (59% vs 34%). Finally, chemoradiation reduced distant metastasis; however, it did not reach sufficient levels. Of the 18 patients with recurrence in the

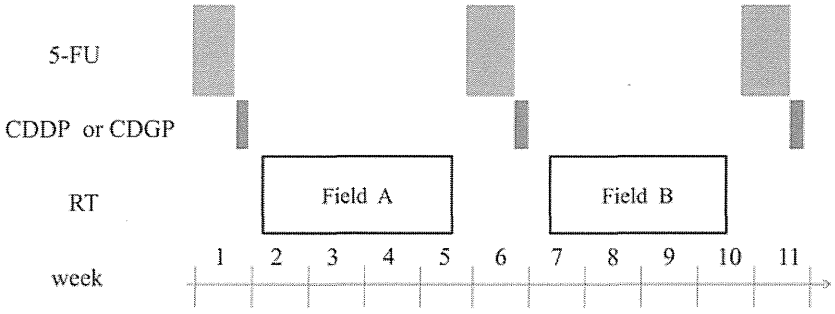

Fig. 1. Study design of alternating chemoradiotherapy. 5-FU = 5-fluorouracil 800 mg/m^2 on Days 1–5 continuous infusion, CDDP = cisplatin 50 mg/m^2 Day 6–7, CDGP = nedapatin 130 mg/m^2 on Day 6, RT = radiotherapy, Field A = large field including from the skull base to supraclavicular fossa, Field B = boost field including the nasopharynx and metastatic lymph nodes.

chemoradiation arm, 10 (56%) developed distant metastasis (DM) in the IGS. A considerable incidence of DM still developed in the IGS due to insufficient dose intensities of chemotherapy, instead of increasing adverse events.

In the Aichi Cancer Center, we conducted alternating chemoradiotherapy for advanced NPC patients from 1987 and reported promising results with sufficiently better compliance (94%), of which the 5-year OAS and PFS rates were 75% and 63%, respectively [6]. In the present study, we analysed the efficacy of alternating chemoradiotherapy for NPC with relatively longer follow-up and sought to refine our treatment strategy according to data regarding failure patterns.

MATERIALS AND METHODS

Patient characteristics

Between 1990 and 2006, a total of 100 consecutive patients with newly diagnosed histology-proven nasopharyngeal carcinoma underwent definitive chemoradiotherapy (CRT) in the Aichi Cancer Center. All patients underwent fiberoptic nasopharyngoscopy and magnetic resonance imaging (MRI) to assess the extent of primary and cervical lymph nodes. Evaluation of distant metastasis was done by chest X-ray, computed tomography (CT), liver ultrasonography, and bone scintigraphy. After 2002, positron emission tomography (PET) or PET-CT was also used to evaluate the extent of the disease. In addition, laboratory data, electrocardiograms, and 24-h creatinine clearance were evaluated to assess general condition. For this analysis, all patients were restaged according to the 6th edition of the American Joint Committee on Cancer (AJCC) staging system [6].

Treatment schedule

Chemotherapy

The treatment scheme is shown in Fig. 1. Details of the treatment regimen have been reported in another article [7]. Chemotherapy regimens were a combination of CDDP and 5-FU (FP) or nedaplatin (CDGP) and 5-FU (FN) regimens. In the FP regimen, 5-FU was administered continuously at a dose of 800 mg/m^2 on Days 1–5 and CDDP at a dose of 50 mg/m^2 on Days 6–7. In the FN regimen, 5-FU was administered continuously at a dose of 800 mg/m^2 on Days 1–5 and CDGP at a dose of 130 mg/m^2 on Day 6. Chemotherapy was performed in principal three times at 4-week intervals. However, when a WBC count <3000/mm^2 or a platelet count <100 000/mm^2 was obtained at the scheduled date of drug administration, chemotherapy was postponed and radiation therapy was alternately prescribed. When hematological data obtained two weeks after radiotherapy did not meet the inclusion criteria (WBC count >3000/mm^2 and platelet count >100 000/mm^2), the next cycle of chemotherapy was withdrawn. When the WBC count decreased to <1000/mm^2 or the platelet count decreased to <25 000/mm^2 after chemotherapy, doses of both 5-FU and CDDP were decreased by 25% at the next cycle. In addition, the dose of CDDP only was decreased by 25% when serum creatinine levels >1.5 mg/dl were noted.

Radiotherapy

Using a 6–10 MV photon beam by linear accelerator, external beam radiotherapy commenced 2–3 d after the completion of previous chemotherapy. At simulation and daily treatment, the head, neck and shoulder were immobilized in a hyperextended position using a thermoplastic mask. Radiotherapy was performed with a daily fraction of 1.8–2.0 Gy. The initial radiation field covered the nasopharynx and upper and middle cervical regions using bilateral opposing portals and lower cervical, and supraclavicular region using anterior single field irradiation at a dose of 36–40 Gy. Then, a shrinking field of 26–30 Gy was boosted to the nasopharynx and involved lymph nodes using the dynamic conformal rotational technique. In the shrinking field, we kept enough margins of primary tumors and involved lymph nodes from the edge of field. Those margins were mainly decided dependent on proximity to

critical structures such as the brain-stem, spinal cord, optic pathway and temporal lobes. During the second period of chemotherapy, radiotherapy was temporarily interrupted to spare the increasingly acute toxicity of 5-FU. Additional boosts of up to 10 Gy with stereotactic multiple arc treatment were also permitted, if residual tumors existed at primary sites.

Follow-up and statistical consideration

Toxicities of CRT were evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 [8]. During the treatment period, complete blood counts and biochemical examinations were performed at least once a week. After completion of CRT, the treatment response was assessed by fiberoptic nasopharyngoscopy, MRI and/or PET/CT. The frequency of follow-up was every month for the first year, once every two months between the second and third post-treatment year, and once every three months after the third post-treatment year. Fiberoptic nasopharyngoscopy was performed at every visit, and post-treatment MRI scans were obtained every three months for the first year and then every six months thereafter. The survival period was calculated from the start of treatment to death or the last follow-up examination, and progression-free survival was defined as the period from the start of treatment to the progression of tumors or death by any cause. Overall survival and progression-free survival curves were calculated by the Kaplan-Meier method [9]. The log-rank test was used to compare survival curves. A Cox-proportional hazard model was used for multivariate analysis. Differences in the ratios between the two groups were assessed by the chi-square test.

RESULTS

Patient characteristics

Between June 1990 and March 2005, 100 patients with NPC received definitive CRT in the Aichi Cancer Center. Table 1 shows patient characteristics in this cohort. We analysed all patients who were treated with CRT. The median age was 55 years old (range, 28–80). Performance status was distributed as 2 of 0, 93 of 1, 3 of 2, and 2 of 3, respectively. Of these, 8 patients (8%) had histopathology with keratinizing squamous cell carcinoma (WHO type I), and 70 patients (70%) had Stage III–IVB disease. During this period the number of patients with NPC who were treated with radiotherapy alone was 13. The common reasons for radiotherapy alone were advanced age or poor general condition.

Table 1. Patient characteristics

Characteristics		*n*
Age, years: median (range)	55 (28–80)	
Gender:		
	Male	72
	Female	28
Performance status		
	0	2
	1	93
	2	3
	3	2
Histology		
	type I	8
	non type I	90
	others	2
T stage		
	1	37
	2a	15
	2b	15
	3	15
	4	18
N stage		
	0	11
	1	31
	2	34
	3a	9
	3b	15
Stage		
	I	4
	IIA	2
	IIB	24
	III	34
	IVA	12
	IVB	24

Treatment contents

The median dose to the primary site was 66.6 Gy (range, 50.4–80.2 Gy), and the median dose to involved lymph nodes was 66 Gy (range, 40.4–82.2 Gy), respectively. The median period of the whole course of alternating CRT was

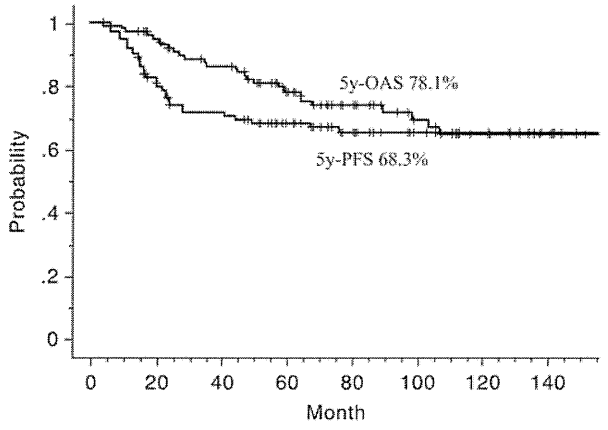

Fig. 2. Overall survival (OAS) and progression-free survival (PFS) curves.

85 days (range, 47–147 days), and the median period of overall treatment time of radiation therapy (OTT) was 69 days (range, 42–110 days).

Treatment outcomes

The 5-year rates of OAS and PFS were 78.1% and 68.3%, respectively (Fig. 2). The 5-year rates of OAS of the group divided by stage were 100, 100, 86.1, 77.6, 91.7 and 60.3% for Stage I, IIA, IIB, III, IVA and IVB, respectively. The 5-year rates of OAS and PFS of 96 patients who received alternating CRT were 78.2% and 68%, respectively. As for initial response after completion of CRT, complete remission (CR) rates of primary and nodal lesions were 86% and 83%, respectively. At a median follow-up of 65.9 months (range, 3.9–22.9 months), 62 were alive without disease, 11 were alive with disease, 18 died from the disease, 2 died from other diseases (both esophagus carcinoma) and 7 died from unknown reasons.

The 5-year rates of loco-regional progression-free survival (LRPFS) and distant metastasis-free survival (DMFS) were 77.9% and 87.8%, respectively.

A total of 32 patients (32%) developed treatment failure at one or more sites. Disease progression developed in 19 for primary, 9 for regional and 11 for distant sites at the last follow-up. Among 11 patients with distant failure, the most frequent site was the lung in 8, followed by bone in 4 and the liver in 2.

Of 21 patients who developed locoregional recurrence, 13 were treated with additional chemoradiation. Of the remainder, 2 patients were re-treated with radiotherapy alone, and 4 with only chemotherapy. One patient received neck dissection for regional failure, and another did not receive any treatment because of the patient's refusal for treatment.

Out of 11 patients who developed distant metastasis, 9 were treated by chemotherapy, and 2 patients received palliative radiotherapy only.

Univariate analysis

Univariate analysis (UVA) results are listed in Table 2.

Elderly age, male, WHO type I histology, and N3 were revealed as significant unfavorable prognostic factors of OAS. The 5-year rate of OAS of the group with WHO type I histology was significantly lower than that with non-type I histology (33.3% vs 81.6%, $P < 0.0001$, Fig. 3). The group with N3 lesions had significantly worse 5-year OAS (60.3%) than that with N0–2 (84%; $P = 0.0017$). The 5-year rates of OAS of patients who received reduced dose and planned dose chemotherapy were 76.6% and 78.6%, respectively ($P = 0.75$).

As for PFS, significantly unfavorable factors were revealed as WHO type I histology, T4 and N3.

The 5-year PFS rate of the group with N3 was significantly lower than that with N0–2 (41.5% vs 76.5%, $P = 0.001$). The 5-year PFS rate of the group with T4 was significantly lower than that with T1–3 (54.5% vs 71.4%, $P = 0.014$). The 5-year rates of PFS of patients who received reduced dose and planned dose chemotherapy were 69.7% and 66.7%, respectively ($P = 0.59$).

The 5-year rate of LRPFS of the group with WHO type I histology was significantly lower than that with non-type I histology (21.4 % vs 84.5 %, $P < 0.0001$).

The 5-year rate of DMFS of patients with N3 was significantly lower than that with N0–2 (62.8% vs 95.1%, $P < 0.0001$). The 5-year LRPFS of patients with T4 was significantly lower than that with T1–3 (63.3% vs 81.1%, $P = 0.027$).

Multivariate analysis

Multivariate analysis (MVA) results are listed in Table 3. On MVA, significantly unfavorable prognostic factors of OAS were elderly age, WHO type I histology and N3, respectively. As for PFS, they were WHO type I histology, T4 and N3, respectively.

Treatment compliance

Regarding the contents of chemotherapy, 82 patients received FP, while 14 received FN. Four patients had other chemotherapy regimens, as described below. One patient with Stage I (cT1N0M0) received two courses of CDDP/5-FU followed by definitive radiotherapy. One patient received six courses of weekly docetaxel (TXT) because of elderly age and poor medical condition. One patient received chemotherapy with both CDGP and TXT because 5-FU was inappropriate due to a past history of myocardial infarction. One patient received concurrent administration with decreased doses of CDGP and 5-FU due to elderly age. Chemotherapy compliance is shown in Table 4. In 96 patients who received alternating CRT, over 90% of patients received three courses of chemotherapy and 70% of patients received the planned dose of three courses. In

Table 2. Univariate analyses for overall survival and progression-free survival

Factors	No.	5-year OAS (%)	*P*-value	5-year PFS (%)	*P*-value
Gender					
Female	28	88.7	0.017	77.9	0.15
Male	72	73.8		64.4	
Age (years)					
<51	48	93.4	0.0006	73.6	0.26
≥51	52	64.2		63.4	
PS					
0, 1	95	79.1	0.148	69.9	0.1
2, 3	5	60		30	
Histology					
WHO non type I	90	81.6	$P<0.0001$	72.1	$P<0.0001$
type I	8	33.3		14.3	
T stage					
T1–3	82	78.2	0.79	71.4	0.014
≥T4	18	77.4		54.5	
N stage					
N0–2	76	84	0.001	76.5	0.001
N3	24	60.3		41.5	
Total treatment duration (day)					
<85	48	69	0.0615	62.3	0.135
≥85	52	85.6		73.8	
OTT (day)					
<69	49	78.2	0.884	72.2	0.36
≥69	51	78.2		64.8	
Dose for primary site (Gy)					
<66	30	76.7	0.712	70	0.7
≥66	70	78.7		67.5	
Dose for metastatic LN (Gy)					
<66	35	77.5	0.683	71.8	0.78
≥66	54	74.8		65.1	

OAS = overall survival, PFS = progression-free survival, PS = performance status, WHO = World Health Organization, OTT = overall treatment time of radiotherapy, LN = lymph node.

detail, 29 patients received reduced dose chemotherapy while 67 patients received the planned dose of three courses. The most common reason for dose reductions was renal dysfunction (47%), followed by severe mucositis (20%). The median total dose of CDDP was 300 mg/m^2 (range, 150–340 mg/m^2), CDGP was 375 mg/m^2 (range, 80–400 mg/m^2), and for 5-FU was 12 000 mg/m^2 (range, 3050–12 000 mg/m^2). In the cohort of patients who received reduced dose chemotherapy, the median total doses of CDDP, CDGP and 5FU were 250 mg/m^2, 330 mg/m^2 and 9400mg/m^2, respectively. Unplanned interruption of RT was experienced in 14 patients (14%), and 2 out of 14 patients required a break in RT over seven days. Severe mucositis (36%) was the most common reason for interruption of RT, followed by infection of the hyperalimentation catheter (29%).

Treatment toxicity

Acute toxicities observed during treatment are listed in Table 5. The most common toxicity was leukopenia. Grade

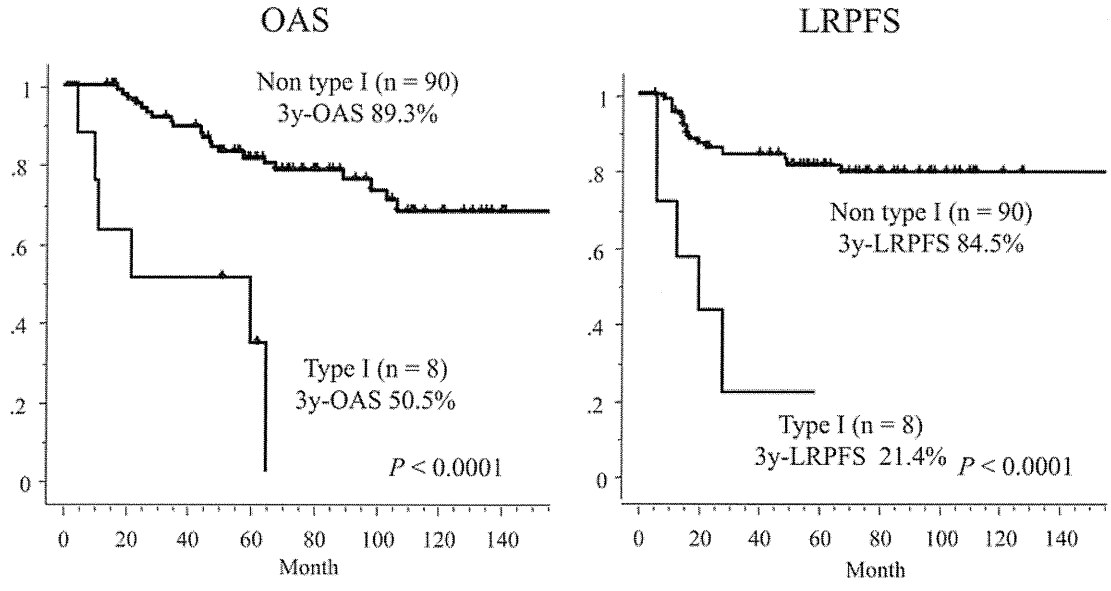

Fig. 3. Overall survival (OAS) and locoregional progression-free survival (LRPFS) curves of groups divided by WHO histopathological types.

Table 3. Multivariate analyses for overall survival and progression-free survival

		OAS		PFS	
Factors	**No.**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**
Gender					
Female	28		0.109		0.5
Male	72	2.76 (0.104–1.257)		1.36 (0.291–1.836)	
Age (years)					
<51	48		0.0018		0.198
≥51	52	4.92 (0.074–0.551)		1.62 (0.294–1.290)	
Histology					
WHO non type I	90		0.0034		0.0004
type I	8	4.62 (0.077–0.603)		5.747 (0.067–0.454)	
T stage					
T1–3	82		0.555		0.023
T4	18	1.36 (0.264–2.047)		2.5 (0.181–0.881)	
N stage					
N0–2	76		0.0076		0.0025
N3	24	3.03 (0.147–0.745)		3.012 (0.163–0.680)	
OTT (day)					
<69	49	1.10 (0.395–2.065)	0.8092		0.605
≥69	51			1.215 (0.393–1.724)	

HR = hazard ratio, CI = confidence intervals, OAS = overall survival, PFS = progression-free survival, WHO = World Health Organization, OTT = overall treatment time of radiotherapy.

3 or higher leukopenia, neutropenia, thrombocytopenia and anemia occurred in 37, 22, 11 and 18 patients, respectively. Grade 3 or higher mucositis and dermatitis developed in 20 and 18 patients, respectively.

Late toxicities are listed in Table 6. Three Grade 3 osteomyelitis of the mandible occurred in this series. One patient died because of late toxicity due to lethal mucosal bleeding. The patient diagnosed as cT3N1M0 with histology of Type I received 80 Gy to the primary site including additional SRT boosts of 10 Gy due to an insufficient response at the planned 70 Gy. The patient developed active mucosal bleeding in the nasopharynx, and died five years later. We experienced no Grade 3 or higher late toxicity of brain necrosis, visual disturbance or swallowing disturbance.

DISCUSSION

A randomized control trial showed survival advantages of concurrent chemoradiotherapy over radiation alone, thus it is believed to be the standard treatment for locally advanced NPC. In the IGS, Stage III–IVB patients with NPC were randomized to CRT or RT, and the combined CRT group was treated with radiation and concurrent triweekly CDDP followed by three adjuvant cycles of FP [1]. The 3-year rate of OAS of the RT-only group was significantly lower than that of the CRT group (46% vs 76%; $P < 0.001$), and the same results were noted for the 3-year rate of PFS (24% vs 69%; $P < 0.001$). However, some problems with the results from the IGS were identified. Firstly, results of the RT arm in the IGS seem to be unacceptably bad because the reported 3-year rates of OAS for the same stages were over 70%. One of the reasons for this discrepancy is that the rate of WHO type I histology in the IGS series (24%) is larger than that of endemic regions, which is believed to have adversely impacted on clinical results. Secondly, the compliance of chemotherapy was insufficient in the IGS. The completion rates of planned chemotherapy of concurrent and adjuvant series were reported as 63% and 55%, respectively. In order to confirm this result, the IGS should be extrapolated in endemic regions [4]. In Hong Kong, the NPC-9901 trial on patients with T1-4N2-3M0 disease was designed to confirm the therapeutic ratio achieved by the IGS regimen. Regarding the compliance of chemotherapy, 65% of patients completed all six cycles, and 79% had five cycles. The CRT arm achieved significantly higher failure-free survival (72% vs 62% at 3 years, $P = 0.027$), mostly as a result of improvements in locoregional control. However, DMFS did not improve significantly (76% vs 73%, $P = 0.47$) and OAS was identical (78% vs 78%, $P = 0.97$). In other RCTs reported by Lin and Chen, the CRT arm significantly improved PFS and OAS [2, 3].

There is also evidence by meta-analysis dealing with eight randomized trials of 1753 patients regarding locally advanced NPC. In this analysis, the pooled hazard ratio of death for adding chemotherapy was 0.82 (95% confidence interval, 0.71–0.94; $P = 0.006$), corresponding to an absolute survival benefit of 6% at 5 years (56% vs 62%). A

Table 4. Compliance of chemotherapy

	n	median (range)
Total cycles given		
1	2	
2	7	
≥3	87	
Total dose given		
Cisplatin (mg/m^2)		300 (150–340)
Nedaplatin (mg/m^2)		375 (80–400)
5-fluorouracil (mg/m^2)		12 000 (3050–12 000)

Table 5. Acute, severe and life-threatening toxicities due to chemoradiotherapy

Toxicity	Gr 0	Gr 1	Gr 2	Gr 3	Gr 4	Gr 5	unknown	≥ Gr 3
Leukopenia	4	12	43	32	5	0	4	37
Granulocytopenia	18	27	28	17	5	0	5	22
Anemia	6	33	39	14	4	0	4	18
Thrombocytopenia	28	37	10	8	3	0	4	11
Liver dysfunction	71	20	5	1	0	0	1	1
Renal dysfunction	71	28	0	0	0	0	1	0
Vomiting	33	14	50	3	0	0	0	3
Mucositis	0	13	67	19	1	0	0	20
Dermatitis	0	37	45	17	1	0	0	18
Salivary gland changes	1	13	86	0	0	0	0	0

Table 6. Late, severe and life-threatening toxicities due to chemoradiotherapy

Toxicity	Gr 0	Gr 1	Gr 2	Gr 3	Gr 4	Gr 5	≥ Gr 3
Swallowing dysfunction	95	4	1	0	0	0	0
Visual dysfunction	99	0	1	0	0	0	0
Hearing impairment	81	5	14	0	0	0	0
Osteomyelitis	96	0	1	3	0	0	3
Brain necrosis	99	1	0	0	0	0	0
Bleeding	99	1	0	0	0	1	1

significant interaction was observed between the timing of chemotherapy and overall survival ($P = 0.005$), with the highest benefit resulting from concomitant chemotherapy [5]. However, increasing acute toxicities caused by administration of chemotherapy were also reported in this analysis. In the IGS, acute toxicities of ≥ Grade 3 were reported as 50% and 76% for RT and CRT arms, respectively. Similarly, in the NPC-9901 trial, toxicities of ≥ Grade 3 were observed as 53% and 84% for RT and CRT arms, respectively ($P < 0.01$). The 3-year actuarial rate of late toxicity was slightly higher in the CRT arm than in that of the RT arm, although it was not significant (28% vs 13%, $P = 0.24$).

In our institute, we adopted alternating CRT for NPC from 1987. In a previous report, 32 patients with NPC received alternating CRT, and the 5-year rates of OAS and PFS were 75% and 63%, respectively. A Phase II study of alternating chemoradiotherapy for patients with NPC was performed in four medical institutions including our institution from 1997 and reported promising results with high compliance (91%), of which the 2-year OAS and PFS rates were 94% and 83%, respectively [10]. In the present study with longer follow-up and a larger cohort, the 5-year rates of OAS and PFS were 78.1% and 68.3%, respectively. We think these data are comparable with previous series. In addition, we believe that acute and late complication rates were sufficiently low according to longer follow-up with 65.9 months.

We believe alternating chemoradiotherapy has several advantages in CRT for NPC. Because the radiation field has to be large, severe mucositis and dermatitis sometimes develops and leads to a treatment break. In addition, late complications, such as disturbances in swallowing or hearing sometimes become significant problems. Alternating chemoradiotherapy has the potential benefit in reducing acute toxicities. As for reported data of the NPC-9901 trial, acute mucositis and skin reactions over Grade 3 were observed in 62% and 20% patients in the CRT arm, respectively. In the present study, acute mucositis or dermatitis of ≥ Grade 3 developed in 20% and 18%, respectively. By alternating chemotherapy and radiotherapy, we could also use intensive multi-agent chemotherapy regimens such as FP or FN without increasing acute and late complications. Although our data is a retrospective analysis in a single institute, the 5-year rate of OAS in the present study (78.1%) was more promising than that of the IGS trial (67%). Regarding the compliance of chemotherapy, over 90% patients in the present study could receive three courses of chemotherapy and 70% of our cohort had completed planned full doses. As a result the total dose of chemotherapy in patients who received a reduced dose was still about 80% of the planned dose. Our data is thought to be more encouraging than that of the IGS, in which only 55% patients completed the planned chemotherapy. Failure patterns in CRT for NPC patients are thought to be both loco-regional, but also in distant sites. In the present study, DMFS at 5-years was 87.8%, which was higher than that of the reported series. The 3-year DMFS rate of the NPC-9901 study was reported as 76%. We believe that it was caused by the advantages of intensive chemotherapy in the present study. An unexpected RT break was needed in 14 patients (14%), of which only 2 patients needed RT breaks longer than one week.

The argument against alternating CRT is that planned RT interruptions may lead to sacrifices in treatment efficacy. In many studies, it is well known that prolongation of overall treatment time negatively influences clinical outcomes. *In vitro*, accelerated repopulation occurred 28 days after the start of RT; thus, prolongation of treatment time led to the development of radiation resistance. In the present study, OTT was not significantly related to clinical outcome. One of the reasons is that the high compliance of the present study would have helped avoid essential prolongation of OTT in our cohort.

In the present series, WHO type I histopathology was a significantly unfavorable factor of both OAS and PFS. The incidence of WHO type I histology in Western countries is very different from East Asian countries. In the IGS series conducted in North America, the rate of WHO type I histology was 22%, which was higher than the rates in studies

conducted in endemic regions. WHO type I histopathology, keratinizing squamous cell carcinoma, was reported to be much less related to EBV infection than non-keratinizing carcinoma. It was also reported to be less sensitive to RT [11]. However, there are not so many reports regarding clinical results. One of the reasons is that the proportion of type I histopathology is very low in endemic regions. In Japan, the proportion of type I histopathology is about 20%, which was similar to North America. Kawashima *et al.* reported a Japanese multi-institutional survey of 333 NPC patients, in which the proportion of type I histopathology was 19% [12]. In that series, type I histopathology proved to be a significantly worse prognostic factor of OAS and PFS on both UVA and MVA. In the present study, the population of type I histopathology was 8%; however, these eight patients had remarkably poor prognosis. Six of the eight patients developed treatment failure. In our series, WHO type I histopathology was a significantly worse factor of both OAS (3-year rates; 50.5% vs 89.3%; $P < 0.0001$) and LRPFS (3-year rates; 21.4% vs 84.5%, $P < 0.0001$). The majority of failure patterns of these patients were in loco-regional sites. In order to improve treatment outcomes of these patients, dose escalation without increasing adverse events is believed to be promising. In recent years, intensity-modulated radiation therapy (IMRT) is widely used for head and neck cancer because of its dose conformity ability for PTV, reducing doses to normal tissue. RTOG 0225, a multi-institutional Phase II trial was conducted to test the feasibility of IMRT with or without chemotherapy for NPC. A 90% LRPF rate was reported as well as an acceptably low incidence of Grade 3 adverse events without xerostomia of Grade 4 [13]. In our institution, we started IMRT for NPC patients using Helical Tomotherapy until June 2006, and we have reported our preliminary clinical results [14]. In the future, dose escalation for patients with type I histopathology using IMRT will be helpful for improving clinical results.

The 5-year rates of PFS and LRPFS of patients with T4 were significantly inferior to those with T1–3, even though there was no significant difference in the 5-year rates of DMFS between these two groups. Because of the proximity of tumors to critical structures such as the brain-stem, spinal cord, optic pathway and temporal lobes, the radiation fields and dose coverages for primary tumors are often compromised. Preliminary results of radiation dose escalation for patients with T3–T4 NPC show good local control (2-year rate of locoregional control; 95.7%) and survival (2-year rate of OAS; 92.1%) [15]. For these patients, dose escalation using IMRT is also promising improved clinical results.

The 5-year rates of OAS and DMFS of patients with N3 were significantly inferior to those with N0–2 in the present series. On the other hand, N3 showed no apparent correlation with worsening LRPF. From this result, patients with N3 are expected to have a higher incidence of distant metastasis. Thus, a more effective regimen of chemotherapy should be considered to overcome limitations. In fact, TAX 324, a randomized Phase III trial, has shown the distinct survival advantages of multi-agent intensive chemotherapy including docetaxel and FP over PF for locally advanced head and neck cancer [16].

We believe that the present results for alternating chemoradiotherapy are promising compared to previously reported series of concurrent chemoradiotherapy. However, several subgroups with some risk factors proved to have insufficient outcomes. In order to refine clinical results without increasing adverse events, there is room for modification especially in patients with high-risk factors. Dose escalation using IMRT for type I histopathology and/or T4 disease and more intensive modifications of chemotherapy for N3 disease should be considered in future.

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Phase II Study of Cetuximab Plus Concomitant Boost Radiotherapy in Japanese Patients with Locally Advanced Squamous Cell Carcinoma of the Head and Neck

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Background: We investigated the tolerability of cetuximab plus radiotherapy in Japanese patients with untreated locally advanced squamous cell carcinoma of the head and neck.
Methods: Patients with epidermal growth factor receptor-expressing locally advanced squamous cell carcinoma of the head and neck received cetuximab (400 mg/m^2 initial dose then 250 mg/m^2 weekly) for 7 weeks plus concomitant boost radiotherapy (weeks 2–7: once daily [1.8 Gy] for 3.6 weeks, then twice daily [1.8 Gy morning and 1.5 Gy afternoon] for 2.4 weeks). The primary endpoint was treatment completion rate (the rate of treated patients completing $\geq$70% of the planned cetuximab dose and the full dose of radiotherapy within 2 weeks over the planned schedule).
Results: Twenty-two patients were evaluable. The treatment completion rate was 100% (95% confidence interval 85–100). The response rate 8 weeks post-radiotherapy was 82% (95% confidence interval 60–95). The most common grade 3/4 treatment-emergent adverse events were mucosal inflammation (73%); dermatitis (27%); and infection, radiation skin injury and stomatitis (23% each).
Conclusions: Cetuximab plus concomitant boost radiotherapy can be safely administered to Japanese patients with locally advanced squamous cell carcinoma of the head and neck. Tolerability and efficacy were in line with those reported in the Phase III Bonner trial in a Western population of patients with locally advanced squamous cell carcinoma of the head and neck.

Key words: cetuximab – concomitant boost – Japanese – locally advanced – radiotherapy – squamous cell carcinoma of the head and neck

INTRODUCTION

Globally, cancers of the lip, oral cavity, pharynx (other than nasopharynx) and larynx account for over 4% of all malignancies, with more than 500 000 new cases worldwide and 300 000 attributed deaths reported in 2008 (1). In Japan alone, in 2007, 7879 people died of head and neck cancer, representing 2.3% of all cancer deaths that year (2).

Patients with locally advanced squamous cell carcinoma (SCC) of the head and neck (LASCCHN) have a number of treatment options available, depending on regulatory authority approvals. These options include concurrently administered chemoradiotherapy with or without surgery and the combination of the EGFR-targeting IgG1 monoclonal antibody cetuximab and radiotherapy (3,4). The use of cetuximab in combination with radiotherapy grew out of the finding that epidermal growth factor receptor (EGFR) is expressed by almost all SCCs of the head and neck (SCCHN) (5,6) and the observation from *in vivo* models that this combination enhanced tumor regression compared with radiation or cetuximab alone (7). Regulatory approval of the combination of cetuximab and radiotherapy in the USA and the EU was based on the results of the large Phase III trial conducted by Bonner et al. in centers in the USA and 14 other countries (8). This trial reported that the addition of cetuximab to once-daily, twice-daily or concomitant boost radiotherapy significantly improved overall survival, progression-free survival and locoregional control compared with radiotherapy alone in patients with LASCCHN. Survival benefits were maintained long term, with 5-year overall survival rates of 46% in the cetuximab plus radiotherapy arm and 36% in the radiotherapy alone arm (9).

It was notable that the addition of cetuximab to radiotherapy in the Bonner trial did not exacerbate the adverse events commonly associated with radiotherapy of the head and neck, including mucositis, xerostomia and dysphagia (8). Among grade $\geq$3 reactions, only acneiform rash and infusion reactions, both with a known association to cetuximab, occurred with a higher incidence in the cetuximab plus radiotherapy arm compared with the radiotherapy arm of the trial.

The Phase II study reported here was initiated to assess the tolerability and feasibility of administering cetuximab together with the concomitant boost radiotherapy regimen used in the Bonner trial to Japanese patients with newly diagnosed LASCCHN. The concomitant boost radiotherapy regimen was chosen because it was the most frequently used in the Bonner trial and the results from our trial would therefore be appropriate for comparison with those from the Bonner trial. Tumor response to treatment was also evaluated in this study.

PATIENTS AND METHODS

Patient Selection

The inclusion criteria used in this study closely followed those used in the Bonner trial to ensure that the patient, disease and treatment characteristics were similar in the two studies. Japanese patients with Stage III or IV (Union for International Cancer Control TNM classification) pathologically proven SCC of the oropharynx, hypopharynx or larynx confirmed by magnetic resonance imaging (MRI) and computed tomography (CT) and with tumor EGFR expression and an expected survival of at least 12 months were eligible for inclusion in the study. Tumor EGFR expression was determined at a single reference laboratory (SRL Medisearch, Inc., Tokyo, Japan) by immunohistochemistry on formalin-fixed or paraffin-embedded tumor tissue using the DAKO pharmDx kit (Glostrup, Denmark). The minimum criterion required to confirm EGFR expression was any intensity of membrane staining above-background level by at least one cell. Other main criteria were: at least bi-dimensionally measurable disease; age $\geq$20 years; Karnofsky performance status (KPS) $\geq$60; adequate bone marrow, kidney and liver function; no distant metastases; no prior chemotherapy within the last 3 years; no prior radiotherapy to the head and neck; and no prior treatment with cetuximab.

The study protocol was approved by institutional review boards and the trial was conducted in accordance with the protocol and with the ethical principles of the Declaration of Helsinki, as well as with the International Conference on Harmonization (ICH) Note for Guidance on Good Clinical Practice (GCP) (ICH Topic E6, 1996), the Japanese ministerial ordinance on GCP, the standard stipulated in Articles 14-3 and 80-2 of the Japanese Pharmaceutical Affairs Law, and applicable regulatory requirements. A quality assurance review of the data was conducted and an independent Radiation Therapy Quality Assurance Committee was set up to ensure compatibility of the type of radiotherapy used at each center with that defined in the protocol. All patients provided written informed consent and were also asked to provide informed consent for investigation of biomarkers other than EGFR in their tumor tissue.

Study Design and Treatment

This was an open-label, Phase II study conducted in patients with newly diagnosed LASCCHN across four centers in Japan. The primary endpoint of the study was tolerability, the main variable of which was treatment completion rate: the rate of patients who completed $\geq$70% of the cetuximab planned dose administration (in terms of relative dose intensity [RDI] of cetuximab) and the full dose of radiotherapy within 2 weeks over the planned schedule of $\leq$8 weeks. The RDI of cetuximab of $\geq$70% was estimated to be equivalent to no more than one missed dose of cetuximab, which, based on calculations on dose intensity data from the Bonner trial, was considered to be the minimum dose level required for cetuximab clinical activity. The selection of an RDI of $\geq$70% as a component of the treatment completion rate was therefore considered to represent tolerability at clinically effective doses. A secondary efficacy endpoint was the best

response 8 weeks after the completion of radiotherapy according to modified World Health Organization criteria as assessed by an independent review committee, the Efficacy and Safety Evaluation Committee (ESEC), and the investigators, using imaging. Tumor response at 8 weeks after completion of radiotherapy was to be confirmed at 12 weeks for the analysis of best tumor response. Determination of tumor *KRAS* mutation status was requested by the Pharmaceuticals and Medical Device Agency, Japan, in order to increase information on the incidence of this type of mutation among Japanese patients with LASCCHN, and response according to tumor *KRAS* mutation status was also assessed. Tumor DNA was screened for the presence of *KRAS* codon 12 and 13 mutations by pyrosequencing at a single laboratory (Biomarker Technologies, Merck Serono RBM, Ivrea, Italy) using a previously validated test (PyroMark KRAS kit; QIAGEN, Hilden, Germany).

All patients received a 7-week course of cetuximab plus concomitant boost radiotherapy. Cetuximab was administered at an initial dose of 400 mg/m^2 (over 120 min), with subsequent weekly doses of 250 mg/m^2 (over 60 min) as an intravenous infusion for 7 weeks of treatment, starting 1 week prior to radiotherapy. Radiotherapy treatment was determined using a 3D treatment planning system. Uninvolved nodal areas of the neck were treated with 54 Gy/30Fr. The primary tumor and gross nodal disease were treated with 72 Gy/42Fr. The irradiation schedule is shown in detail in Fig. 1.

On-study tumor response assessments were performed 8 and 12 weeks after completion of radiotherapy using MRI scanning of the neck and, at week 12, CT of the chest and abdomen. Where progressive disease (PD) was confirmed 8 weeks after completion of radiotherapy, imaging at 12 weeks was not performed. In cases where cetuximab therapy was discontinued before PD was confirmed, radiotherapy was to continue as planned, and assessments including imaging were to be performed at 8 and 12 weeks after completion of radiotherapy.

Treatment-emergent adverse events (TEAEs) (i.e. those events with an onset on or after the first dosing day of treatment and up until 60 days after the last treatment administration) were assessed weekly during treatment and at 4, 8 and 12 weeks after completion of radiotherapy. TEAEs were assessed by National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE, version 3.0) and coded by the Medical Dictionary for Regulatory Activities (MedDRA version, 13.0): composite categories for the special adverse events skin reactions, acne-like rash and infusion-related reactions (IRRs), were based on MedDRA terms.

Statistical Considerations

All statistical analyses were performed on data recorded until the follow-up visit at week 12 after completion of the last radiotherapy dose for the last patient in the intention-to-treat (ITT)/safety population (defined as all patients receiving at least one dose of the study treatment). The clinical cut-off date was 11 June 2010.

Patients completing $\geq$70% of the cetuximab planned dose were those with a cetuximab RDI (from the second infusion onwards) $\geq$70%. Cetuximab RDI was calculated only for patients who received at least two doses of cetuximab. Patients receiving the full dose of radiotherapy within 2 weeks over the planned schedule were those receiving 42 fractions (thirty 1.8 Gy fractions [total dose 54.0 Gy] and twelve 1.5 Gy fractions [total dose 18.0 Gy]), at a total dose of 72.0 Gy and with a duration of exposure to radiotherapy of $\leq$56 days. The completion rate was defined as the proportion of patients who completed the planned cetuximab and radiotherapy schedules relative to the number of subjects in the ITT/safety population.

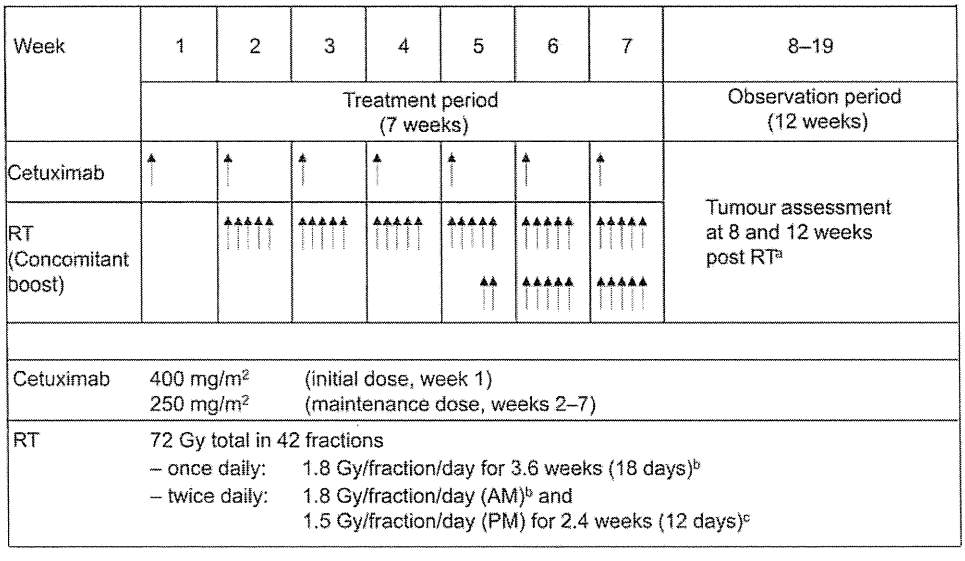

Figure 1. Schedule of irradiation treatment. [a]Imaging at week 12 (i.e. 4 weeks post-RT) was not to be performed for patients with progressive disease at week 8. [b]1.8 Gy/Fr (large field): The primary tumor, gross nodal area and uninvolved nodal area. [c]1.5 Gy/Fr (small field): The primary tumor and gross nodal area. Fr, fraction; Gy, Gray; RT, radiotherapy.

Descriptive statistics were used to summarize the data. A sample size of 20 patients was selected based on a completion rate of 94% reported for concomitant boost radiotherapy in the Bonner trial. The assumption was that at least 80% of the 20 patients would complete treatment, giving two-sided 95% confidence intervals of 68–99%, thereby encompassing the rate in the Bonner trial. The small sample size did not have any power to test statistical hypotheses but was considered to be sufficient for the evaluation of the tolerability (as primary endpoint), safety and efficacy in Japanese patients, in compliance with regulatory requirements. For completion and response rates, two-sided 95% confidence intervals (according to Clopper–Pearson) were calculated. All statistical analyses were performed using SAS® (SAS Institute, Inc., Cary, NC, USA), version 9.1.

RESULTS

Patient Characteristics

Between 6 March 2009 and 4 January 2010, 27 patients were enrolled. Five were ineligible for the study and therefore did not receive protocol-related treatment: due to investigator's decision, withdrawal of consent and interstitial lung disease ($n = 1$ patient each) and protocol-defined radiotherapy unable to be administered (because the required dose was out of the range of that defined by the protocol) ($n = 2$). Thus, 22 patients were enrolled and treated (ITT/safety population). Patient characteristics are summarized in Table 1. Most of the patients (95%) were male and 64% had a KPS of 100. The primary tumor sites were mainly the hypopharynx and larynx (36% each) and 45% of the patients had stage IV disease.

Table 1. Demographics and disease characteristics at baseline: ITT/safety population ($n = 22$)

Characteristic	
Age (years)	
Median	67
Range	(53–81)
Sex, *n* (%)	
Male	21 (95)
Female	1 (5)
Karnofsky performance status, *n* (%)	
100	14 (64)
90	8 (36)
Primary tumor site, *n* (%)	
Hypopharynx	8 (36)
Larynx	8 (36)
Oropharynx	6 (27)
Histology of squamous cell carcinoma, *n* (%)	
Well differentiated	5 (23)
Moderately differentiated	10 (45)
Poorly differentiated	3 (14)
Not known	4 (18)
TNM classification, *n* (%)	
T1–T2	9 (41)
T3–T4	13 (59)
N0	7 (32)
N+	15 (68)
UICC stage, *n* (%)	
Stage III	12 (55)
Stage IV	10 (45)

TNM, tumor node metastasis; UICC, Union for International Cancer Control.

Treatment Completion Rate

The treatment completion rate was 100% (95% CI 85–100) (Table 2). All 22 patients completed ≥70% of the cetuximab RDI and the full radiotherapy dose within 2 weeks over the planned schedule.

Treatment Exposure

One patient discontinued the study due to PD observed at 8 weeks after completion of treatment. The median duration of cetuximab treatment was 8 weeks, the median number of infusions administered was 8 and the median cumulative dose administered was 2169 mg/m^2 (Table 3). All but two patients (91%) received an RDI of ≥90%. The minimum observed cetuximab RDI was 80 to ≤90%. The dose of cetuximab was reduced in one patient, due to a TEAE (grade 3 dry skin). Most of the patients received cetuximab with fewer than 3 days delay in treatment, but two (9%) required cetuximab delays of 3–8 days (infection, $n = 1$; other reason, $n = 1$).

The median duration of radiotherapy was 44 days. All 22 patients received a total dose of 72.0 Gy radiotherapy divided into 42 fractions, i.e. 30 fractions of 1.8 Gy and 12 fractions of 1.5 Gy. The maximum radiotherapy delay which occurred in each patient is categorized as no delay or ≤5 days delay, 6–10 days delay, 11–15 days delay and ≥16 days delay. All patients were able to receive each fraction of radiotherapy with no or ≤5 days delay. In total, all patients completed their scheduled radiotherapy within ≤56 days, in accordance with the protocol-specified full radiotherapy dose criteria (Table 3).

Response Rate

According to the central review by the ESEC, the response rate 8 weeks after completion of radiotherapy was 82%, with a complete response rate of 41% (Table 4). The corresponding results based on the investigator assessment were 86 and 50%, respectively.

Table 2. Completion rate ($n = 22$)

Parameter	Patients, *n* (%)
Completion of ≥70% of cetuximab relative dose intensity	22 (100)
Completion of full dose of radiotherapy with a delay ≤2 weeks	22 (100)
Treatment completion rate [95% CI]	22 (100) [85–100]

CI, confidence interval.

Treatment completion rate and efficacy according to tumor *KRAS* mutation status

All 20 patients who underwent tumor *KRAS* mutation status testing had *KRAS* wild-type tumors. The completion rate among this group was 100% (95% CI 83–100). According to ESEC, 16 patients had a tumor response, giving a response rate of 80% (95% CI 56–94).

Safety

The most common TEAEs (≥50% patients) were mucosal inflammation (86%); dry mouth (77%); constipation, dry skin and dysgeusia (68% each); acne (64%); and dermatitis and pyrexia (50% each). Grade 3/4 TEAEs were reported in 21 (95%) patients. The most common (≥20% of patients) grade 3/4 TEAEs (Table 5) were mucosal inflammation (73%); dermatitis (27%); and infection, radiation skin injury and stomatitis (23% each). In terms of the special adverse events, all 22 patients experienced skin reactions and acne-like rash: three patients (14%) experienced a grade 3 reaction but there were no grade 4 TEAEs in these categories. There was one IRR (blood pressure increase, grade 1). No adverse events led to permanent discontinuation of either cetuximab or radiotherapy. No TEAE leading to death was reported.

DISCUSSION

In this study, we confirmed the feasibility of using a combination of cetuximab and concomitant boost radiotherapy for the treatment of Japanese patients with LASCCHN. The combination of cetuximab and concomitant boost radiotherapy has previously demonstrated efficacy benefits compared with concomitant boost radiotherapy alone in a subgroup of patients in the Phase III Bonner trial in a Western population. The characteristics of patients and their disease at baseline in the study reported here were generally similar to those observed in patients receiving cetuximab plus radiotherapy (once daily, twice daily and concomitant boost) in the Bonner trial, but with a few differences. In the present study, patients were slightly older versus those in the Bonner trial (8) (median age 67 versus 56 years), all had a good performance status (KPS ≥90, 100% versus 70%) and the proportion of patients with oropharynx as the primary tumor site was lower (27% versus 56%) whereas the proportion with primary hypopharyngeal tumors was higher (36% versus 17%). Patients with oropharyngeal tumors appeared to benefit particularly well from cetuximab plus radiotherapy in the Bonner trial (9).

Five patients enrolled to the trial were subsequently considered to be ineligible for protocol-defined treatment, and thus did not receive any study treatment. For two of these patients, the radiotherapy dose calculated to be required for effective treatment was outside the range specified by the

Table 3. Treatment exposure: ITT/safety population ($n = 22$)

Treatment	
Cetuximab	
Duration (weeks)	
Median	8
Range	7–9
Number of infusions	
Median	8
Range	7–9
Cumulative dose (mg/m^2)	
Median	2169
Range	1910–2415
Relative dose intensity,[a] *n* (%)	
≥90%	20 (91)
80 to <90%	2 (9)
Maximum dose delay, *n* (%)	
No delay or <3 days delay	20 (91)
3–8 days	2[b] (9)
Radiotherapy	
Duration[c] (days)	
Median	44
Range	40–52
Number of fractions	
Median	42
Range	42–42
Total dose administered (Gy)	
Median	72
Range	72–72
Maximum delay in each patient, [d] *n* (%)	
No delay or ≤5 days delay	22 (100)

[a]Relative dose intensity calculated only for patients who received at least two doses of cetuximab, with the initial cetuximab dose excluded from the calculation.
[b]One patient due to infection, one due to a reason other than an adverse event.
[c]Duration of radiotherapy exposure is defined as: the date of the last dose of radiotherapy − (date of the first dose of radiotherapy + 1).
[d]The maximum radiotherapy delay in each patient is categorized as follows: no delay or ≤5 days delay; 6–10 days delay; 11–15 days delay, and ≥16 days delay.