during brachytherapy tough it is adjacent to target volume and radioactive sources [8].

The purpose of this study was to retrospectively analyze the incidence of vaginal morbidities after HDR-ISBT for gynecological cancers and to find clinical and dosimetric factors which affect the incidence of the vaginal morbidities.

Methods

The inclusion criteria of this single institutional retrospective study were patients with gynecological malignancies who were treated by HDR-ISBT with or without external beam radiation therapy (EBRT) with a follow-up length exceeding 6 months or more. Patients with distant metastasis outside of pelvis were excluded from current study. HDR-ISBT was applied for both primary and salvage intents. Patients with superficial vaginal disease with thickness less than 5 mm were treated with HDR-ICBT and did not treated by HDR-ISBT; therefore these patients were not included in this analysis. Also HDR-ISBT was not applied for those patients who had distant metastasis or for those patients with far advanced tumors which had not responded to EBRT performed before HDR-ISBT. These patients were treated with EBRT alone. One patient who succumbed to progressive cancer in 5.5 months after ISBT was also excluded in this analysis. The medical records of all patients with gynecological malignancies treated with HDR-ISBT at the National Cancer Center Hospital, Tokyo, Japan, between 2008 and 2011 were retrieved and 44 patients were included in this study.

In the patients without prior pelvic irradiation, pelvic EBRT was delivered before HDR-ISBT. The common EBRT portals were whole pelvic irradiation including gross tumor volume (GTV) with adequate margin as well as the pelvic lymph nodes basin up to the level of the common iliac (L4/5 junction). If the tumor involved the lower third of the vagina, or there were clinically palpable inguinal nodes, inguinal regions were also included in the EBRT portals. The initial 20-40 Gy was delivered to the whole pelvis with a 4-fields box technique and then pelvic irradiation was administered with a central shield being employed to reduce exposure of organs at risk (OAR). The total dose delivered to the pelvic side wall was up to 50 Gy in a conventional fractionation. In patients with a history of prior pelvic radiation therapy or in feeble elderly patients, no EBRT or smaller EBRT fields with a reduced total dose were employed. HDR-ISBT was basically performed after the central shield was inserted. However for those patients treated without EBRT, HDR-ISBT was applied as solitary radiotherapy modality. The detailed procedure of gynecological HDR-ISBT was described elsewhere [9]. In brief, transperineal needle applicator insertion was performed under either general or local anesthesia with the patients in lithotomy position and guided by trans-rectal ultrasound (TRUS) or CT which can be taken with the patients lying in lithotomy position with the applicators in place. For advanced large disease, a Syed-Neblett perineal template (Best Medical International, Inc., Springfield, VA) was used in order to sufficiently cover lateral disease extent. For rather localized small disease, with limited parametrial and/or paracolpial invasion, free-hand needle applicator insertion with or without a vaginal applicator was used with fewer needles inserted compared to the Syed-Neblett perineal template. Treatment planning was performed with brachytherapy planning system (Oncentra® Nucletron, Veenendaal, The Netherlands) using CT images taken by the large bore CT simulator (Aquilion LG®, Toshiba, Tokyo, Japan), which allows imaging of the patients in lithotomy position. Although different applicator was used throughout the patients, the calculation method applied was the same. The clinical target volume (CTV) was defined based on the CT image obtained after needle insertion, as well as physical examination immediately before needle insertion, the intra-operative TRUS image and the most recent MRI were also taken into account. Reference points were set on the surface of CTV and prescribed dose was delivered to those points. HDR-ISRT treatment plan was calculated initially by geometrical optimization or volume optimization and then manual graphical modification was followed to enclose the CTV by the prescription dose while minimizing high dose to OAR. The median HDR-ISBT dose was 24 Gy (range, 18-54 Gy), and median HDR-ISBT dose per fraction was 6 Gy (range, 4-6 Gy). HDR-ISBT was performed twice daily with each fraction 6 hours apart. HDR-ISBT was performed with MicroSelectron HDR (Nucletron, Veenendaal, The Netherlands) using Ir-192.

At the discretion of the attending physician, weekly CDDP 40 mg/m^2 was used in 10 patients concurrently with EBRT. In general, patients with bulky disease, good performance status and adequate organ function were selected for the candidate for the administration of concurrent chemoradiation. Patients were seen in follow up 1 week after HDR-ISBT for a skin check, then every 1-2 months for 2 years, every 3-4 months for 5 years, and every 6-12 months thereafter.

When adding doses of EBRT, HDR-ISBT, and HDR-ICBT, we used the equivalent dose in 2 Gy fractions (EQD_2) according to the LQ model [10,11]. For re-irradiated patients, prior central pelvic EBRT doses were also added to EQD_2 for OARs. For those who had prior HDR-ICBT without DVH parameters of OARs because of lack of three dimensional dose calculations, it was difficult to estimate EQD_2 for OARs. Therefore, prescribed dose for tumor in EQD_2 ($\alpha/\beta = 10$) was converted to EQD_2 for late responding tissue ($\alpha/\beta = 3$) and added

together. Time interval between prior RT and the current RT was not taken into consideration in this analysis.

Rectum and bladder were contoured as a whole organ. Vaginal wall was extracted with a thickness of 4 mm on all CT images according to the Vienna group [12]. As for rectum and bladder, dosimetric parameter of D_{2cc} was used because these values have been validated by several studies [6-8]. On the other hand, there is no validated parameter for vaginal dose; therefore $D_{0.5cc}$, D_{1cc}, D_{4cc}, D_{6cc}, and D_{8cc} were calculated along with D_{2cc} for vaginal wall dose volume parameters.

Late vaginal morbidities were retrospectively evaluated according to LENT-SOMA scales [13]. Because morbidity scores were evaluated retrospectively in this study, we focused on only vaginal ulcer which could be regarded as one of the severest symptoms and could be retrieved accurately from medical records.

Student's unpaired t-test was used to compare the continuous variables and Pearson's chi-square test to compare categorical variables. A p value of < 0.05 was considered as statistically significant. In addition, calculation of the area under the curve (AUC) of receiver operating characteristics (ROC) was used to determine the most predictive dosimetric parameter of vaginal ulcer. The predictive values of parameters were evaluated based on the AUC. The optimal threshold for each parameter was defined as the point yielding the minimal value for $(1 - \text{sensitivity})^2 + (1 - \text{specificity})^2$, which is the point on the ROC curve closest to the upper left-hand corner [14]. The obtained cutoff point was used for dividing patients into two groups and the incidences of vaginal ulcer were calculated by Kaplan-Meier method with the difference evaluated by log-lank test. All statistical analyses were performed using SPSS Statistics version 18.0 (SAS Institute, Tokyo, Japan).

This retrospective study was approved by the institutional review board of the National Cancer Center.

Results

There were 44 patients who met the eligibility criteria and 36 patients were alive at the time of the analysis (May 2012). The median follow-up length of living patients was 18.3 months (range, 7.6-39.5 months). The pretreatment characteristics of the 44 patients included in this study are summarized in Table 1. Median age was 56 years (range, 25-89 years). HDR-ISBT was applied as the primary therapy in 14 patients (31.8%) and as the salvage therapy in 30 patients (68.2%). Eight patients (18.2%) had previously received pelvic irradiation, in the form of EBRT and/or ICBT. Twenty four patients were treated with Syed-Neblett perineal template, 17 with free-hand with vaginal applicator and three with free-hand without vaginal applicator. Treatment details are summarized in Table 2. Ten patients underwent concurrent chemotherapy. In most cases HDR-ISBT dose per fraction was 6 Gy. Median total EQD_2 of CTV D_{90} was 67.7 Gy. Median EQD_2 of D_{2cc} for rectum and bladder was 60.8 Gy and 58.1 Gy, respectively. Median EQD_2 of $D_{0.5cc}$, D_{1cc}, D_{2cc}, D_{4cc}, D_{6cc}, and D_{8cc} for vaginal wall were 210.7 Gy, 167.3 Gy, 131.5 Gy, 111.6 Gy, 100.0 Gy, and 83.2 Gy, respectively. Table 3 shows EQD_2 of rectum, bladder and vaginal wall for the patients with or without prior pelvic radiation therapy. For re-irradiation patients, median EQD_2 of D_{2cc} for rectum and bladder, $D_{0.5cc}$, D_{1cc}, D_{2cc}, D_{4cc}, D_{6cc} and D_{8cc} for vaginal wall was 91.1 Gy, 100.9 Gy, 260.3 Gy, 212.3 Gy, 170.1 Gy, 117.1 Gy, 105.2 Gy, and 94.7 Gy, respectively. For those without prior radiation therapy, median EQD_2 of D_{2cc} for rectum and bladder, $D_{0.5cc}$, D_{1cc}, D_{2cc}, D_{4cc}, D_{6cc} and D_{8cc} for vaginal wall was 56.3 Gy, 54.3 Gy, 147.4 Gy, 126.2 Gy, 108.0 Gy, 103.5 Gy, 94.7 Gy, and 80.7 Gy, respectively (Table 3). In EQD_2 of D_{2cc} for rectum, bladder and vaginal wall the difference was statistically significant ($p < 0.001$, $p < 0.001$, and $p = 0.001$, respectively).

As for late morbidities of vagina, five patients experienced vaginal ulcer after HDR-ISBT. All of vaginal ulcer occurred within two years after completion of the HDR-ISBT. Patient characteristics and objective/management

Table 1 Patients characteristics (n = 44)

		Patients (n)
Median age (years, range)		56 (25-89)
Primary site	Cervix	24 (54.6%)
	Vagina	12 (27.3%)
	Corpus	5 (11.3%)
	Ovary	2 (4.5%)
	Vulva	1 (2.3%)
Primary therapy		14 (31.8%)
	Cervical cancer	4 (9.1%)
	Vaginal cancer	10 (22.7%)
Salvage therapy		30 (68.2%)
	Post ope regidual tumor	5 (11.4%)
	Post ope recurrent tumor	21 (47.7%)
	Post RT recurrent tumor	4 (9.1%)
Histology	Scc	25 (56.8%)
	Adeno	16 (36.4%)
	Others	3 (6.8%)
Prior pelvic RT*	Yes	8 (18.2%)
	No	36 (81.8%)
Median tumor size (cm, range)		3.6 (1.0-8.0)
Pelvic LN† metastais	Yes	11 (25%)
	No	33 (75%)

*RT radiation therapy.
†LN lymph node.

Table 2 Treatment details (n = 44)

	Median range
Central pelvic dose of EBRT* (Gy)	30 (0-50)
No. of needles used in HDR-ISBT†	15 (5-29)
HDR-ISBT† fractions	4 (3-9)
HDR-ISBT† dose per fraction (Gy)	6 (4-6)
CTV†† (ml)	35.1 (2.4-142.1)
CTV†† D_{90} in EQD_2‖ (Gy)	67.7 (48.8-94.2)
Rectum D_{2cc}¶ in EQD_2‖ (Gy)	60.8 (30.5-114.3)
Bladder D_{2cc}¶ in EQD_2‖ (Gy)	58.1 (7.3-120.3)
Vaginal wall $D_{0.5cc}$¶ in EQD_2‖ (Gy)	210.7 (51.5-468.1)
Vaginal wall D_{1cc}¶ in EQD_2‖ (Gy)	167.3 (49.9-352.1)
Vaginal wall D_{2cc}¶ in EQD_2‖ (Gy)	131.5 (43.7-294.4)
Vaginal wall D_{4cc}¶ in EQD_2‖ (Gy)	111.6 (34.0-200.8)
Vaginal wall D_{6cc}¶ in EQD_2‖ (Gy)	100.0 (20.4-173.7)
Vaginal wall D_{8cc}¶ in EQD_2‖ (Gy)	83.2 (10.3-144.4)
Concurrent chemotherapy	
Yes	10 patients
No	34 patients

*EBRT: external beam radiation therapy.
†HDR-ISBT: high-dose-rate interstitial brachytherapy.
††CTV: clinical target volume.
‖EQD2: equivalent dose in 2 Gy fractions.
¶D0.5cc, D1cc, D2cc, D4cc, D6cc, D8cc: most exposed 0.5, 1, 2, 4, 6 and 8 cm3 of tissue.

Table 3 DVH parameters for bladder and vaginal wall with or withour prior radiation therapy

	Prior pelvic RT[J] (+) (n = 8)	Prior pelvic RT[J] (-) (n = 36)	*p* value
eMedian rectum D_{2cc}† (EQD_2*, Gy, range)	91.1 (71.0-114.3)	56.3 (30.5-82.7)	< 0.001*
Median bladder D_{2cc}† (EQD2*, Gy, range)	100.9 (69.7-120.3)	54.3 (7.3-82.7)	< 0.001*
Median vaginal wall $D_{0.5cc}$† (EQD2*, Gy, range)	260.3 (59.9-349.3)	147.4 (47.9-267.3)	0.109
Median vaginal wall D_{1cc}† (EQD2*, Gy, range)	212.3 (58.2-277.5)	126.2(33.6-182.7)	0.013
Median vaginal wall D_{2cc}† (EQD2*, Gy, range)	170.1 (56.6-247.5)	108.0 (31.7-150.9)	0.001*
Median vaginal wall D_{4cc}† (EQD2*, Gy, range)	117.1 (34.0-200.8)	103.5 (39.1-139.4)	0.139
Median vaginal wall D_{6cc}† (EQD2*, Gy, range)	105.2 (33.0-173.7)	94.7 (20.4-138.7)	0.097
Median vaginal wall D_{8cc}† (EQD2*, Gy, range)	94.7 (32.4-144.4)	80.7 (10.3-130.4)	0.105

[J]RT: radiation therapy.
*EQD2: equivalent dose in 2 Gy fractions.
†D0.5cc, D1cc, D2cc, D4cc, D6cc, D8cc: most exposed 0.5, 1, 2, 4, 6, and 8 cm3 of tissue.

scores of vaginal ulcer according to LENT-SOMA are summarized in Table 4. Two patients had superficial and > 1 cm^2 vaginal ulcer and three had vaginal fistula (two vesicovaginal fistulae and one vesicovaginorectal fistula). Three out of the five patients had prior pelvic irradiation and the interval between prior pelvic irradiation and secondary pelvic irradiation was 15, 27, and 40 months, respectively. All of the three patients with vaginal fistula received hyperbaric oxygen therapy without success. Two underwent surgical intervention (one total cystectomy for massive hematuria and one nephrostomy) for their vesicovaginal fistula, while one was followed up conservatively with a persistent vesicovaginal fistula. The other two patients with grade 2 vaginal ulcer were treated conservatively. The overall 2-year actuarial incidence of vaginal ulcer was 11.4%; 37.5% for re-irradiation patients and 5.6% for those without prior radiation therapy (Figure 1a). Comparison of dose-volume parameters of the vaginal wall is shown in Table 5 for the patient with and without vaginal ulcer. It was shown that the incidence of vaginal ulcer in the patients with prior pelvic irradiation was statistically higher than that of the patients without prior pelvic irradiation ($p = 0.035$). It was also shown that the mean EQD_2 of vaginal wall D_{2cc} of patients with or without vaginal ulcer was statistically different ($p = 0.025$). There was no relationship between administration of concurrent chemotherapy and manifestation of vaginal ulcer ($p = 0.256$), number of needles used in HDR-ISBT ($p = 0.293$) nor bladder D_{2cc} EQD_2 ($p = 0.091$). The ROC analysis revealed that vaginal wall D_{2cc} was the best dosimetric parameter predicting the incidence of vaginal ulcer and the cutoff value of 145 Gy in vaginal wall D_{2cc} provided the lowest p value in log-rank test (Table 6). Figure 1b shows Kaplan-Meyer curve for the incidence of vaginal ulcer stratified by vaginal wall D_{2cc} 145 Gy in EQD_2. The 2-year incidence rates of vaginal ulcer in the patients with vaginal wall D_{2cc} equal to or less than 145 Gy in EQD_2 and over 145 Gy were 3.7% and 23.5%, respectively, with a statistically significant difference ($p = 0.026$).

Discussion

Although the Manchester method of ICBT for the cervical cancer was developed to avoid the occurrence of radiation induced vaginal ulcer and necrosis, vaginal ulcer is now very rarely encountered because vaginal wall is relatively radioresistant and typical ICBT delivers radiation dose less than the tolerance of the relatively radioresistant vaginal wall. In a retrospective study of cervical cancer patients using EBRT and the film based low-dose rate (LDR) brachytherapy, Samuel et al. showed that vaginal tolerance dose was above 150 Gy [15]. In recent advancement of image guided brachytherapy (IGBT), rectum and bladder doses were recommended to be

Table 4 Patient characteristics who developed vaginal ulcer

Patient no.	Age at HDR-ISBT*	Primary site	Prior pelvic RT	Interval between prior RT and HDR-ISBT* (mo)	HDR-ISBT* with/without EBRT††	Total vaginal wall $D_{0.5cc}$#/D_{1cc}#/D_{2cc}# in EQD_2## (Gy)	LENT SOMA¶ objective score	LENT SOMA¶ management score
1	40	Cervix	WPRT† 45 Gy/25fr + EBRT†† boost 15 Gy/5fr	27	HDR-ISBT* 36 Gy/9fr	272.1/202.6/169.1	4	4
2	51	Cervix	None	None	WPRT† 30 Gy/15fr + CS‖ 20 Gy/10fr + HDR-ISBT* 24 Gy/4fr	215.2/171.8/145.4	4	3
3	64	Corpus	None	None	WPRT† 30 Gy/15fr + HDR-ISBT* 30 Gy/5fr	196.6/141.5/109.1	2	1
4	64	Cervix	WPRT† 40 Gy/20fr + CS 10 Gy/5 + HDR-ICBT 18 Gy/3fr	40	HDR-ISBT* 48 Gy/8fr	465.4/352.1/294.4	2	1
5	67	Cervix	WPRT† 50 Gy/50fr + HDR-ICBT 12 Gy/3fr	15	HDR-ISBT* 42 Gy/7fr	234.0/211.1/193.5	4	3

*HDR-ISBT: high-dose-rate interstitial brachytherapy.
†WPRT: whole pelvis radiation therapy.
††EBRT: external beam radiation therapy.
‖CS: radiation therapy with center shielding.
¶LENT-SOMA: Late Effects of Normal Tissues - Subjective, Objective, Management, Analytic.
#D0.5cc, D1cc, D2cc: most exposed 0.5, 1 and 2 cm3 of tissue.
##EQD2: equivalent dose in 2 Gy fractions.

reported in the treatment of ICBT for cervical cancer but vagina was not mentioned as OAR [9]. In the GEC-ESTRO working group (II) or American Brachytherapy Society guidelines, vagina was taken into consideration for OAR but it was stated that the vaginal dose volume parameters still need to be defined [16,17]. Dimopoulos et al. reported clinical result of primary vaginal cancer treated with IGBT and they experienced two vaginal fistulae and one periurethral necrosis. However they did not specify DVH parameters of vaginal wall with vaginal complication [18]. Lee et al. reported in detail the toxicity analysis of CT based HDR-ISBT for gynecologic malignancies. They reported that D_{2cc} for the rectum was a reliable predictor of late rectal complication; however because of limited number of events it was not able to explore the DHV parameters for vaginal complication [5]. Recently, Vienna group tried to find out DVH parameters that correlate with vaginal late morbidities but vaginal D_{2cc} did not relate with the vaginal morbidities [12]. The calculation method was the same as

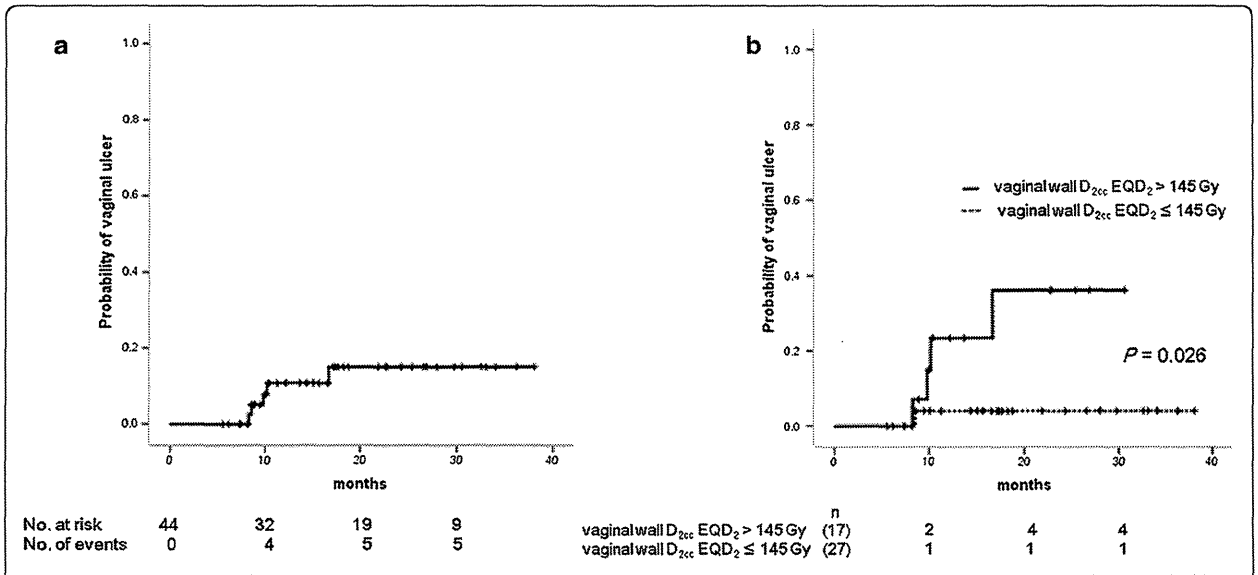

Figure 1 Cumulative incidene of vaginal ulcer. a: Cumulative incidence of vaginal ulcer. **b**: Cumulative incidence of vaginal ulcer stratified by vaginal wall D_{2cc} 145 Gy in EQD_2.

Table 5 Clinical predictors of vaginal ulcer

Characteristic		Vaginal ulcer (+) (n = 5)	Vaginal ulcer (-) (n = 39)	p value
Prior pelvic RT*		3	5	
	Yes	2	34	0.035*
	No			
Concurrent chemotherapy				
	Yes	0	10	0.256
	No	5	29	
Median number of needles used in HDR-ISBT[ʃ] (range)		14 (10-24)	15 (5-29)	0.293
Median CTV[†] (ml, range)		54.7 (17.7-114.0)	34.7 (2.4-142.1)	0.271
Median rectum D_{2cc}[††] (EQD_2[‖], Gy, range)		84.2 (34.0-100.7)	57.9 (30.5-114.3)	0.118
Median bladder D_{2cc}[††] (EQD_2[‖], Gy, range)		69.3 (37.4-113.5)	57.7 (7.3-120.3)	0.091
Median vaginal wall $D_{0.5cc}$[††] (EQD_2[‖], Gy, range)		206.4 (106.6-349.3)	149.4 (47.9-310.1)	0.243
Median vaginal wall D_{1cc}[††] (EQD_2[‖], Gy, range)		169.1 (91.6-277.5)	127.9 (33.6-220.8)	0.096
Median vaginal wall D_{2cc}[††] (EQD_2[‖], Gy, range)		152.5 (71.1-247.5)	109.0 (31.7-201.9)	0.025*
Median vaginal wall D_{4cc}[††] (EQD_2[‖], Gy, range)		115.5 (83.8-200.8)	110.6 (34.0-153.2)	0.152
Median vaginal wall D_{6cc}[††] (EQD_2[‖], Gy, range)		102.5 (60.4-173.7)	99.5 (20.4-146.3)	0.266
Median vaginal wall D_{8cc}[††] (EQD_2[‖], Gy, range)		82.0 (47.6-144.4)	84.3 (10.3-140.3)	0.511

*RT: radiation therapy.
[ʃ]HDR-ISBT.
[†]CTV: clinical target volume.
[‖]EQD2: equivalent dose in 2 Gy fractions.
[††]D0.5cc, D1cc, D2cc, D4cc, D6cc, D8cc: most exposed 0.5, 1, 2, 4, 6, and 8 cm3 of tissue.

the current study, which was composed of EBRT and ICBT/ISBT and normalized to 2 Gy per fraction (EQD_2) using the linear-quadratic model with α/β of 3 Gy for the vaginal morbidities [10-12,16]. The difference between Vienna group and the current study was that there were more patients with severe vaginal morbidities in the current study, presumably because there were more patients who received re-irradiation and current study excluded the patients treated with HDR-ICBT. HDR-ISBT delivers higher dose to the vaginal wall than HDR-ICBT because the multiple needle applicators directly contact vaginal wall. According to the current results, after vaginal wall $D_{0.5cc}$, D_{1cc}, D_{2cc}, D_{4cc}, D_{6cc}, and D_{8cc} having been compared, vaginal wall D_{2cc} was found to be the most relevant DVH parameter predicting the incidence of vaginal ulcer. ROC analysis also showed that vaginal wall D_{2cc} of 145 Gy in EQD_2 can be used as clinical cutoff dose predicting vaginal ulcer. This figure is quite similar to the vaginal tolerance dose of 150 Gy derived from a retrospective study of LDR brachytherapy which was previously mentioned [15]. The current report is the first one concerning about vaginal DVH parameter and complication using modern era of three-dimensional image-guided brachytherapy. It was also found in this study that the history of prior pelvic irradiation was another significant predictive factor for vaginal ulcer (Table 5). Lee et al. reported a patient with colovaginal fistula with previous EBRT [5,7]. As shown in Table 3,

Table 6 Dosimetric predictors for the development of vaginal ulcer

Parameter	ROC[†] AUC*	Cutoff[¶]	2-y incidence ofvaginal ulcer (%)	P value[#]
Vaginal wall $D_{0.5cc}$[‖] (EQD_2[††])	0.667	≤195 Gy	0.0	0.058
		>195 Gy	18.5	
Vaginal wall D_{1cc}[‖] (EQD_2[††])	0.682	≤171 Gy	4.2	0.091
		>171 Gy	20.0	
Vaginal wall D_{2cc}[‖] ($EQD2$[††])	0.733	≤145 Gy	3.7	0.026*
		>145 Gy	23.5	
Vaginal wall D_{4cc}[‖] (EQD_2[††])	0.618	≤83 Gy	0.0	0.119
		>83 Gy	15.6	
Vaginal wall D_{6cc}[‖] (EQD_2[††])	0.569	≤86 Gy	5.6	0.323
		>86 Gy	15.4	
Vaginal wall D_{8cc}[‖] (EQD_2[††])	0.559	≤75 Gy	5.6	0.323
		>75 Gy	15.4	

*AUC: area under the curve.
[†]ROC: receiver operator characteristic.
[††]EQD2: equivalent dose in 2 Gy fractions.
[‖]D0.5cc, D1cc, D2cc, D4cc, D6cc, D8cc: most exposed 0.5, 1, 2, 4, 6, and 8 cm3 of tissue.
[¶]Cutoff refers to the most predictive value from the AUC of ROC curve.
[#] Univariate analysis by log-rank test.

both rectum and bladder D_{2cc} was significantly higher in patients with prior pelvic irradiation than those without prior pelvic irradiation. However both rectum and bladder D_{2cc} was not in itself a significant prognostic factor for vaginal ulcer and could not be used as a surrogate indicator (Table 5).

There were several limitations in this study. Contouring of the vagina was not based on MRI but CT, which is inferior to MRI in tissue contrast. However because 41 out of 44 patients were inserted either cylinder or mold into their vagina, contouring of vagina was considered to be precise. The time interval between the prior pelvic RT and HDR-ISBT was not taken into consideration for the calculation of the total dose for OARs. Additionally, this study was a retrospective study with small number of patients with heterogeneous tumor origin, heterogeneous treatment applied, small number of events, and with short follow-up period. Therefore we should be cautious about the results of the current study. However even tumor origin differed greatly in current cohorts of study, it is considered to be feasible because the main concern in current study was focused on only the vaginal toxicity.

It should be stressed that with the introduction of HDR-ISBT in gynecological malignancies and increment of vaginal dose, vaginal tolerance dose must be taken into consideration. Further discussion and validation of vaginal DVH parameters in image-guided brachytherapy in a multicenter prospective study is needed.

Conclusions

The DVH parameters for vagina are essential for treatment planning and optimization in image based HDR-ISBT in gynecological malignancies. Vaginal wall D_{2cc} in EQD_2 should be monitored and be kept under 145 Gy in order to avoid vaginal ulcer. Also in patients with prior pelvic irradiation, vaginal wall dose including the prior radiation dose should be kept lower than 145 Gy.

Consent

Written informed consent was obtained from the patient for the publication of this report and any accompanying images.

Abbreviations

HDR-ISBT: High-dose rate interstitial brachytherapy; EQD_2: Dose in equivalent in 2 Gy fractions; ICBT: Intracavitary brachytherapy; ISBT: Interstitial brachytherapy; DVH: Dose volume histogram; EBRT: External beam radiation therapy; GTV: Gross tumor volume; CTV: Clinical target volume; OAR: Organ at risk; AUC: Area under the curve; ROC: Receiver operating characteristics; IGBT: Image guided brachytherapy; PDR: Pulsed dose rate.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

TK, MS, RY, KH, MK, SS, KT, KY, KI, MM, and YI performed the treatment. NM and JI analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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Lobular Endocervical Glandular Hyperplasia Is a Neoplastic Entity With Frequent Activating *GNAS* Mutations

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Abstract: To clarify the significance of *GNAS* mutations in cervical tumorigenesis, we performed mutational analyses in a total of 154 lesions and in 22 normal tissues of the uterine cervix. Activating *GNAS* mutations were found in 8 of the 19 lobular endocervical glandular hyperplasias (LEGH; 42%) and 4 of the 79 endocervical-type mucinous adenocarcinomas (5%) but were never seen in the normal endocervical tissue, minimal deviation adenocarcinomas, endometrioid adenocarcinomas, or squamous cell carcinomas. We further examined the presence of human papillomavirus (HPV) DNA and p16 expression to probe the relationship between *GNAS* mutations and HPV infection in LEGHs and carcinomas. All the *GNAS*-mutated LEGHs were negative for HPV DNA and p16 expression, whereas all the *GNAS*-mutated adenocarcinomas were positive for HPV DNA and/or p16 expression, implicating *GNAS* mutations in the development of LEGH and a minor subset of HPV-related cervical adenocarcinomas. Additional mutational analyses of LEGH identified *KRAS* and *STK11* mutations in 1 and 2 cases, respectively. The *GNAS*, *KRAS*, and *STK11* mutations were mutually exclusive; thus, a total of 11 LEGHs (58%) had 1 of these genetic alterations. Although LEGH has been regarded as a metaplastic lesion, the frequent presence of genetic alterations suggests a neoplastic nature.

Key Words: lobular endocervical glandular hyperplasia, uterine cervix, *GNAS*, *KRAS*, *STK11*

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The incidence of cervical carcinoma has been declining, but it still remains to be one of the most common cancers in women.[1,2] Human papillomavirus (HPV) plays a major role in carcinogenesis in the uterine cervix.[1] Previous studies have shown that HPV is involved in virtually all squamous cell carcinomas and the majority of adenocarcinomas of the uterine cervix.[1,3] Because of the common involvement of HPV in cervical carcinogenesis, the detection of HPV DNA and the overexpression of p16, which is a consequence of the inactivation of Rb by HPV E7 protein, are used as markers for cervical neoplasia.[4,5]

In contrast, a subset of adenocarcinomas, including minimal deviation adenocarcinoma (MDA) and some endocervical-type mucinous adenocarcinomas, are not associated with HPV infection.[6,7] Although the histogenesis and molecular basis for the HPV-negative cervical cancers largely remains to be clarified, previous studies have suggested that lobular endocervical glandular hyperplasia (LEGH) is a potential precursor of HPV-negative cervical adenocarcinomas.[8–10] LEGH consists of lobular proliferation of cervical glands with gastric-type mucin expression and is regarded as a hyperplastic/metaplastic lesion; however, it is sometimes difficult to distinguish from adenocarcinomas, particularly MDA, in clinical situations.[11,12]

GNAS encodes the α-subunit of the stimulatory guanine nucleotide–binding protein (Gsα), which transduces signals from a 7-transmembrane receptor by stimulating adenylyl cyclase, leading to elevated intracellular cAMP levels.[13] Previous studies have shown that activating *GNAS* mutations result in the constitutive activation of adenylyl cyclase and the elevation of cAMP levels regardless of the presence or absence of receptor agonists.[13,14] Oncogenic *GNAS* mutations were first reported in 1989 in pituitary adenomas, but the mutations had long been identified in very limited types of lesions, including some endocrine tumors and fibrous dysplasia.[13–16] However, other authors and our group have recently identified the frequent presence of activating *GNAS* mutations in several glandular neoplasms of digestive organs.[17–21] In the current study, we examined the mutational status of *GNAS* in a variety of cervical lesions, including LEGH, different subtypes of adenocarcinoma, and squamous cell carcinoma.

MATERIALS AND METHODS

Tissue Samples

This study was approved by the Ethics Committee of the National Cancer Center, Tokyo, Japan. The present study involved 22 normal endocervical tissues and 154 lesions of the uterine cervix, including 19 LEGHs, 3 MDAs, 79 endocervical-type mucinous adenocarcinomas, 10 endometrioid adenocarcinomas, and 43 squamous cell carcinomas. No intestinal-type, signet ring cell, or villoglandular-type mucinous adenocarcinoma was identified in our case files. Cases related to Peutz-Jeghers Syndrome (PJS) were not included. Normal endocervical tissue samples were obtained from surgical specimens of endometrial or ovarian cancers. All the tissue samples were obtained by surgical resection at the National Cancer Center Hospital, Tokyo, Japan. All the specimens were routinely fixed in 10% formalin, embedded in paraffin, and subjected to hematoxylin-eosin staining. LEGH, MDAs, and endocervical-type mucinous adenocarcinomas were also stained with Alcian blue/periodic-acid Schiff (PAS). The histologic classification of MDA, adenocarcinomas, and squamous cell carcinomas was made on the basis of the current World Health Organization classification.[1] LEGH was diagnosed on the basis of previous reports using the following criteria: small to medium-sized endocervical glands with a lobular architecture, pale eosinophilic cytoplasm with abundant PAS-positive mucin, no or minimal cellular atypia, and no evidence of stromal invasion.[11,22,23]

Mutational Analysis and Detection of HPV DNA

Sections of the tumor specimens of 10 μm thickness were deparaffinized and stained briefly with hematoxylin and then subjected to DNA extraction. The lesions and normal tissues were separately microdissected using sterilized toothpicks under a microscope. The dissected samples were incubated in 50 μL of DNA extraction buffer (50 mM Tris-HCl, pH 8.0, 1 mM ethylenediaminetetraacetic acid, 0.5% [vol/vol] Tween 20, 200 μg/mL proteinase K) at 50°C overnight. Next, the samples were heated at 100°C for 10 minutes to inactivate proteinase K and then directly subjected to a polymerase chain reaction (PCR) using pairs of primers encompassing exons 8 and 9 of *GNAS* (Supplemental table, Supplemental Digital Content 1, http://links.lww.com/PAS/A185).[20] When the amplification of exon 8 of *GNAS* was unsuccessful, additional PCR reactions using another pair of primers were also performed. LEGH specimens were also analyzed for mutations in exons 1 to 9 of *STK11* and exon 2 of *KRAS*.[19,20]

To test the presence of HPV DNA, we performed PCR using general HPV primers targeting the L1 region of HPV (GP5+/6+), as described previously.[24] Cases negative for this analysis were further examined using HPV genotype–specific PCRs targeting the E7 regions of HPV16, 18, 33, 52, and 58.[3] Samples with unsuccessful PCR results using the first primer set for *GNAS* exon 8, which amplifies 189 bp products, were considered to have low-quality DNA and were excluded from the analysis for HPV to avoid false-negative results.

The PCR products were electrophoresed in a 2% (wt/vol) agarose gel and were recovered using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). Isolated PCR products were sequenced using an Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems Inc., Foster, CA). The sequencing results for the products obtained by PCR using GP5+/6+ were analyzed using BLAST (http://blast.ncbi.nlm.nih.gov) to identify specific HPV genotypes.

Immunohistochemistry

Deparaffinized 4-μm-thick sections from each paraffin block were exposed to 0.3% hydrogen peroxide for 15 minutes to block endogenous peroxidase activity. Antigen retrieval was performed by autoclaving in 10 mM citrate buffer (pH6.0) for 10 minutes. Anti-p16 antibody (G175-405, 1:10 dilution; BD Biosciences, Franklin Lakes, NJ) was used as the primary antibody. For staining, we used an automated stainer (Dako, Glostrup, Denmark) according to the vender's protocol. ChemMate EnVision (Dako) methods were used for detection. Cases exhibiting diffuse staining (>50% of areas) with moderate or strong intensities were regarded as p16 positive.

The correlation between HPV DNA and p16 expression was analyzed by the Fisher exact test. A P value < 0.05 was considered statistically significant.

RESULTS

Clinicopathologic Findings

The average ages of the patients with LEGH, adenocarcinomas, and squamous cell carcinomas were 51 (range, 37 to 65), 48 (range, 30 to 70), and 45 (range, 30 to 64) years, respectively (Table 2, Fig. 1). Alcian blue/PAS staining showed that all the LEGHs and MDAs were positive for neutral mucin. Among the 79 endocervical-type mucinous adenocarcinomas, 11 (14%) and 14 (18%) cases had neutral and acidic mucin expressions, respectively. The remaining 54 cases (68%) showed mixed acidic and neutral mucin expressions.

Mutational Analysis for *GNAS*

GNAS mutations were identified in 8 of the 19 LEGHs (42%) and 4 of the 79 endocervical-type mucinous adenocarcinomas (5%; Table 1). No *GNAS* mutations were found in normal endocervical tissues, MDAs, endometrioid adenocarcinomas, or squamous cell carcinomas (Tables 1 and 2, Fig. 2). All the mutations were heterozygous missense mutations affecting codon 201. None of the normal tissue samples exhibited *GNAS* mutations, confirming the somatic nature of the mutations.

Detection of HPV DNA

We examined the presence of HPV DNA to probe the potential relationship between *GNAS* mutation and HPV infection. Fifty-six endocervical-type mucinous adenocarcinomas (71%), 6 endometrioid adenocarcinomas (60%), and 32 squamous cell carcinomas (74%) were positive for HPV DNA in an analysis using universal HPV primers (GP5+/6+) (Tables 1 and 2). Sequencing of the PCR products identified the HPV genotypes 16, 18,

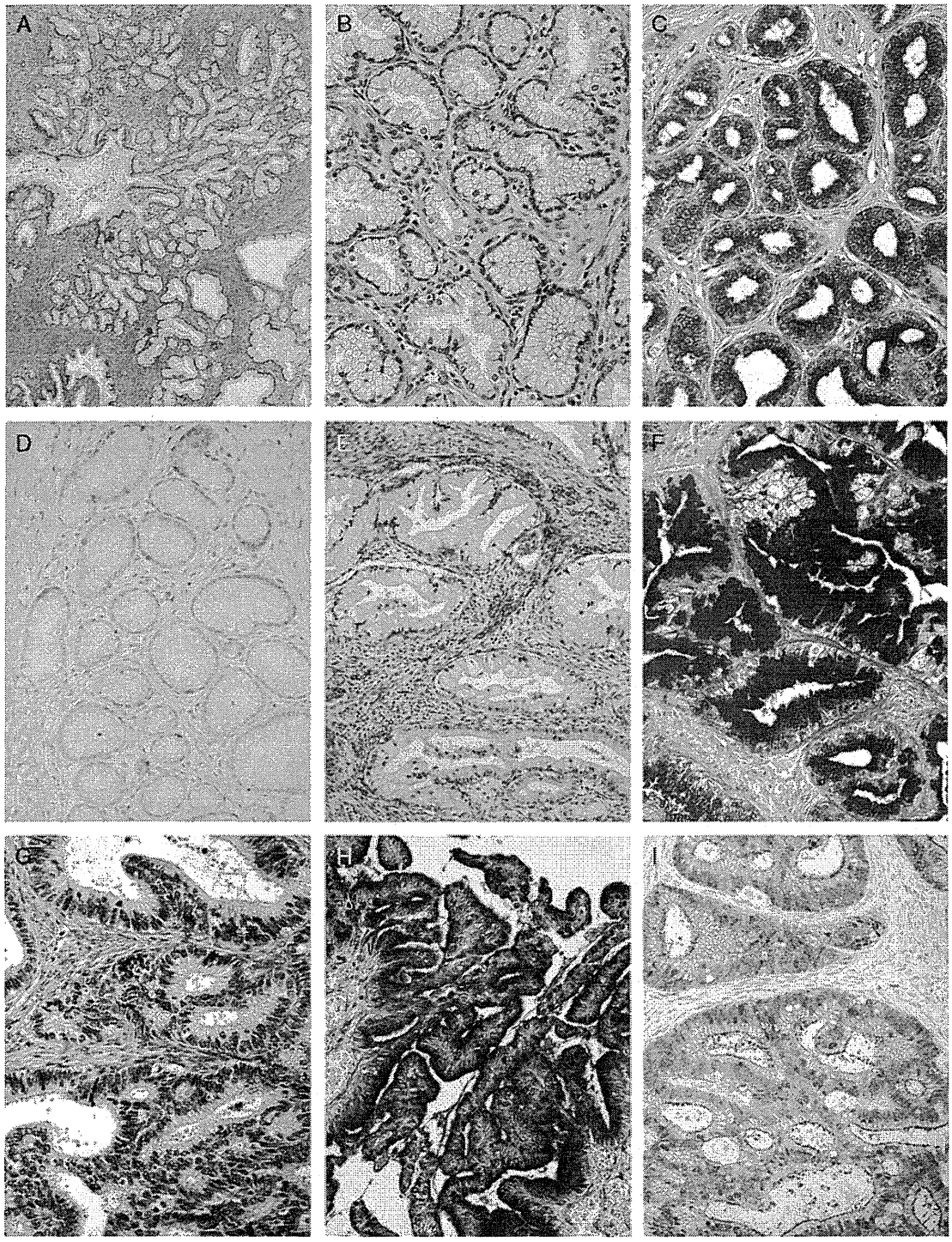

FIGURE 1. Representative histologies of the lesions. A–D, LEGH. A, Small to medium-sized cervical glands with a lobular architecture surrounding a dilated gland. B, The glands are lined by mucin-rich columnar cells without nuclear atypia. The glands express PAS-positive neutral mucin (C) and are negative for p16 (D). E and F, MDA. E, Proliferation of irregular-shaped glands associated with desmoplastic stromal reaction. F, MDA expressing PAS-positive neutral mucin. G–I, Endocervical-type mucinous adenocarcinoma. G, Complex glands lined by columnar cells with significant nuclear atypia. The glands express predominantly Alcian blue-positive acidic mucin (H) and are diffusely positive for p16 (I).

TABLE 1. *GNAS* Mutations, HPV DNA, and p16 Expression in Cervical Lesions

		GNAS				HPV DNA			p16
	Total Analyzed	Total Mutated, n (%)	Cases Mutated	Nucleotide	Amino Acid	Total Positive, n (%)	Cases Positive	Genotype	Positive, n (%)*
Normal endocervical tissue	22	0	0			NA			NA
LEGH	19	8 (42)	4	c.601C > T	p.R201C	0†			1 (5)
			4	c.602G > A	p.R201H				
Adenocarcinoma									
Minimal deviation	3	0	0			0†			1 (33)
Endocervical-type mucinous	79	4 (5)	3	c.601C > T	p.R201C	59 (75)	31	16	64 (81)
			1	c.602G > A	p.R201H		27	18	
							1	45	
Endometrioid	10	0	0			9 (90)	4	16	9 (90)
							5	18	
Squamous cell carcinoma	43	0	0			37 (86)	23	16	43 (100)
							9	18	
							1	33	
							1	45	
							1	52	
							1	56	
							1	58	

*Cases with diffuse (>50%), and moderate or strong staining.
†The GP5+/6+ and HPV type-specific PCR were not done in 2 LEGH and 2 MDA cases.
NA indicates not analyzed.

45, and 56. We further analyzed cases that tested negative for the universal HPV primers using type-specific PCRs encompassing the E7 regions of HPV16, 18, 33, 52, and 58 and additionally identified HPV DNA in 3 endocervical-type mucinous adenocarcinomas, 3 endometrioid adenocarcinomas, and 5 squamous cell carcinomas. Two LEGHs and 2 MDAs were excluded from the HPV DNA analysis because of poor DNA quality. Overall, our analysis detected HPV DNA in 59 of the 79 endocervical-type mucinous adenocarcinomas (75%), 9 of the 10 endometrioid adenocarcinomas (90%), and 37 of the 43 squamous cell carcinomas (86%) but in none of the LEGHs or MDAs that were examined.

p16 Immunohistochemistry and Comparison With HPV DNA Status

Positive p16 expression was observed in 1 LEGH (5%), 1 MDA (33%), 64 endocervical-type mucinous

TABLE 2. Mutational Status of LEGH

LEGH No.	Age	*GNAS*	*STK11*	*KRAS*	HPV DNA	p16 Expression*
1	45	–	c.597+24_38del	–	–	–
2	46	c.601C > T	–	–	–	–
3	47	–	–	c.35G > A	–	–
4	47	c.601C > T	–	–	–	–
5	55	–	–	–	–	+
6	52	–	c.del509A	–	–	–
7	65	c.602G > A	–	–	–	–
8	58	–	–	–	–	–
9	46	–	–	–	–	–
10	50	c.602G > A	–	–	–	–
11	45	c.601C > T	–	–	–	–
12	41	–	–	–	–	–
13	37	c.602G > A	–	–	–	–
14	46	–	–	–	–	–
15	63	c.602G > A	–	–	–	–
16	61	c.601C > T	–	–	–	–
17	61	–	–	–	–	–
18	51	–	ND	–	ND	–
19	48	–	ND	–	ND	–

*Cases with diffuse (>50%), and moderate or strong staining.
ND indicates not done.

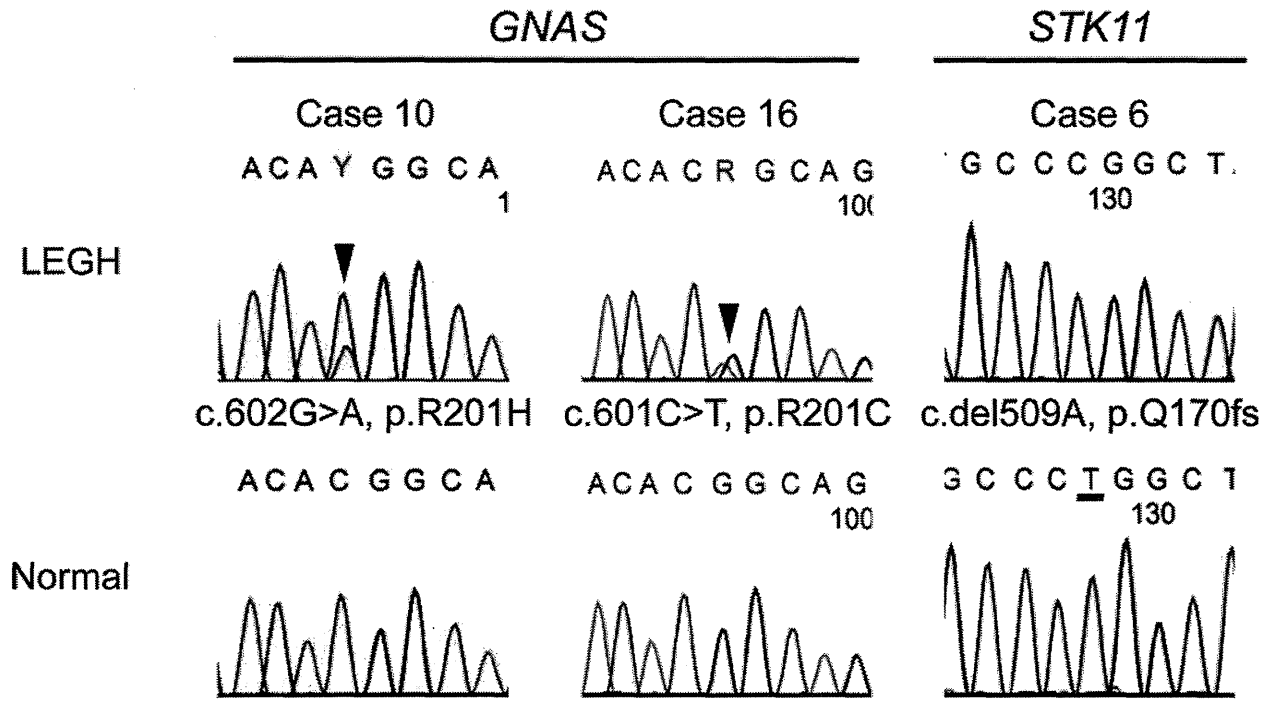

FIGURE 2. *GNAS* and *STK11* mutations in LEGH. The missense mutations in *GNAS* are indicated by the arrowheads. A deletion in *STK11* is underlined. Note the absence of a normal sequence in *STK11* in the LEGH specimen, indicating the homozygosity of this mutation. All the samples were sequenced using reverse primers.

adenocarcinomas (81%), 9 endometrioid adenocarcinomas (90%), and all squamous cell carcinomas (Tables 1 and 2, Fig. 1). As expected, a close correlation existed between p16 expression and the presence of HPV DNA ($P = 8.2 \times 10^{-17}$).

Collectively, 1 LEGH (5%), 1 MDA (33%), 66 endocervical-type mucinous adenocarcinomas (84%), 9 endometrioid adenocarcinomas (90%), and all the squamous cell carcinomas (100%) were positive for HPV DNA and/or p16 expression. All the *GNAS*-mutated LEGHs were negative for HPV DNA and p16 expression. In contrast, all 4 *GNAS*-mutated endocervical-type mucinous adenocarcinomas were positive for p16, and 2 of them were also positive for HPV DNA.

Mutational Analysis for *KRAS* and *STK11* in LEGH

One LEGH had a heterozygous *KRAS* mutation affecting codon 12 (Table 2). *STK11* mutations were found in 2 LEGHs: a single nucleotide deletion involving codon 509 and a 15-nucleotide deletion within intron 4 (Table 2, Fig. 2). Both *STK11* mutations were homozygous. Analyses of normal tissue samples of *KRAS*-mutated or *STK11*-mutated cases did not identify mutations, indicating their somatic nature. The *STK11* mutation analysis was not performed for 2 LEGH specimens (LEGH 18, 19) because of the poor DNA quality.

DISCUSSION

The current study identified *GNAS* mutations in 8 of 19 LEGHs. The *GNAS* mutations that were identified have been previously reported in various tumors and have been shown to act as activating mutations.[13–21] Recent studies have reported the frequent presence of activating *GNAS* mutations in several types of tumors of digestive organs, including pancreatic intraductal papillary mucinous neoplasm, colorectal villous adenoma, low-grade appendiceal mucinous neoplasms, and pyloric gland adenoma of the stomach and duodenum.[17–21] Interestingly, these lesions with *GNAS* mutations mostly show a low-grade morphology and frequent gastric-type mucin expression, similar to LEGH.[17–20,25–28] We have also shown that the presence of *GNAS* mutation is correlated with the expression of MUC5AC, a gastric foveolar mucin, in low-grade mucinous appendiceal tumors.[21] Considering the close correlation between this genetic alteration and the gastric phenotype, *GNAS* mutations may play a role in gastric epithelial differentiation in these lesions.

Watery vaginal discharge is a common manifestation of LEGH.[11,25,29,30] It is noteworthy that the activation of Gsα, which is encoded by *GNAS*, leads to elevated cAMP levels and the induction of water and electrolyte secretion in some epithelial cell types.[31,32] Consistently, massive secretory diarrhea is a known complication of colorectal villous adenomas, which also have frequent *GNAS* mutations.[19,33–35] Considering the regulatory role of the Gsα-cAMP pathway in epithelial secretion, the activation of this pathway might be responsible for the hypersecretory phenotype of LEGH and colorectal villous adenoma.

In addition to LEGH, our study also identified activating *GNAS* mutations in 4 of 79 endocervical-type mucinous adenocarcinomas. Some recent studies have reported an association between LEGH and endocervical-type adenocarcinoma and have suggested that LEGH might be a precursor of HPV-negative adenocarcinomas.[8–10,29,30] To elucidate the relationship between *GNAS* mutation and HPV infection, we analyzed the presence of HPV DNA and p16 expression, which is closely correlated with HPV infection.[4] Consistent with previous reports, HPV infection and p16 expression were rare among LEGH and MDA but were highly prevalent in adenocarcinomas and squamous cell carcinomas.[1,3,4,8–10,36] All 4 *GNAS*-mutated endocervical-type adenocarcinomas were also positive for HPV DNA and/or p16 expression. These results indicate that *GNAS* mutation is involved in a minor proportion of HPV-related cervical carcinogenesis. In contrast, no *GNAS* mutations were identified in 13 endocervical-type adenocarcinomas that were negative for HPV DNA and p16 expression, which likely represent HPV-unrelated tumors. These results imply that *GNAS*-mutated LEGH may not be a major precursor of HPV-negative cervical adenocarcinomas. However, as we analyzed only 3 MDAs because of their rarity, further studies of larger numbers of cases are needed to exclude the involvement of *GNAS* mutations in their histogenesis.

We additionally performed mutational analyses of *KRAS* and *STK11* in LEGH. We expected the presence of *KRAS* mutations in LEGH because *GNAS*-mutated tumors of digestive organs were frequently associated with *KRAS* mutations.[17–21] However, our analysis identified activating *KRAS* mutations in only 1 LEGH, which had no *GNAS* mutation. Unlike tumors of digestive organs, *GNAS* mutations were not associated with *KRAS* mutation in LEGH.

STK11, also known as *LKB1*, is a tumor suppressor responsible for PJS.[37] PJS is an autosomal dominant hereditary disorder characterized by mucocutaneous pigmentation, gastrointestinal hamartomatous polyposis, and an occasional association with LEGH.[1,38,39] Even though *STK11* mutations have been described in cervical adenocarcinoma and squamous cell carcinoma,[40,41] to our knowledge, a mutational analysis for *STK11* has not been reported in sporadic LEGH. Our analysis identified *STK11* mutations in 2 LEGH specimens: a frameshift mutation, and an intronic deletion. Although the significance of this intronic deletion remains unclear, the somatic nature and homozygosity of the mutation suggest that it is potentially pathologic.

LEGH has generally been regarded as a non-neoplastic lesion.[11,25] However, our analyses identified a total of 11 (58%) LEGHs with mutations in either *GNAS*, *STK11*, or *KRAS*. These findings indicate that more than half of the LEGH specimens represented clonal lesions associated with alterations to oncogenes or tumor suppressor genes. Indeed, some reports have shown that LEGH can exhibit mild nuclear atypia and can be associated with adenocarcinoma.[8,9,29,30] Kawauchi et al[10] have also reported that some LEGHs have chromosomal imbalances. On the basis of the present and previous observations, we suggest that LEGH might be better regarded as a neoplastic entity. At the same time, our observations also revealed that LEGH is a genetically heterogenous group of lesions.

The current study demonstrated the presence of activating *GNAS* mutations in LEGH and a minor subset of endocervical-type mucinous adenocarcinomas. *GNAS*-mutated adenocarcinomas were positive for HPV DNA and/or p16 expression, suggesting that *GNAS* mutations are involved in a minor subset of HPV-related lesions. Our study also showed that more than half of the LEGHs had genetic alterations of classic oncogenes or suppressor genes, suggesting a neoplastic nature of this entity.

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Status of treatment for the overall population of patients with stage IVb endometrial cancer, and evaluation of the role of preoperative chemotherapy: A retrospective multi-institutional study of 426 patients in Japan

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HIGHLIGHTS

- Only 66% of patients with stage IVb endometrial cancer underwent primary surgery.
- Hysterectomy and chemotherapy may prolong overall survival in selected patients with stage IVb EMCA.
- Primary chemotherapy followed by surgery may be a useful treatment choice in patients not suitable for primary surgery

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ABSTRACT

Objective. We previously reported on the role of cytoreduction in 248 patients with surgical stage IVb endometrial cancer (EMCA). This study aimed to evaluate the clinical characteristics, prognosis according to initial treatment, and impact of preoperative chemotherapy in the overall population of patients with clinical and surgical stage IVb EMCA.

Methods. A multi-institutional retrospective analysis was performed in 426 patients diagnosed with clinical and surgical stage IVb EMCA from 1996 to 2005. Factors associated with overall survival (OS) were identified using univariate and multivariate analyses.

Results. The median OS for all 426 patients was 14 months. Patients were divided into three groups according to their initial treatment: primary surgery group ($n = 279$), primary chemotherapy group ($n = 125$), and palliative care group ($n = 22$). The median OS times for these groups were 21, 12, and 1 month, respectively ($p < 0.0001$). Patients in the primary surgery group had better performance status (PS) and lower numbers of extra-abdominal metastases than those in the primary chemotherapy group. Multivariate analysis identified good PS, endometrioid histology, absence of clinical intra-abdominal stage IVb metastasis, hysterectomy, and chemotherapy as independent predictors of OS. In the primary chemotherapy group, 59 patients subsequently underwent surgery, and these patients had similar OS to those in the primary surgery group.

Conclusions. Hysterectomy and chemotherapy may prolong OS in selected patients with stage IVb EMCA. Our data suggest that primary chemotherapy followed by surgery may be a useful treatment choice in patients not suitable for primary surgery.

Introduction

Most cases of endometrial cancer (EMCA) present in the early stages and have a favorable prognosis, but patients occasionally present with

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stage IVb disease which has a very poor prognosis [1,2]. Therapeutic decision making for patients with stage IVb EMCA remains challenging because of the lack of available data for this group.

Several retrospective studies and a meta-analysis have reported that surgical cytoreduction, as performed in patients with ovarian cancer, was also useful in patients with stage IVb EMCA [3–11]. We conducted a multicenter retrospective study of patients with stage IVb EMCA who were treated at Japan Clinical Oncology Group-related institutions. We previously reported the detailed clinicopathological characteristics and role of cytoreductive surgery in 248 patients with surgical stage IVb EMCA [12].

Some patients with stage IVb EMCA do not undergo surgery for initial treatment. In patients with unresectable intra- or extra-abdominal metastases, surgery may not be the preferred initial treatment. Furthermore, some patients with stage IVb EMCA are medically inoperable. According to the International Federation of Gynecology and Obstetrics (FIGO) Annual Report [13], the 4-year survival rate is 22.3% in patients with surgical stage IVb EMCA and 7.0% in patients with clinical stage IVb EMCA. The prognosis of patients with stage IVb EMCA who do not undergo initial surgery is extremely poor, but detailed data about the treatments received by these patients are lacking.

Neoadjuvant chemotherapy (NAC) and interval debulking surgery followed by chemotherapy (NAC-setting treatment [NACT]) has emerged as an alternative treatment for patients with advanced ovarian cancer who have unresectable disease or poor performance status (PS). A phase III study found that NACT had a comparable outcome to primary debulking surgery followed by chemotherapy, but with less surgery-related adverse effects [14]. The first prospective study of NACT in patients with serous EMCA with transperitoneal spread reported that NACT resulted in a high rate of optimal debulking surgery [15].

One of the reasons for the difficulty in establishing therapeutic algorithms for stage IVb EMCA is the small numbers of patients in most series. Another reason is that few studies have evaluated treatment of the overall population of patients with stage IVb EMCA, including non-surgical patients.

In the current study, we analyzed all patients who were diagnosed with stage IVb EMCA, including non-surgical cases. The primary objectives of this study were to clarify the current status of treatment, and to analyze the clinicopathological characteristics and prognostic factors in this population. The secondary objective was to evaluate the role of preoperative chemotherapy in patients who did not undergo initial surgical intervention.

Methods

Patients

We performed a retrospective review of all patients diagnosed with clinical or surgical FIGO 1988 stage IVb EMCA from 1996 to 2005, who were treated in 30 Gynecologic Cancer Study Group of Japan Clinical Oncology Group-related institutions. Patients with sarcoma were excluded.

A case report form was developed using data software (FileMaker-pro Version 6 or 8) to obtain equivalent data from multiple institutions. The investigation protocol, including the case report form, was approved by the Institutional Review Board of each institution.

Complete clinical data were collected by reviewing inpatient charts, operative records, and outpatient records from each institution. Pathological information was collected from the pathology reports of the endometrial biopsy specimen and the hysterectomy specimen. The sites of metastases, surgical procedures, and sites and maximum diameter of residual disease after surgery were collected from radiology reports, intraoperative findings, and pathology reports. Treatment data included initial treatment, adjuvant treatment after surgery, and surgical treatment after chemotherapy. Regular follow-up was performed at each institution. Follow-up information included the date and disease status at the last follow-up, or the date and cause of death.

Patients were divided into the following three groups according to their initial treatment: primary surgery group, primary chemotherapy group, and palliative care group. Patients who underwent primary radiotherapy only were classified into the palliative care group. The primary chemotherapy group was subdivided into a group who underwent laparotomy after chemotherapy, and a group who did not. Stage IVb metastases were divided into intra- and extra-abdominal disease. Metastasis to the liver parenchyma was classified as extra-abdominal disease. Because patients with non-surgical treatment were included, we defined intra-abdominal lesions that were detectable on pretreatment imaging studies as clinical intra-abdominal stage IVb metastases. In patients of the primary chemotherapy group who subsequently underwent surgery, the response to preoperative chemotherapy was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST). Patient outcomes were analyzed by overall survival (OS), calculated from the date when initial treatment was started to the date of death or last contact.

Statistical analyses

Differences in the distributions of clinicopathological characteristics among groups were analyzed by Fisher's exact test for qualitative variables. OS curves were estimated using the Kaplan–Meier method, and were compared among groups using the log-rank test. A two-sided p value of <0.05 was considered statistically significant. Independent prognostic factors were identified using multivariate Cox proportional hazards regression analyses. All analyses were performed using SPSS statistical software (11.0.1J; SPSS Inc., Chicago, IL).

Results

Initial treatment and patient characteristics

We identified a total of 426 patients with stage IVb EMCA. Patient grouping by initial treatment was as follows: 279 patients (66%) in the primary surgery group, 125 (29%) in the primary chemotherapy group, and 22 (5%) in the palliative care group (Fig. 1).

The primary surgery group included 149 patients who had stage IVb disease detected by preoperative imaging examinations and 130 patients diagnosed as stage IVb EMCA only after they underwent laparotomy. Of the 125 patients in the primary chemotherapy group, 59 (47%) underwent subsequent laparotomy and the remaining 66 did not undergo any subsequent surgery.

Table 1 shows the clinicopathological characteristics of patients according to the initial treatment. The median age of patients was 59 years, and 84% of patients had a pretreatment Eastern Cooperative Oncology Group (ECOG) PS of 0 or 1. The mean body mass index was 23 kg/m^2 (range: 14–39 kg/m^2). Medical comorbidities included hypertension in 23% of patients and diabetes in 12%. The most common histological subtype was endometrioid, accounting for 58% of cases.

The majority of patients in the palliative care group were old, emaciated, hypertensive, and had two or more extra-abdominal metastases. Patients in the primary surgery group had a better PS ($p = 0.002$) and lower rates of comorbidities (hypertension, $p = 0.049$; diabetes, $p = 0.003$) than those in the primary chemotherapy group. Tumor histology was not significantly different among the three groups.

Extra-abdominal stage IVb disease was documented in 229 patients (54%), including 82% of patients in the primary chemotherapy group and 86% in the palliative care group. Patients in these two groups were more likely to have metastases in two or more anatomical regions than those in the primary surgery group. The most common sites of extra-abdominal metastases were the lungs (28%), liver (13%), mediastinal lymph nodes (9%), and bone (8%). Clinical intra-abdominal stage IVb disease was documented in 134 patients (31%). Of the 220 patients with intra-abdominal stage IVb disease in the primary surgery group,

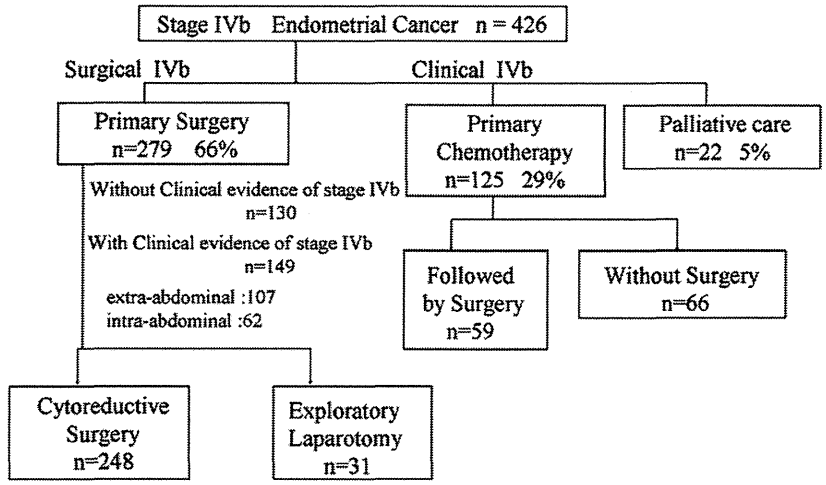

Fig. 1. Patients with stage IVb endometrial cancer, grouped according to initial treatment.

Table 1
Clinicopathological characteristics ($n = 426$).

Characteristics	Primary surgery		Primary chemotherapy		Palliative care		Total		Fisher's exact-test
	n	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*p*-Value[c]
Median age, years (range)	59	(30–89)	58	(30–83)	73	(53–84)	59	(30–89)	
ECOG performance status									
0/1	253	(91)	97	(77)	7	(32)	357	(84)	0.002
2–4	25	(9)	26	(21)	15	(68)	66	(15)	
Unknown	1	(<1)	2	(2)	0	(0)	3	(1)	
Body mass index									
<20	51	(18)	32	(26)	9	(40)	92	(22)	0.311
20 ≤ 25	135	(48)	57	(45)	5	(23)	197	(46)	
≥25	80	(29)	34	(27)	6	(28)	120	(28)	
Unknown	13	(5)	2	(2)	2	(9)	17	(4)	
Diabetes mellitus									
Yes	23	(8)	23	(18)	4	(18)	50	(12)	0.003
No	251	(90)	95	(76)	17	(77)	364	(85)	
Unknown	5	(2)	7	(6)	1	(5)	13	(3)	
Hypertension									
Yes	54	(19)	35	(28)	10	(45)	99	(23)	0.049
No	219	(78)	84	(67)	11	(50)	314	(74)	
Unknown	6	(3)	6	(5)	1	(5)	13	(3)	
Histology									
Endometrioid	163	(58)	68	(54)	14	(64)	245	(58)	0.514
Non-endometrioid	116	(42)	57	(46)	8	(36)	181	(42)	
Extra-abdominal metastases									
Negative	172	(62)	22	(18)	3	(14)	197	(46)	<0.001
Positive	107	(38)	103	(82)	19	(86)	229	(54)	
Number of regions									
1	83	(29)	49	(39)	7	(32)	139	(33)	
≥2	24	(9)	54	(43)	12	(54)	90	(21)	
Clinical intra-abdominal stage IVb metastases									
Negative	217	(78)	60	(48)	11	(50)	288	(68)	<0.001
Positive	62	(22)	65	(52)	11	(50)	138	(32)	
Treatment									
Surgery	279	(100)	59	(47)	0	(0)	338	(79)	
Hysterectomy	248	(89)	53	(42)			301	(70)	
No hysterectomy	31	(11)	6	(5)			37	(9)	
Chemotherapy	238	(85)	125	(100)	0	(0)	363	(86)	
Taxanes + platinum	135	(48)	78	(63)			213	(50)	
AP ± α	84	(30)	38	(30)			122	(29)	
Others	19	(7)	9	(7)			28	(7)	
Radiotherapy	42	(15)	28	(22)	9	(41)	79	(19)	
ERT: Pelvis	28	(10)	19	(15)	8	(36)			
ERT: PAN	16	(6)	4	(3)	3	(14)			
ERT: Others[a]	12	(4)	18	(15)	3	(15)			
ICRT	3	(1)	3	(2)	2	(9)			
Chemotherapy + radiotherapy[b]	31	(11)	28	(22)	0	(0)	59	(14)	

AP ± α, doxorubicin + platinum ± others; ERT, external radiotherapy; ICRT, intracavitary irradiation.
[a] Others included ERT to whole abdomen, supraclavicular, bone and brain.
[b] These patients are also included in the chemotherapy group and the radiotherapy group.
[c] *p*-Value, primary surgery group vs. primary chemotherapy group.

Table 2
Univariate analyses for overall survival.

Variable	n	(%)[a]	Median OS (months)	
			(95% CI)	Log-rank p^b
Age				
≤59	220	(52)	22 (17–27)	
≥60	206	(48)	12 (11–14)	0.0013
ECOG performance status				
0–1	357	(84)	19 (15–23)	
2–4	66	(15)	4 (2–5)	<0.0001
Diabetes mellitus				
Yes	50	(12)	13 (11–15)	
No	363	(85)	5 (3–7)	0.3761
Hypertension				
Yes	99	(23)	17 (12–23)	
No	314	(74)	14 (9–19)	0.7702
Histology				
Endometrioid	245	(58)	24 (19–29)	
Non-endometrioid	181	(42)	9 (8–11)	<0.0001
Extra-abdominal metastasis				
Positive	229	(54)	11 (9–14)	
Negative	197	(46)	21 (16–26)	0.0494
Clinical intra-abdominal stage IVb metastasis				
Positive	138	(32)	9 (6–12)	
Negative	288	(68)	20 (15–26)	<0.0001
Initial treatment				
Primary surgery	279	(65)	21 (17–26)	
Primary chemotherapy	125	(29)	12 (9–15)	
Palliative care	22	(6)	1 (0–4)	<0.0001
Hysterectomy				
Yes	301	(71)	24 (20–28)	
No	125	(29)	5 (4–7)	<0.0001
Chemotherapy				
Yes	363	(85)	18 (14–22)	
No	63	(15)	5 (3–7)	<0.0001
Radiotherapy				
Yes	79	(19)	12 (6–17)	
No	347	(81)	15 (12–19)	0.8958

ECOG, Eastern Cooperative Oncology Group.
[a] Numbers may not add up to totals because some data are unknown.
[b] Patients with unknown status were excluded in the calculation of log-rank p values.

only 62 (28%) had clinical intra-abdominal stage IVb disease detected by preoperative imaging examinations.

Treatment modalities

In the primary surgery group, 249 patients (89%) underwent postoperative adjuvant therapy, including 207 who underwent chemotherapy alone, 11 who underwent radiotherapy alone, and 31 who underwent both chemotherapy and radiotherapy. In the primary chemotherapy group, 59 patients underwent subsequent surgery, of which 52 also received postoperative radiotherapy or chemotherapy. Of the 66 patients who did not undergo surgery after primary chemotherapy, 21 also received radiotherapy.

The most common chemotherapy regimen was taxanes + platinum ± doxorubicin, followed by doxorubicin + cisplatin ± cyclophosphamide/ifosfamide. The treatment details are shown in Table 1.

Treatment outcomes

The median follow-up time among the censored patients was 41 months, and the median OS for all stage IVb EMCA patients was 14 months (95% confidence interval [CI]: 11–18). The causes of death were EMCA in 301 patients, other disease in 4, and unknown in 6. At the last follow-up, 62 patients were alive with no evidence of disease, 45 were alive with disease, and 8 were alive with unknown disease status. There were no treatment-related deaths.

Univariate and multivariate analyses

Univariate analyses of the relationships between OS and the demographic, clinicopathological, and therapeutic variables found that age, PS, histology, extra-abdominal metastasis, clinical intra-abdominal stage IVb metastasis, initial treatment, hysterectomy, and chemotherapy were significantly associated with OS (Table 2).

The median OS was 21 months (95% CI: 17–26) in the primary surgery group, 12 months (95% CI: 9–15) in the primary chemotherapy group, and 1 month (95% CI: 0–4) in the palliative care group ($p < 0.0001$; Fig. 2A).

Cox multivariate analysis showed that PS, histology, clinical intra-abdominal stage IVb metastasis, hysterectomy, and chemotherapy were independent prognostic factors for OS (Table 3).

Subgroup analysis

To explore the impact of preoperative chemotherapy, we analyzed the clinicopathological characteristics of patients who underwent primary chemotherapy followed by surgery, and compared the OS in this group with the OS in the primary surgery group.

Patients who underwent primary chemotherapy followed by surgery had a better PS and a lower rate of two or more extra-abdominal

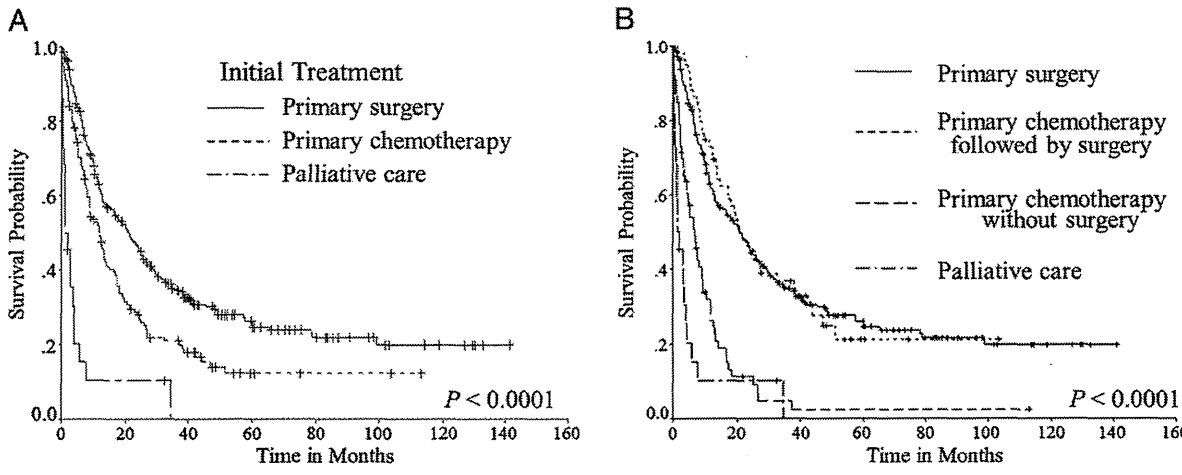

Fig. 2. Kaplan–Meier curves for overall survival (OS). A: Median OS according to initial treatment in patients with stage IVb endometrial cancer ($n = 426$): primary surgery group (solid line), 21 months; primary chemotherapy group (dotted line), 12 months; and palliative care group (dash-dot line), 1 month. B: Median OS of patient groups, showing the two primary chemotherapy subgroups: primary chemotherapy followed by surgery (dotted line), 21 months; and primary chemotherapy without surgery (dashed line), 7 months.

Table 3
Multivariate analyses.

Variable	Hazard ratio	95% CI	*p*-Value
ECOG performance status			
0–1 vs. 2–4	1.837	1.353–2.495	<0.001
Histology			
Endometrioid vs. non-EM	2.015	1.601–2.537	<0.001
Clinical intra-abdominal stage IVb metastasis			
Negative vs. positive	1.514	1.175–1.951	0.001
Hysterectomy			
No vs. yes	0.324	0.250–0.420	<0.001
Chemotherapy			
No vs. yes	0.449	0.327–0.618	<0.001

ECOG, Eastern Cooperative Oncology Group.

metastases than those who underwent primary chemotherapy without subsequent surgery ($p < 0.0001$) (Table S1), and had similar characteristics to those who underwent primary surgery, except for disease distribution. Of the 59 patients who underwent primary chemotherapy followed by surgery, 24 (40%) had non-endometrioid histology. Preoperatively, 57 patients (97%) received chemotherapy alone, and 2 patients received both chemotherapy and radiotherapy. Chemotherapy with taxane + platinum was administered to 58% of patients. The response to preoperative chemotherapy was as follows: complete or partial response in 40 patients, stable disease in 9 patients, progressive disease in 7 patients, and not evaluable in 3 patients. The median time from the start of chemotherapy to surgery was 99 days (range: 29–297 days). Postoperative therapy was as follows: chemotherapy alone in 44 patients, radiotherapy alone in 2 patients, both chemotherapy and radiotherapy in 5 patients, and no adjuvant treatment in 8 patients.

Table 4 shows the surgical procedures performed in the primary surgery group and in the group who underwent primary chemotherapy

Table 4
Surgical procedures and outcomes of surgery in the primary surgery group and the primary chemotherapy group.

	Primary surgery		Primary CT followed by surgery	
	($n = 279$)		($n = 59$)	
	n	(%)	*n*	(%)
Procedures performed				
Hysterectomy + BSO	248	(89)	53	(90)
Type of hysterectomy				
Simple	184	(66)	29	(49)
Subtotal	9	(3)	0	(0)
Modified-radical	49	(18)	24	(41)
Radical	6	(2)	0	(0)
Omentectomy/biopsy	180	(65)	35	(60)
Pelvic lymphadenectomy	158	(57)	31	(53)
Para-aortic lymphadenectomy	83	(30)	25	(42)
Resection of peritoneum	95	(34)	19	(32)
Appendectomy	30	(11)	5	(9)
Resection of colon/ileum	17	(6)	3	(5)
Colostomy/ileostomy	3	(2)	1	(2)
Diaphragm peritonectomy	1	(<1)	0	(0)
Resection of internal iliac artery	1	(<1)	0	(0)
Splenectomy	1	(<1)	1	(2)
Resection of liver	0	(0)	1	(2)
Mastectomy	1	(<1)	0	(0)
Resection of umbilicus/skin meta	4	(1)	0	(0)
Resection of supraclavicular LN	3	(1)	0	(0)
Resection of inguinal LN	7	(3)	0	(0)
Results of surgery				
Postoperative residual disease				
None	61	(22)	19	(32)
≤1 cm	65	(23)	15	(25)
>1 cm	153	(55)	25	(43)

BSO, bilateral salpingo-oophorectomy; CT, chemotherapy.

followed by surgery. Hysterectomy was performed in 89% ($n = 248$) of the primary surgery group and 90% ($n = 53$) of the group who underwent primary chemotherapy followed by surgery. In addition to hysterectomy and bilateral salpingo-oophorectomy, most patients underwent cytoreductive procedures with the intent of achieving maximum cytoreduction, including omentectomy, lymph node biopsy, resection of peritoneal dissemination, and colonic resection. The procedures performed were similar in both groups. Optimal cytoreduction (≤1 cm residual disease) was achieved in 45% ($n = 126$) of the primary surgery group and 57% ($n = 34$) of the group who underwent primary chemotherapy followed by surgery ($p = 0.087$).

Three patients in the primary surgery group and one patient who underwent primary chemotherapy followed by surgery died within 30 days after surgery, all because of disease progression. Three of these four patients underwent exploratory laparotomy without further surgery, and the other patient had >2 cm residual disease. One life-threatening postoperative complication (pulmonary embolism) was reported in a patient who underwent primary chemotherapy followed by surgery.

The survival curves of the group who underwent primary chemotherapy followed by surgery and the primary surgery group were almost the same ($p = 0.8351$) (Fig. 2B).

Discussion

Few studies to date have included both surgically and non-surgically treated patients with stage IVb EMCA [16–19]. In our multicenter study of patients with stage IVb EMCA, we previously evaluated the role of treatment with cytoreductive surgery in 248 patients [12]. In the current study, we analyzed all 426 patients with stage IVb EMCA, including those who did not undergo surgery. This is the largest study of the overall population of patients with stage IVb EMCA.

The relationships between clinicopathological data and survival in the overall population of patients with stage IVb EMCA have not been well evaluated. The largest previously reported study was conducted by Aalders et al. [20] in 1984 and included 83 patients. In contrast to our study population, their patients were treated primarily by radiotherapy. They found that patients with well- and moderately differentiated adenocarcinoma had a better response rate than those with poorly differentiated adenocarcinoma [20]. Our previous analysis of 248 patients who underwent cytoreductive surgery examined the precise distribution of intra-abdominal disease and histopathological factors, and found that lower grade endometrioid type EMCA was an independent prognostic factor [12]. As the present study included patients who did not undergo surgery, we could not obtain detailed clinicopathological data such as the grade and precise distribution of disease in all cases, but we still found that endometrioid histology was an independent favorable prognostic factor in the overall population of those with stage IVb EMCA.

Our study identified some factors that should be considered during therapeutic decision making in stage IVb EMCA. First, clinical intra-abdominal stage IVb metastasis was a poor prognostic factor. Landrum et al. [21] conducted a case control study that compared intra-abdominal stage IVb EMCA with stage IIIc ovarian cancer, which has a similar metastatic pattern. They found that the 2-year OS was lower in EMCA than in ovarian cancer (52% vs. 76%, $p = 0.008$). However, as these two tumors may have different biological characteristics and chemosensitivities, the treatment strategies for stage IIIc ovarian cancer may not be equally suitable for intra-abdominal stage IVb EMCA.

Second, in our study of the overall population with stage IVb EMCA, 54% of patients had extra-abdominal metastasis. Most of the extra-abdominal metastases in EMCA were unresectable, in contrast to stage IV ovarian cancer where the most common site of stage-IV defining disease is pleural effusion [22]. The National Comprehensive Cancer Network Guidelines recommend palliative hysterectomy with or without

chemotherapy, radiotherapy, or hormonal therapy for extra-abdominal disease [23]. A systematic review of the Cochrane collaboration reported that combination chemotherapy improves survival in advanced EMCA [24]. Our previous analysis found that intra-abdominal optimal cytoreductive surgery and adjuvant therapy were prognostic factors even in the presence of extra-abdominal metastasis [12]. In the present study, chemotherapy and hysterectomy were identified as independent prognostic factors in patients with stage IVb EMCA. Our findings suggest that hysterectomy may be useful even in patients with unresectable extra-abdominal metastasis.

Similar to the direction of treatment for advanced ovarian cancer, several studies have reported that cytoreductive surgery was useful in advanced EMCA [3–10,25,26]. However, many patients do not undergo primary surgery for stage IVb EMCA, including 36% of the patients in the study by Numazaki et al. [27] and 34% of the patients in our study. In previous small series, 17–59% of patients with stage IV EMCA did not undergo surgery [16–19]. In these patients, surgery may not be selected as the initial treatment for various reasons including extra-abdominal metastasis, unresectable intra-abdominal disease, and medical comorbidities. Goff et al. [5] reported that surgical cytoreduction was not selected because of extra-abdominal metastasis in 8 of the 18 patients who did not undergo surgical cytoreduction. In our retrospective study, varying criteria were used for selecting chemotherapy as the initial treatment. It seems that chemotherapy was more likely to be chosen as the initial treatment than surgery in patients with poor PS, complications, and two or more extra-abdominal metastases.

In advanced ovarian cancer, NACT was first used as an alternative to primary debulking surgery in patients with apparently unresectable tumors or poor PS [28,29]. The indications for NACT were subsequently extended to include all cases of advanced disease, including patients with resectable tumors and good PS. The phase III NACT trial reported noninferior survival with less serious morbidity in the NACT arm [14].

Vandenput et al. [15] conducted the first prospective study of NACT in patients with serous EMCA with transperitoneal spread diagnosed by laparoscopy. Thirty patients received 3–4 cycles of NAC, of whom 24 underwent optimal cytoreduction. The median OS was 23 months. NACT therefore resulted in a high rate of optimal debulking surgery in these patients. In our study, only 59 patients (47%) in the primary chemotherapy group subsequently underwent surgery. The primary chemotherapy group included patients who were treated with the intent of progressing to NACT as well as patients who underwent chemotherapy with palliative intent only. Interestingly, the OS was comparable between the group who underwent primary chemotherapy followed by surgery and the primary surgery group. Of the patients who underwent chemotherapy or radiotherapy without surgery, only a few survived for a long time.

The study of NACT by Vandenput et al. [15] was limited to patients with intra-abdominal stage IVb EMCA, whereas in our study, 40 (68%) of the patients who underwent primary chemotherapy followed by surgery had extra-abdominal disease. Our findings suggest that NACT may be a useful treatment option for highly selected patients with intra-abdominal or extra-abdominal stage IVb EMCA. However, EMCA is less chemosensitive than ovarian cancer, and NACT is not necessarily suitable for all patients with advanced EMCA. Preoperative chemotherapy can show whether the tumor is chemosensitive, and the morbidity associated with surgery can then be avoided in patients with chemoresistant tumors. Preoperative chemotherapy can therefore be used to determine which patients are suitable candidates for NACT.

This study may have a selection bias and has several limitations. First, the quality of data may not be uniform because of the retrospective, multi-center design. We made a considerable effort to standardize data collection using a case report form. Second, interpretation of the results is difficult because decisions regarding initial treatment may vary among institutions. Third, it is unknown which patients in the primary chemotherapy group received initial chemotherapy with the intent of proceeding to NACT. Therefore, the group who underwent primary chemotherapy followed by surgery is not the same as a NACT group. Only a prospective trial can definitively determine the role of NACT in advanced EMCA.

In conclusion, our results show the current status of treatment for the overall population of patients with stage IVb EMCA in Japan. Good PS, endometrioid histology, absence of clinical intra-abdominal stage IVb disease, hysterectomy, and chemotherapy were identified as independent prognostic factors for survival. These findings suggest that combined hysterectomy and chemotherapy may benefit selected patients with stage IVb EMCA. Subgroup analysis found that OS was similar between patients who underwent primary chemotherapy followed by surgery and those who underwent primary surgery. Our data suggest that preoperative chemotherapy may be a useful treatment choice for patients with stage IVb EMCA who are not suitable for primary surgery.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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