Conclusion

This case indicates that high cardiac output induced by high-level HIV viremia might in part be involved in the pathogenic mechanism of HIV-related heart failure. Comprehensive and prompt treatments against high HIV load and optimal cardiovascular assessment by cardiologists could lead to the successful improvement of cardiac function associated with the remission of high-level HIV viremia.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

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Case report

A case of AIDS-associated oral Kaposi's sarcoma of the tongue

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ABSTRACT

Kaposi's sarcoma (KS) is one of the most common diseases seen in patients presenting with acquired immunodeficiency syndrome (AIDS); however, it is rare in Japan. We herein report a case of AIDS-associated KS of the tongue, which was initially misdiagnosed as recurrent hemangioma according to the initial histopathological diagnosis. The patient is a 42-year-old male who had been suffering from a painful vascular neoplasm-like mass on the dorsum of the tongue. The patient did not complain of any other distinct symptoms and a debulking operation was planned based on the clinical diagnosis of hemangioma. However, preoperative blood tests revealed the presence of syphilis and the human immunodeficiency virus and the patient was therefore diagnosed to have full-blown AIDS. Therefore, the patient's oral lesion was then instead suspected to be oral KS (OKS). A histopathological examination of the tongue biopsy specimen showed the typical findings of KS. Combination active antiretroviral therapy (cART) combined with liposomal doxorubicin was administered and the patient achieved a complete remission (CR). In conclusion, clinicians including oral surgeons, should take OKS into account in the diagnosis of vascular neoplasm-like masses of the tongue in adults since this complication may occur as a result of AIDS.

1. Introduction

Kaposi's sarcoma (KS) is a well known vascular tumor first described by Moriz Kaposi in 1872 [1]. This angioproliferative disorder, which is characterized by the proliferation of spindle-shaped cells, neoangiogenesis, inflammation, and edema [2], is categorized as an intermediate neoplasm due to the absence of conventional features of malignancy [3].

Four major forms of KS have so far been identified: classic, African endemic, immunosuppression-associated or transplant-associated, and AIDS-associated [4]. The histopathologic and immunohistochemical features are similar in all of the clinical forms. In addition, human herpesvirus 8 (HHV-8) DNA is found in almost all cases of KS and in all of the clinical forms [5].

AIDS-associated KS (AIDS-KS), the most aggressive form of the disease, is found in human immunodeficiency virus-1 (HIV-1) infected individuals and it is particularly frequent in homosexual and bisexual men. Although the relative risk of acquiring KS for AIDS patients is >10,000 fold [6], the incidence of KS has been reduced with the advent of combination active antiretroviral therapy (cART) [7].

Head and neck involvement in AIDS-KS is common [8]. Oral lesions represent the first sign of KS in 22% of HIV-positive individuals, and ultimately, 71% of these patients will develop AIDS-associated oral KS (AIDS-OKS) [2,9]. AIDS-OKS has been reported to occur most frequently on the hard palate, followed by the gingiva and the tongue [5,10–12].

In this report, we present a rare case of AIDS-OKS which occurred on the tongue that led to the discovery of AIDS in a Japanese man.

2. Case report

A 42-year-old Japanese male was referred to our hospital for evaluation of a painful mass on the dorsal surface of the tongue that had slowly increased in size. The patient underwent a resection of a neoplastic lesion on the dorsal surface of the tongue at another hospital approximately 5 months before the initial examination at our hospital. The resected specimen was histopathologically diagnosed at the previous hospital as hemangioma. After the operation, the patient suffered from throat pain and began to suspect a recurrence

☆ AsianAOMS: Asian Association of Oral and Maxillofacial Surgeons; ASOMP: Asian Society of Oral and Maxillofacial Pathology; JSOP: Japanese Society of Oral Pathology; JSOMS: Japanese Society of Oral and Maxillofacial Surgeons; JSOM: Japanese Society of Oral Medicine; JAMI: Japanese Academy of Maxillofacial Implants.

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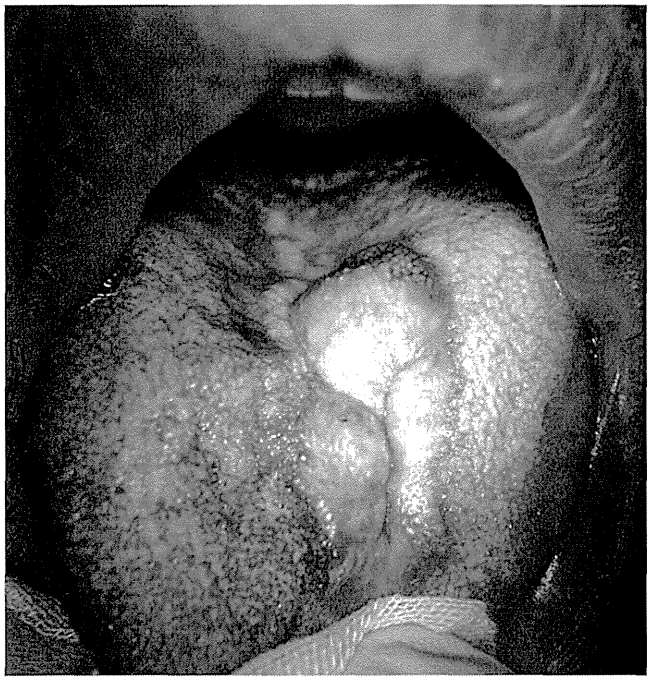


Fig. 1. The appearance of the tongue lesion at the initial examination. A blue to purplish, nodular, exophytic mass measuring 2 cm × 2 cm was found to occupy the left side of the doral surface of the tongue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

of the neoplasm. The patient's past history included diet-controlled diabetes mellitus diagnosed at 30 years of age and hepatitis B contracted at 33 years of age.

No facial asymmetries, cervical lymphadenopathies, or other distinct extraoral findings were detected. Upon intraoral examination, a blue to purplish, exophytic, and vascular neoplasm-like mass measuring 2 cm × 2 cm was found to occupy the left side of the dorsal surface of the tongue (Fig. 1). The findings of the remaining oral cavity and oropharynx were within normal limits. MRI scans of the head and neck revealed only a small superficial lesion on the dorsal surface of the tongue without regional metastasis or infiltration into the deep tissue (Fig. 2). Laboratory data at the first examination are shown in Table 1. All of the hemogram data showed subtle decreases, and the biochemistry data were within normal limits except subtle increase in C-reactive protein.

A debulking operation was planned based on a clinical diagnosis of recurrent hemangioma. Since preoperative blood tests revealed an elevated HbA1c level (7.8%) and the presence of syphilis, the

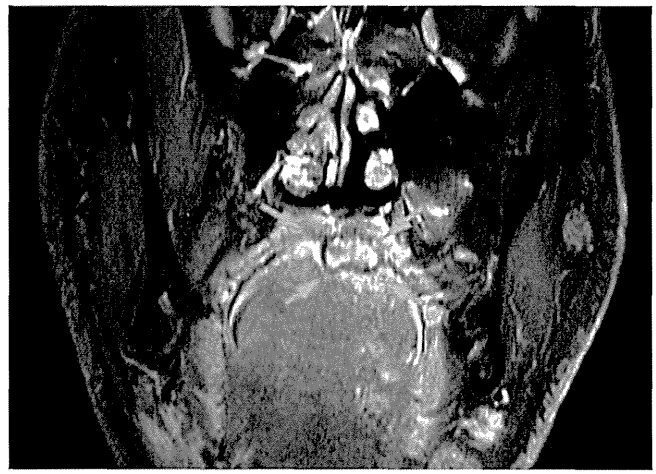


Fig. 2. MRI scan of the tongue lesion at the initial examination. A contrast-enhanced T1-weighted MRI scan showed only a small superficial lesion (arrows) on the dorsal surface of the tongue without infiltration into the deep tissue.

Table 1
Laboratory data at the first examination.

Variable	Result
WBC ($\times 10^3/\mu L$)	3.2
Baso (%)	0.0
Eosin (%)	6.6
Neut (%)	64.0
Lymp (%)	14.7
Mono (%)	14.7
RBC ($\times 10^6/\mu L$)	3.74
Hgb (g/dL)	11.4
Hct (%)	33.6
PLT ($\times 10^3/\mu L$)	114
TP (g/dL)	7.5
Alb (g/dL)	3.9
Na (mEq/dL)	142
K (mEq/dL)	3.9
Cl (mEq/dL)	108
BUN (mg/dL)	15.3
Crea (mg/dL)	0.71
T-Bil (mg/dL)	0.3
AST (U/L)	12
ALT (U/L)	13
LD (U/L)	144
ALP (U/L)	231
CRP (mg/dL)	0.48
P-Glu (mg/dL)	101

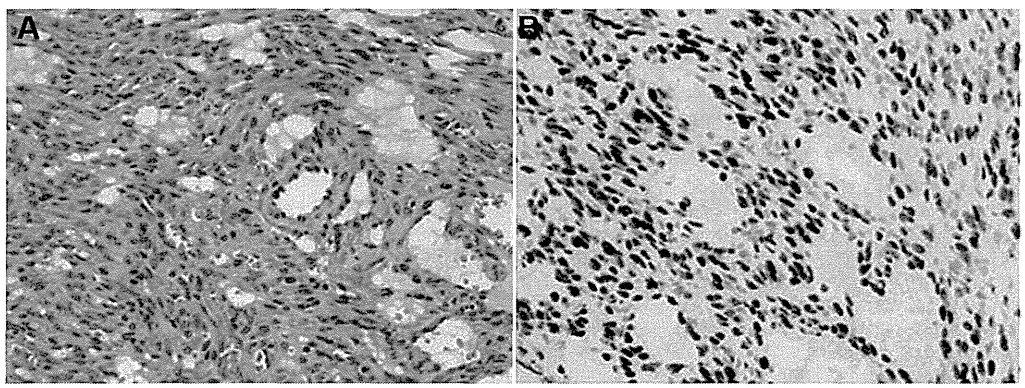


Fig. 3. Histopathological findings of the biopsy specimen. (A) HE staining. A histopathological examination showed proliferation of monomorphic spindle cells with slit-like vascular containing erythrocytes. Original magnification ×100. (B) Immunohistochemical staining for HHV-8. Positive reactions were seen in the tumor cells. Original magnification ×100.

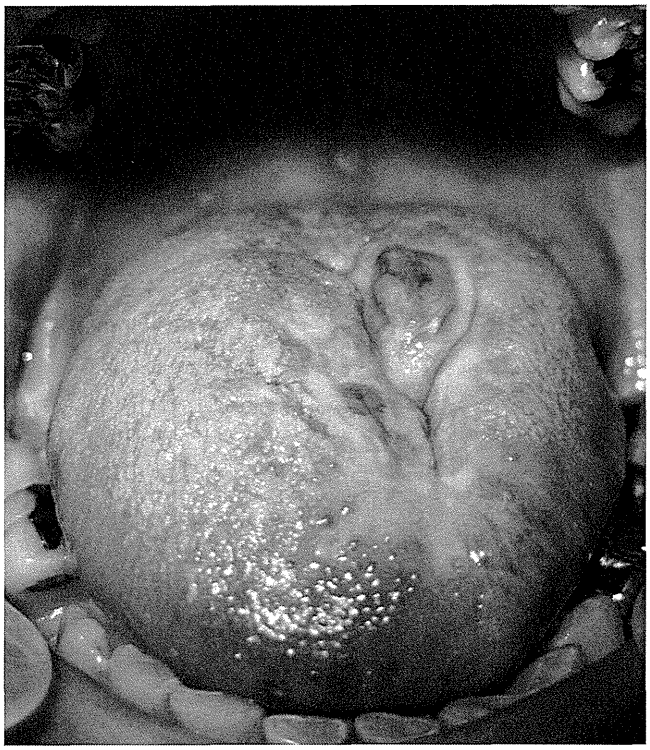


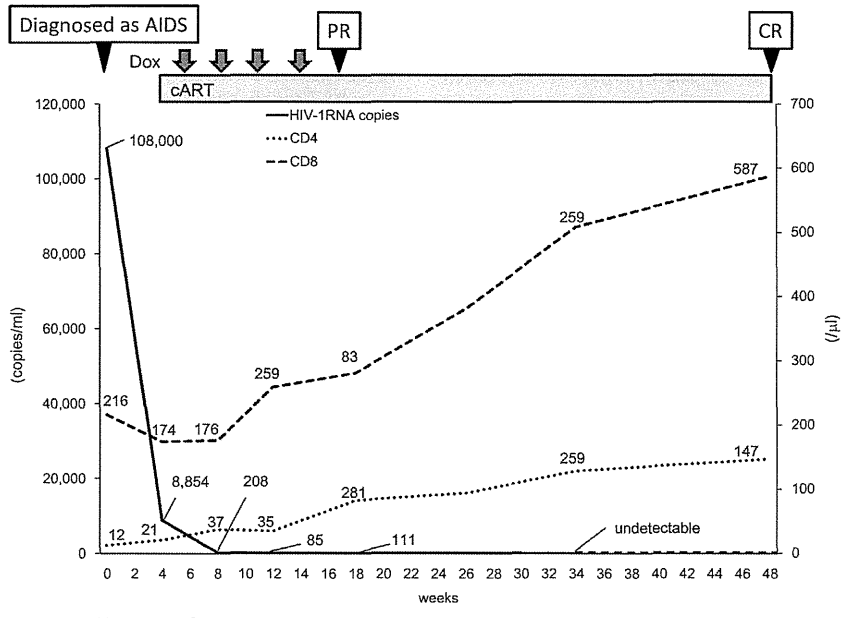
Fig. 4. The appearance of the tongue lesion at 4 weeks after the initiation of cART. AIDS-OKS was significantly reduced and in a partial remission (PR) 4 weeks after the initiation of cART.

patient was referred to a general internist at our hospital. In addition, because the patient was strongly suspected to be infected with HIV due to a history of commercial sex, he was referred to a hematologist at our hospital. The patient was found to have a CD4-positive T-lymphocyte count/percentage of 12 cells/μL/39.6%, a CD4/CD8 ratio of 0.05, and an HIV-1 RNA viral load of 108,000 copies/mL. Therefore, he was diagnosed with AIDS.

Fig. 5. The appearance of the tongue lesion at 1 year after the initiation of cART. The mass of AIDS-OKS completely disappeared.

To obtain an accurate diagnosis for the neoplastic tongue lesion, an incisional biopsy was performed under local anesthesia. A histopathological examination showed proliferation of monomorphic spindle cells with slit-like vascular containing erythrocytes (Fig. 3A). A strong reaction for antibodies against HHV-8 was also seen (Fig. 3B). As a result, the histopathological examination led to a final diagnosis of AIDS-OKS.

Regarding the curative approach, cART comprising lamivudine/abacavir sulfate, darunavir ethanolate, and ritonavir was administered. At the same time, chemotherapy was done with liposomal doxorubicin once every 3 weeks. Four weeks after the initiation of the treatment, the oral lesion was found to have



Dox: doxorubicin 20 mg/m²
cART: lamivudine/abacavir sulfate 600 mg/300 mg/day, darunavir ethanolate 1200 mg/day, ritonavir 200 mg/day
PR: partial remission, CR: complete remission

Fig. 6. Time-dependent changes of CD4 and CD8 cell counts and HIV load during the clinical course of the patient with AIDS-OKS.

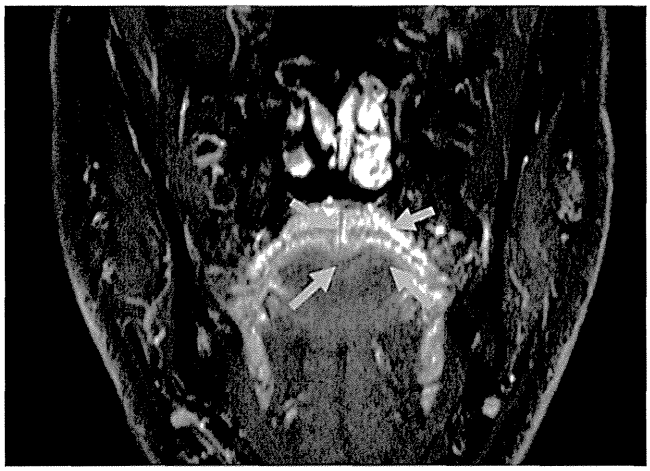


Fig. 7. MRI scan of the tongue lesion at 1 year after the initiation of cART. A contrast-enhanced T1-weighted MRI scan revealed that OKS was in a complete remission (CR).

significantly decreased in size (Fig. 4). At present 1 year after the initiation of cART, the patient is still alive in good health condition, and HIV viral load cannot be ascertained (Fig. 6). His OKS is in complete remission (CR) and no recurrent sign of OKS has been identified in clinical and imaging follow-up examinations (Figs. 5 and 7).

3. Discussion

In this paper, we report the case of a patient who presented with AIDS-KS of the tongue, which led to a diagnosis of AIDS. AIDS-KS of the head and neck occurs in 63% of all cases. Eighty percent of patients with AIDS-KS are asymptomatic [13], and this disease may be the first manifestation of AIDS [14,15]. The most typical site of AIDS-KS is the skin. It has been reported that lymph nodes, visceral organs, and mucosal membranes, such as the oral mucosa, may be affected, sometimes without skin involvement [10]. On the other hand, OKS is locally aggressive but rarely fatal and is characterized by multifocal lesions affecting the palate and tonsils [14]. In Japan, there have been some reports focused on AIDS-OKS [16–25]. OKS most frequently occurs on the palate and gingiva, and rarely occurs on the buccal mucosa or the tongue [26]. To the best of our knowledge, there has been only one report in Japan of OKS occurring on the tongue without any other characteristic AIDS-related symptoms.

The clinical differential diagnoses of OKS include oral purpura, bacillary angiomatosis, and pyogenic granulomas, which can be distinguished from each other using microscopy [27]. In most cases, these diseases are easily distinguished from AIDS-OKS based on information obtained from clinical findings and clinical histories. However, having insufficient clinical data may cause misdiagnoses on occasion [25]. Therefore, the final diagnosis must be made based on careful history-taking and sufficient physical examinations [28]. In the present case, the previous histopathological diagnosis and insufficient clinical data led to the misdiagnosis of recurrent hemangioma of the tongue.

Numerous approaches have been adopted to treat KS lesions of the oral cavity, including local radiotherapy [29], intralesional injection of vinblastine or 3% sodium tetradecyl sulfate [30], laser therapy, surgical excision, and the use of cytotoxic alkaloids, bleomycin, anthracycline, paclitaxel [31], or cART with liposomal doxorubicin [32]. The incidence of AIDS-KS has been reduced with the advent of cART [7]. Therefore, it is recommended that most, if not all, patients, be treated with antiretroviral drugs [33,34]. It has also been reported that KS patients with poor prognostic indices should be treated initially with cART and systemic chemotherapy [33,35]. Liposomal doxorubicin is one of the effective chemotherapeutic options for treating KS because of its high anti-tumor effect and low toxicity for non-target organs [36]. Since AIDS-OKS in the present case completely disappeared without any severe side effects after treatment with cART and systemic chemotherapy using liposomal doxorubicin, this treatment seemed to be effective for AIDS-OKS.

In conclusion, if any vascular neoplasm-like lesions of the tongue are seen in adult patients, clinicians, including oral surgeons, should obtain a thorough medical history and consider AIDS-OKS in the differential diagnosis.

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Impact of HIV Infection on Colorectal Tumors: A Prospective Colonoscopic Study of Asian Patients

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Background: Non-AIDS defining cancer has recently become a major problem in HIV-infected patients. Little has been reported on whether HIV infection is a risk factor for colorectal adenoma, especially in Asians.

Methods: The study was conducted under a prospective cross-sectional design and included all adults who underwent colonoscopy. Subjects were matched by age and sex to compare the prevalence of colorectal adenoma, adenocarcinoma, polyps, and other tumors. Detailed risk factors were assessed, including lifestyle habits, medications, comorbidities, gastrointestinal symptom rating scale, HIV-associated factors, and human papillomavirus infection. To evaluate the effects of HIV infection on adenoma, the odds ratio (OR) was estimated by multivariate logistic regression.

Results: A total of 177 HIV-infected patients and 177 controls were selected for analysis. No significant difference was noted in the prevalence of adenoma (n = 29 vs. 40, $P = 0.14$). Multivariate analysis adjusted by baseline demographics and risk factors showed that HIV is not associated with increased risk of adenoma (adjusted OR = 0.66, $P = 0.16$). Kaposi's sarcoma was more common in HIV-infected patients (n = 6 vs. 0, $P = 0.03$). Among HIV-infected patients, advanced age was an independent and significant risk factor for adenoma (adjusted OR = 2.28, $P < 0.01$). CD4 count, HIV-RNA, history of antiretroviral treatment, and oncogenic human papillomavirus infection were not risk factors for adenoma.

Conclusions: HIV infection was not identified as risk for adenoma in Asian patients. However, advanced age was independently associated with increased risk of adenoma. HIV-infected patients should not miss screening opportunity for colorectal adenoma and other gastrointestinal malignancies.

Key Words: colorectal cancer, colorectal adenoma, oncogenic HPV infection, Japan, gastrointestinal malignancy

(*J Acquir Immune Defic Syndr* 2014;65:312–317)

INTRODUCTION

The introduction of highly active antiretroviral therapy (HAART) has significantly improved the morbidity and mortality of HIV-infected patients.[1,2] However, the incidence of non-AIDS defining cancer has increased with prolongation of life expectancy of HIV-infected patients.[3–6]

Colorectal cancer is the third most commonly diagnosed cancer in male patients and the second in female patients, and a major cause of death worldwide.[7] To prevent the development of colorectal cancer and death, removal of premalignant lesion, adenoma, is effective, and screening is recommended in patients aged 50 years and older.[8–11] Recent studies from western countries have suggested that higher incidence of colonic adenoma in patients with HIV infection compared with the general population.[12–14] Furthermore, HIV-infected patients are at high risk of oncogenic human papillomavirus (HPV) infection, and the potential role of HPV infection in the development of colorectal cancer has been suggested.[15–17] However, little is known about the risk of adenoma in HIV-infected patients compared with the general population.

In the past, the incidence of colorectal cancer was lower in Asia compared with Western countries.[18] However, the incidence has increased lately in Asian countries, including Japan, and is currently comparable with that in western countries.[18,19] Nevertheless, all previous studies on colorectal adenoma in HIV-infected patients were only from the United States, and there are no available data in Asia.[12–14] This study reports the findings of a prospective cross-sectional colonoscopic study that compared the prevalence of colorectal

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The authors have no conflicts of interest to disclose.

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adenoma in HIV-infected patients with HIV-negative patients in Japan.

METHODS

Study Design, Setting, and Participants

We conducted a prospective cross-sectional single-center study in adults who underwent colonoscopy between September 2009 and July 2012 at the endoscopy unit of the National Center for Global Health and Medicine (NCGM). NCGM has one of the largest clinics for patients with HIV infection in Japan with more than 3500 registered patients as of May 2013. The institutional review board at NCGM approved this study. The study was conducted according to the principles expressed in the Declaration of Helsinki.

The following inclusion criteria were used in this study: (1) aged 18 years or older, (2) independent in activities of daily living, (3) able to understand written documents and to write, and (4) asymptomatic but desired screening for colorectal cancer, or presented with continuous or intermittent lower gastrointestinal (GI) symptoms. The following exclusion criteria were used in this study: (1) contraindication or patient refusal of total colonoscopy, (2) colonoscopy for follow-up evaluation during the study period, and (3) previous diagnosis of either adenoma or adenocarcinoma. All inclusion and exclusion criteria were fulfilled before patients were enrolled in the study. Each HIV-infected patient was matched with 1 HIV-negative patient based on age in 5-year age-bands and sex.

Clinical Factors

A detailed questionnaire was completed at the endoscopy unit on the same day of colonoscopy. Patients were asked about their (1) lifestyle habits (smoking history and alcohol consumption), (2) medications [nonsteroidal anti-inflammatory drugs (NSAIDs) and low-dose aspirin], and (3) comorbidities (hypertension, diabetes mellitus, and coronary heart disease) in a face-to-face interview with the medical staff. With regard to medication history, prescriptions and medical records were reviewed in addition to information provided by the patients to avoid omissions. The survey form included photographs of all these oral drugs, which are approved in Japan. Regular use of medication was defined as oral administration starting at least 1 year before the interview. The smoking index was evaluated among ever and daily smokers and was defined as the number of cigarettes per day multiplied by the number of smoking years. Then, smoking index was categorized into nil, <400, 400–799, and >800. Alcohol consumption was calculated and categorized into nondrinker, light (1–180 g/wk), moderate (181–360 g/wk), and heavy drinker (>360 g/wk). To evaluate lower GI symptoms, the GI symptom rating scale rating on a 7-graded Likert scale was used.[20,21] The GI symptom rating scale consists of 15 questions covering lower GI symptoms: increased flatus, decreased passage of stools, increased passage of stools, loose stools, hard stools, urgent need for defecation, and feeling of incomplete evacuation. Positive symptoms were defined as score ≥ 3.

For HIV-infected patients, CD4 cell count, HIV viral load, history of HAART, and sexual behavior were also obtained. CD4 cell counts within 1 week and HIV-RNA viral load within 1 month were used in the analysis, and positive result for real-time HIV-RNA was defined as $\geq$40 copies per milliliter. Sexual behavior was defined as men who have sex with men or heterosexuality. Furthermore, immediately following colonoscopy, rectal swabs (DNAPAP cervical sampler; Qiagen, Gaithersburg, MD) were obtained. Rectal samples were analyzed for HPV-DNA and genotyping by means of polymerase chain reaction-invader assay as described previously.[22] HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 were defined as oncogenic HPV.[23]

Diagnosis of Colorectal Adenoma, Adenocarcinoma, and Non-Neoplastic Polyps

After intestinal lavage with 2 L of solution containing polyethylene glycol, colonoscopy was performed by experienced gastroenterologists by using an electronic high-resolution video endoscope (model CFH260; Olympus Optical, Tokyo, Japan). The location of all lesions was recorded in electronic endoscopic database (Olympus Medical Systems; Solemio Endo). All visualized lesions were biopsied and histologically assessed by experienced pathologists.

Statistical Analysis

Baseline characteristics were compared using the unpaired Student t test or χ^2 test (Fisher exact test) for quantitative or qualitative variables, respectively. To estimate the effect of HIV infection on adenoma, multivariate logistic regression analysis was performed adjusted for age, sex, and possible risk factors for adenoma (these included smoking and alcohol consumption, diabetes mellitus, coronary artery diseases, and NSAIDs and aspirin use). In addition, we conducted uni- and multivariate logistic regression analysis in HIV-infected patients to elucidate the impact of other factors on adenoma related to HIV-infected patients (CD4 count, HIV-RNA, history of HAART, sexual behavior, and oncogenic HPV infection).

Statistical significance was defined at 2-sided P values < 0.05. We estimated the odds ratios (ORs) and 95% confidence intervals (CIs). All statistical analyses were performed using the Statistical Package for Social Sciences version 17.0 (SPSS, Chicago, IL).

RESULTS

Participants

A total of 177 HIV-infected patients and 177-HIV-negative controls were selected for analysis after the application of the aforementioned exclusion criteria and age matching (Fig. 1). The baseline characteristics are listed in Table 1. The study subjects were mostly men, Asians, and comparatively young. HIV-infected patients were more likely to be smokers

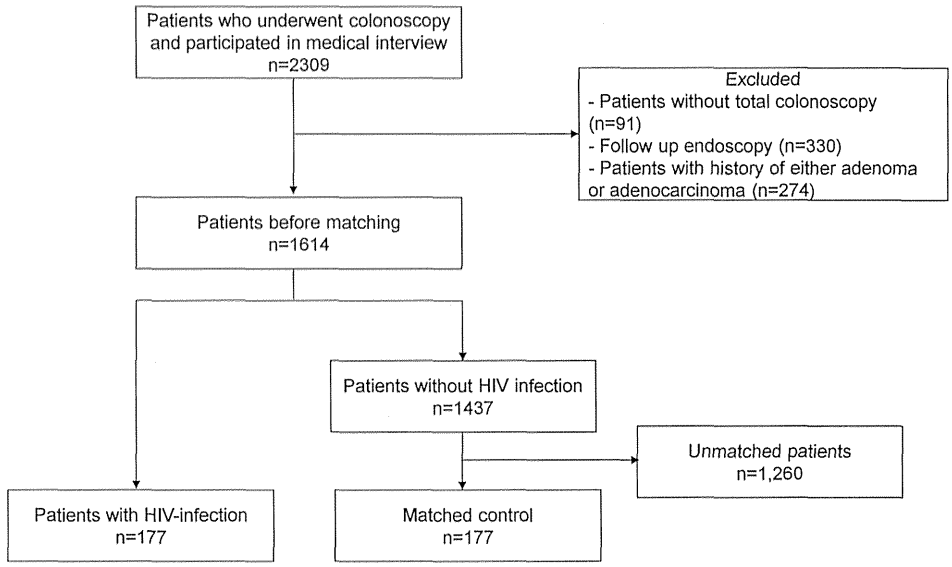

FIGURE 1. Flow diagram of patient selection.

and on treatment with NSAIDs. In contrast, aspirin was mostly used by the control subjects. All other major background parameters were similar in the 2 groups. With regard to the clinical symptoms, there was no difference in GI symptom scores other than increased passage of stools but there was no difference in the proportion of asymptomatic patients between the 2 groups. In patients with HIV infection, the median CD4 count was 371/μL (interquartile range, 121–579), 29.4% of the patients were treatment naive, 75.4% had HPV infection, and 71.5% were infected with oncogenic HPV. The most frequently identified HPV types were type 16 (41%), followed by type 58 (35%), 59 (33%), 52 (27%), 31 (25%), 33 (25%), 51 (19%), 18 (18%), 35 (14%), 39 (13%), 56 (11%), and type 45 (7%).

Prevalence of Colorectal Adenoma, Adenocarcinoma, Non-Neoplastic Polyps, and Other Tumors

Adenomas were identified in 29 (16.4%) patients with HIV infection and in 40 (22.6%) control subjects, and the incidence was not significantly different between the 2 groups (Table 2). Classification of the adenoma according to size (<5, 5–9, and ≥10 mm) showed that HIV-negative subjects tended to have mainly adenomas measuring <5 mm (P = 0.08) although this difference did not reach statistical significance. The incidences of adenocarcinoma and hyperplastic polyps were higher in patients without HIV infection, although the differences in the rates were not statistically significant ($P > 0.05$). In contrast, Kaposi's sarcoma was diagnosed only in HIV-infected patients ($P = 0.03$).

Uni- and multivariate analyses showed that HIV infection did not correlate with higher prevalence of adenoma (Table 3, adjusted OR = 0.66; 95% CI: 0.37 to 1.18; P = 0.16). Multivariate analysis identified age as an independent and significant factor associated with increased risk of adenoma (adjusted OR = 1.72; 95% CI: 1.29 to 2.29; $P <$ 0.01). All other factors did not correlate with adenoma by multivariate analysis.

Factors Associated With Colorectal Adenoma in Patients With HIV Infection

Age was an independent factor associated with increased risk of adenoma by uni- and multivariate analysis (adjusted OR = 2.28; 95% CI: 1.37 to 3.80; $P < 0.01$; Table 4). High CD4 count, low HIV-RNA, and history of HAART were associated with prevalence of adenoma by univariate analysis, although these factors were not significant on multivariate analysis. Oncogenic HPV infection was not associated with adenoma.

DISCUSSION

This study demonstrated that HIV infection was not an independent risk for colorectal adenoma after adjustment for variables known to be related to adenoma. In HIV-infected patients, only age was associated with increased risk of colorectal adenoma, whereas CD4 count, HIV-RNA, and HPV infection were not associated with adenoma by multivariate analysis. To our knowledge, this is the first study that compared the prevalence of colorectal adenoma between patients with and without HIV infection in Asia.

Previous reports suggested possible relation between HIV infection and increased risk of colorectal adenoma.[12–14] Bini et al[12] investigated the prevalence of adenoma in 2382 patients (165 HIV-infected patients and 2217 controls) who underwent screening sigmoidoscopy. Their study identified a high incidence of adenoma in HIV-infected patients and that the risk of such lesion was higher in patients with low CD4 count and long-term HIV infection. The same group also conducted a prospective study of 408 patients who underwent total colonoscopy in the United States.[13] They included only

TABLE 1. Clinical Characteristics of Patients With and Without HIV Infection

	HIV-Positive Patients (n = 177)	HIV-Negative Patients (n = 177)	*P*
Age, yr (IQR)	42 (37–50)	42 (37–50)	0.99
Male gender (%)	167 (94.4)	167 (94.4)	1.00
Asian (%)	171 (96.6)	176 (99.4)	0.12
Cigarette smoking (%)			
Never smoker	58 (32.8)	78 (44.1)	
Smoking index			
<400	89 (50.3)	60 (33.9)	
400–799	22 (12.4)	25 (14.1)	
>800	8 (4.5)	14 (7.9)	0.02*
Alcohol consumption (%)			
Nondrinker	77 (43.5)	59 (33.3)	
Light drinker	82 (46.3)	86 (48.6)	
Moderate drinker	13 (7.9)	24 (13.6)	
Heavy drinker	5 (2.8)	8 (4.5)	0.09
Current NSAIDs use (%)†	27 (15.3)	13 (7.3)	0.02*
Current aspirin use (%)	3 (1.7)	11 (6.2)	0.03*
Diabetes mellitus (%)	9 (5.1)	17 (9.6)	0.10
Coronary vascular disease (%)	6 (3.4)	5 (2.8)	0.76
Asymptomatic, %	33.5‡	36.6‡	0.55
GI symptoms score			
Increased flatus (SD)	1.9 (1.1)	2.0 (1.4)	0.80
Decreased passage of stools (SD)	1.8 (1.3)	1.8 (1.3)	0.76
Increased passage of stools (SD)	2.7 (2.0)	2.2 (1.6)	0.03*
Loose stools (SD)	2.4 (1.6)	2.1 (1.4)	0.13
Hard stools (SD)	1.7 (1.2)	1.6 (1.0)	0.84
Urgent need for defecation (SD)	2.3 (1.7)	2.1 (1.6)	0.14
Feeling of incomplete evacuation (SD)	2.2 (1.3)	2.2 (1.4)	0.61
CD4 count (IQR)	371 (121–579)	NA	NA
HIV-RNA $\log_{10}$/mL (IQR)	1.6 (1.6–3.8)	NA	NA
Treatment naive (%)	52 (29.4)	NA	NA
MSM (%)	135 (76.3)	NA	NA
HPV infection (%)	98/130 (75.4)	NA	NA
Oncogenic HPV (%)	93/130 (71.5)	NA	NA

*$P < 0.05$.

†None of the patients was on selective cox-2 inhibitor.

‡There were 1 missing data in HIV-positive group and 2 in HIV-negative group, thus comparisons were made between 59/176 (33.5%) of HIV-positive and 64/175 (36.6%) of HIV-negative patients.

IQR, interquartile range; SD, standard deviation; MSM, men who have sex with men; NA, not applicable.

TABLE 2. Prevalence of Colorectal Adenoma, Adenocarcinoma, Non-Neoplastic Polyps, and Other Tumors

	HIV-Positive Patients (n = 177)	HIV-Negative Patients (n = 177)	*P*
Any adenoma	29 (16.4%)	40 (22.6%)	0.14
Adenoma, <5 mm	21 (11.9%)	33 (18.6%)	0.08
Adenoma, 5–9 mm	12 (6.8%)	10 (5.6%)	0.66
Adenoma, ≥10 mm	0	4 (2.3%)	0.12
Adenocarcinoma	0	5 (2.8%)	0.06
Hyperplastic polyp	17 (9.6%)	28 (15.8%)	0.08
Other tumors	6 (33.9%)	3 (17.0%)	0.502
Kaposi's sarcoma	6 (33.9%)	0	0.03*
Malignant lymphoma	0	0	1.00
Carcinoid tumor	0	1 (0.6%)	1.000
Lipoma	0	2 (1.1%)	0.499

*$P < 0.05$.

asymptomatic patients aged 50 years or older and found a high rate of colonic neoplasm, including adenoma, in HIV-infected patients. They also reported that patients with HIV infection who were not on treatment with HAART and those with a positive family history of colorectal cancer were at higher risk for colonic neoplasm. In contrast, the study of Kothari et al,[14] which included 130 HIV-infected patients and 779 controls who underwent screening colonoscopy, did not find significant difference in the prevalence of adenoma between the 2 groups. Similarly, our study showed similar prevalence of adenoma in patients with and without HIV infection. These differences may be explained by differences in sample size, populations, and different inclusion criteria. The abovementioned previous studies included only asymptomatic patients whereas this study included many patients with GI symptoms. Taken together, these results suggest lack of consensus on this issue. Thus, it is still unclear whether HIV infection is truly associated with increased risk of colorectal adenoma. Bini et al[12] suggested that the low immune status associated with HIV infection may enhance the development of adenoma; however, CD4 count did not correlate with adenoma in our study. Furthermore, HIV itself is also suggested to play a role in oncogenesis.[24] There is limited information on this issue, and further studies are needed to clarify the association between HIV infection and colorectal adenoma.

In this study, advanced age correlated with increased risk of adenoma in HIV-infected patients. Excision of adenoma prevents colon cancer and screening colonoscopy is recommended for individuals aged 50 years or older.[8,10,11] However, it has been suggested that colorectal cancer screening is underused in HIV-infected patients.[25] In addition, patients with HIV infection are at higher risk for other GI malignancies such as Kaposi's sarcoma, anal cancer, and GI lymphoma than general population,[26–28] and these patients are sometimes asymptomatic.[28–31] Therefore, we believe that screening colonoscopy is important in HIV-infected patients, especially those aged 50 years or older.

The association between HPV infection and colorectal cancer is controversial.[32] Although 2 recent studies argued against such association, a recent meta-analysis study demonstrated increased risk of colorectal cancer with HPV infection.[17,33,34] Because previous reports suggested increased prevalence of colorectal adenoma in HIV-infected patients, in whom the prevalence of HPV infection is known to be higher than that in the general population,[15] we hypothesized

TABLE 3. Uni- and Multivariate Analysis to Estimate the Risk for Adenoma

	Unadjusted OR (95% CI)	*P*	Adjusted OR (95%CI)	*P*
HIV infection	0.67 (0.39 to 1.14)	0.14	0.66 (0.37 to 1.18)	0.16
Age per 10 yrs	1.96 (1.53 to 2.53)	<0.01*	1.72 (1.29 to 2.29)	<0.01*
Male gender	0.71 (0.25 to 2.03)	0.52	0.92 (0.28 to 3.05)	0.89
Smoking	1.60 (1.19 to 2.13)	<0.01*	1.35 (0.98 to 1.86)	0.06
Alcohol consumption	0.89 (0.63 to 1.26)	0.51	0.83 (0.56 to 1.22)	0.34
Current NSAIDs use	0.70 (0.28 to 1.75)	0.45	0.94 (0.35 to 2.53)	0.91
Current aspirin use	4.48 (1.52 to 13.3)	<0.01*	11.8 (0.52 to 6.44)	0.35
Diabetes mellitus	2.37 (1.00 to 5.56)	0.05	1.39 (0.54 to 3.60)	0.49
Coronary heart disease	3.63 (1.08 to 12.3)	0.04	1.30 (0.30 to 5.54)	0.72

*$P < 0.05$.

that oncogenic HPV infection may be a risk factor for adenoma in patients with HIV. However, our results did not find such association.

Fecal occult blood test is a useful screening tool for the detection of colorectal cancers.[10] However, fecal blood test is also positive in various GI diseases such as asymptomatic colitis and Kaposi's sarcoma.[35,36] Thus, the diagnostic accuracy of fecal occult blood test may be less than ideal in HIV-infected patients and accordingly was not used in all subjects in this study. Instead, we assessed the clinical symptoms because we hypothesized that differences in GI symptoms might affect the prevalence of colorectal adenoma. Nevertheless, the proportion of asymptomatic patients was not different between the 2 groups.

Important strengths of this study includes its prospective study design, detailed assessment of GI symptoms and other GI tumors, first study in Asia, and conducting total colonoscopy in all subjects. However, there are several limitations to our study. First, because our study population was younger than those in previous studies, the prevalence might have been underestimated compared with other studies. It is well known that the risk of colorectal cancer increases with age.[37] Thus, the young age of our study subjects and the small sample size of our study could have masked any association between HIV infection and colorectal adenoma. Similar to the study by Bini et al,[13] which examined the relation between HIV infection and colorectal adenoma, larger studies on patients aged 50 years or older will be needed in Asia. Second, because we included both symptomatic and asymptomatic patients who underwent diagnostic colonoscopy, a selection bias could not be ruled out in our study. As a result, it is possible that the control group could have included patients suspected to have colon cancer, whereas HIV-infected patients tended to include those who were referred for colonoscopy based on the suspicion of opportunistic infections, which might have led to the higher prevalence of adenoma in the control group. However, the background characteristics and proportion of asymptomatic patients were similar between the 2 groups. Third, although we collected detailed information on risk factors of adenoma, we could not collect data on factors such as obesity and family history of colon cancer as reported previously,[38,39] and these might have influenced the results.

In conclusion, the incidence of adenoma was not significantly different between patients with and without HIV infection. However, it should be noted that 16.4% HIV-infected patients had adenoma and its risk increased with age. As the issue of aging in patients with HIV infection is growing, the results of this study carry certain significance. Thus, HIV-infected patients should not miss screening opportunities for colorectal adenoma and other HIV-related malignancies.

TABLE 4. Uni- and Multivariate Analyses to Estimate the Risk for Adenoma in HIV-Infected Patients

	Unadjusted OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
Age	2.49 (1.66 to 3.79)	<0.01*	2.28 (1.37 to 3.80)	<0.01*
CD4 count per 10/μL	1.02 (1.00 to 1.03)	0.02*	1.01 (0.99 to 1.03)	0.54
HIV-RNA log10/mL	0.40 (0.21 to 0.76)	<0.01*	0.50 (0.18 to 1.37)	0.18
Treatment naive	0.15 (0.03 to 0.64)	0.01*	1.31 (0.12 to 14.49)	0.83
MSM	0.52 (0.22 to 1.24)	0.14	0.66 (0.19 to 2.26)	0.51
Oncogenic HPV	0.25 (0.10 to 0.65)	<0.01*	0.50 (0.17 to 1.47)	0.21

*$P < 0.05$.
MSM, men who have sex with men.

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DNA methylation profiling can classify HIV-associated lymphomas

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Background: HIV-positive patients have a 60-fold to 200-fold increased incidence of non-Hodgkin lymphomas, including Burkitt lymphoma, diffuse large B-cell lymphoma, and primary central nervous system lymphoma. HIV-associated lymphomas frequently have features such as extranodal involvement, decreased responses to standard chemotherapy, and high relapse rates, which indicate a poor prognosis. General pathological features do not clearly differentiate HIV-associated lymphomas from non-HIV lymphomas.

Methods: To investigate the features of HIV-associated lymphomas, we performed genome-wide DNA methylation profiling of HIV and non-HIV lymphomas using Illumina GoldenGate Methylation Cancer Panel I and Illumina Infinium HumanMethylation450 BeadChip microarrays. DNA methylation profiles in HIV-associated and non-HIV lymphomas were characterized using unsupervised hierarchical clustering analyses.

Results: The analyses of promoter regions revealed unique DNA methylation profiles in HIV-associated lymphomas, suggesting profile differences compared with non-HIV lymphomas, which implies specific gene regulation in HIV-associated lymphoma involving DNA methylation. Based on HumanMethylation450 BeadChip data, 2541 target sites were selected as differing significantly in comparisons between HIV-associated and non-HIV-associated lymphomas using Wilcoxon's rank-sum test ($P < 0.05$) and $\Delta\beta$ values more than 0.30. Recurrent cases of HIV-associated lymphoma had different profiles compared with nonrecurrent HIV lymphomas.

Conclusion: DNA methylation profiling indicated that 2541 target sites differed significantly in HIV-associated lymphoma, which may partly explain the poor prognosis. Our data indicate that the methylation profiles of target genes have potential in elucidating HIV-associated lymphomagenesis and can serve as new prognostic markers.

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Keywords: CpG islands, DNA methylation microarray, HIV, HIV-associated lymphomas, poor prognosticators

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Introduction

The incidence of non-Hodgkin's lymphoma is 60-fold to 200-fold higher in patients with HIV infection [1,2]. Most HIV-associated lymphomas are high-grade B-cell lymphomas such as diffuse large B-cell lymphoma, Burkitt lymphoma, and primary central nervous system lymphoma. The clinical course is often aggressive, with a poor prognosis [2]. Since the introduction of highly active antiretroviral therapy, the risk for opportunistic infections and the incidence of AIDS-defining malignancies, including HIV-associated lymphomas, have declined, and prognoses have improved. Nevertheless, lymphomas remain a major cause of death for HIV-infected patients [3]. It is important to identify differences between HIV-associated lymphomas and non-HIV lymphomas, as their clinical and general pathological features do not clearly distinguish them [2]. Recent studies have revealed that the DNA methylation patterns can differentiate among disease subtypes, suggesting that epigenetic DNA alterations are related to carcinogenesis [4,5]. Epigenetic silencing of functionally important genes may contribute to the development of lymphomas [5,6], and promoter hypermethylation of CpG islands (CGIs) in some genes has been reported in aggressive-phenotype lymphoma with a poor prognosis [7]. In this study, we examined DNA methylation of CGIs in a promoter region clustered with HIV-associated lymphomas and non-HIV lymphomas, and investigated the prognostic significance of DNA methylation. Our findings contribute to an understanding of the lymphomagenesis of HIV-associated lymphomas and suggest specific DNA methylation as a useful prognostic biomarker.

Methods

Patients

HIV-associated lymphoma is a pathologically diagnosed malignant lymphoma in HIV patients. Two cohorts were studied. Cohort I consisted of 11 HIV-associated and 18 non-HIV lymphoma patients who visited Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital (CICK), and two non-HIV lymphoma patients who visited the National Center for Global Health and Medicine Hospital (NCGM). Cohort II included nine HIV-associated and 12 non-HIV lymphoma patients who visited NCGM. Formalin-fixed, paraffin-embedded tissues and fresh-frozen tissues were collected from NCGM and CICK, following approval by the ethics committees of both hospitals and in accordance with the Declaration of Helsinki. All patients gave written informed consent for their tissue to be used and for review of their clinical records. Diagnosis was made using the 2008 WHO classification [2]. Hematologists reviewed the tumor specimens and classified them histologically as diffuse large B-cell lymphoma, Burkitt lymphoma, primary central nervous system lymphoma, follicular lymphoma, or Hodgkin's lymphoma. Non-HIV lymphoma samples were randomly selected from among the Burkitt lymphomas, diffuse large B-cell lymphomas, follicular lymphoma, and Hodgkin's lymphoma. Epstein–Barr virus (EBV) status was determined by Epstein–Barr encoded RNA (EBER) *in situ* hybridization and Southern blotting. BCL2 expression was examined by immunostaining.

HumanMethylation450 microarray analysis

Cohort I was analyzed using an Infinium HumanMethylation450 BeadChip microarray [8], which covered 485 577 methylation sites. Genomic DNA was isolated using a DNeasy mini kit (QIAGEN, Valencia, California, USA) according to the manufacturer's protocol. After 1 μg of DNA was ligated at 24°C for 30 min, the reaction was stopped by 5 min at 95°C (REPLI-g FFPE kit; QIAGEN) [9]. The DNA was subjected to genome-wide DNA methylation profiling using an Infinium HumanMethylation450 BeadChip (Illumina, San Diego, California, USA) [8], according to the manufacturer's instructions. The methylation status of specific cytosines is indicated by the β value, with 1 indicating complete methylation and 0 indicating no methylation. We first filtered the probes and samples using the Bioconductor IMA package to load files created by Illumina GenomeStudio software, using the IMA.methy450R function. With this package, we performed filtering steps using the IMA.methy450PP function. The inclusion criteria were as follows: sample call rate, more than 99.5%; detection P value, <0.05; site call rate, more than 90%; probes with no SNPs based on snpsite.txt provided in the IMA package [10]; and probes outside the XY chromosomes. We converted the initial file created by Illumina GenomeStudio to a new file to reflect the filtering results. The data were normalized by entering the filtered data into the Bioconductor lumi package [11]. Using the lumi package, methylation data were first analyzed by the color balance check and then scaled based on the mean of all probes, using methylation simple scaling normalization (SSN) implemented in the lumi package. The Infinium array methylation data are available in the Gene Expression Omnibus database under the accession number GSE42372.

Cancer Panel I microarray analysis

Cohort II was analyzed using the Illumina GoldenGate Methylation Cancer Panel I microarray, a cancer-focused methylation analysis covering 1505 CpG loci from 807 genes (Illumina) [12]. Genomic DNA was isolated (Agencourt FormaPure kit; Beckman Coulter, Brea, California, USA), subjected to sodium bisulfite conversion, labeled with fluorescent dyes, and hybridized to the microarrays according to the manufacturer's protocol. The methylation status of specific cytosines was indicated by the β value (1, complete methylation; 0, no methylation). Only probes with detection P value at

<0.01 were used for the analyses. The X chromosome loci were removed from the analysis, leaving 1421 CpG loci. Raw average β values were not normalized and were used for analyses as per the manufacturer's recommendations. The GoldenGate array methylation data are available in the Gene Expression Omnibus database under the accession number GSE42626.

For the statistical analysis, enrichment analysis of target genes, validation by combined bisulfite restriction analysis (COBRA), and bisulfite DNA sequences, see the Supplementary Methods, http://links.lww.com/QAD/A441.

Results

To identify differences between HIV-associated and non-HIV lymphomas, genome-wide DNA methylation array analyses were performed using Infinium HumanMethylation450 BeadChip technology. DNA from formalin-fixed and paraffin-embedded or fresh-frozen lymphoma tissues collected from the 11 HIV-positive and 20 HIV-negative Asian patients in Cohort I was analyzed (Table 1). DNA methylation throughout the genome was examined using probes targeting six gene regions (Fig. 1a): within 1500 bps of a transcription start site (TSS1500), within 200 bps of a transcription start site (TSS200), and the 5′ untranslated region (5′UTR), first exon (1stExon), body, and 3′ untranslated region (3′UTR) and intergenic regions. Three HIV-negative lymphomas were excluded from the analyses in the filtering steps (see Methods for details). The differences in methylation status between HIV-associated and non-HIV lymphomas were significantly greater for CGIs in the various target regions, compared with non-CGI methylation (Supplementary Fig. 1, http://links.lww.com/QAD/A441). Hierarchical clustering analysis of CGI methylation markers of TSS1500, TSS200, 5′UTR, and 1stExon (Fig. 1b) produced roughly two groups that distinguished HIV-associated lymphomas from non-HIV lymphomas (Groups 1 and 2; Fig. 1b, upper left), with a few exceptions. By contrast, the analysis of non-CGI methylation and CGI methylation in the body and 3′UTR and intergenic gene targets did not give clear groupings (Fig. 1b, upper right and lower images, Supplementary Fig. 2, http://links.lww.com/QAD/A441). As all HIV patients in this study were men (Table 1), we next analyzed male patients only. The CGI results for TSS1500, TSS200, 5′UTR, and 1stExon again clustered into two groups (Supplementary Fig. 3, http://links.lww.com/QAD/A441), suggesting that gender does not affect the results. Generally, patients with HIV-associated lymphomas were younger than patients with non-HIV lymphomas (Table 1) [13]. When we excluded age-related target sites, as previously suggested [14], the analysis of CGI methylation in TSS1500, TSS200, 5′UTR, and 1stExon again produced two groups that distinguished between HIV-associated and non-HIV lymphomas (Supplementary Fig. 4, http://links.lww.com/QAD/A441). These results suggest that DNA methylation of CGIs in promoter regions (TSS1500, TSS200, 5′UTR, and 1stExon) probably distinguishes HIV-associated from non-HIV lymphomas. Among the targets measured, those with a significant absolute difference between HIV-associated and non-HIV lymphomas were used for further analyses (Supplementary Methods, http://links.lww.com/QAD/A441). Compared with non-HIV lymphoma DNA, HIV-associated lymphoma DNA tended to be hypomethylated (Fig. 1c). Representative genes were used to validate the array analyses. Using COBRA, three of the five non-HIV lymphomas cases were methylated as positive controls, whereas none of the HIV-associated lymphomas was detected as methylated at either *RARRES1* or *FGF5* (Fig. 1d, upper). Bisulfite DNA sequencing gave consistent results (Fig. 1d, lower), confirming this tendency toward hypomethylation in Group 1 (Fig. 1d). These findings encouraged us to examine previously analyzed cases in Cohort II.

Table 1. Patient characteristics of lymphoma samples for Human Methylation450 (450K) microarray analysis in Cohort I.

Items examined		HIV	Non-HIV	*P* value (HIV vs. non-HIV)
Sex	Female	0	10	
	Male	11	10	0.0049*
Age	Mean	45.27	64.35	
	SD	16.92	10.60	0.018*
Histology	BL	2	3	
	DLBCL	8	17	
	HD	1	0	0.57
Stage	I & II	3	5	
	III & IV	8	12	
	ND	0	3	0.63
EBV	+	3	7	
	−	8	9	
	ND	0	4	0.22

The statistical significance of differences in the categorical variables was calculated by Fisher's exact test or Wilcoxon's rank-sum test. BL, Burkitt lymphoma; DLBCL, diffuse large B-cell lymphoma; EBV, Epstein–Barr virus; HD, Hodgkin's disease; ND, not determined; SD, standard deviation.
*P<0.05

Data from nine HIV-associated lymphoma samples derived from the first visit of Cohort II, which had been previously analyzed using Illumina GoldenGate Methylation Cancer Panel I (see Methods), were used for hierarchical clustering analyses. The results showed two apparent methylation profiles for HIV-associated lymphomas (Groups 3 and 4, Fig. 2a). The genes with a significant absolute difference between two clusters were used for further analyses (Supplementary Method, http://links.lww.com/QAD/A441). Group 3 tended to be hypermethylated compared with Group 4 (Fig. 2b). COBRA indicated that all of the Group 3 cases were

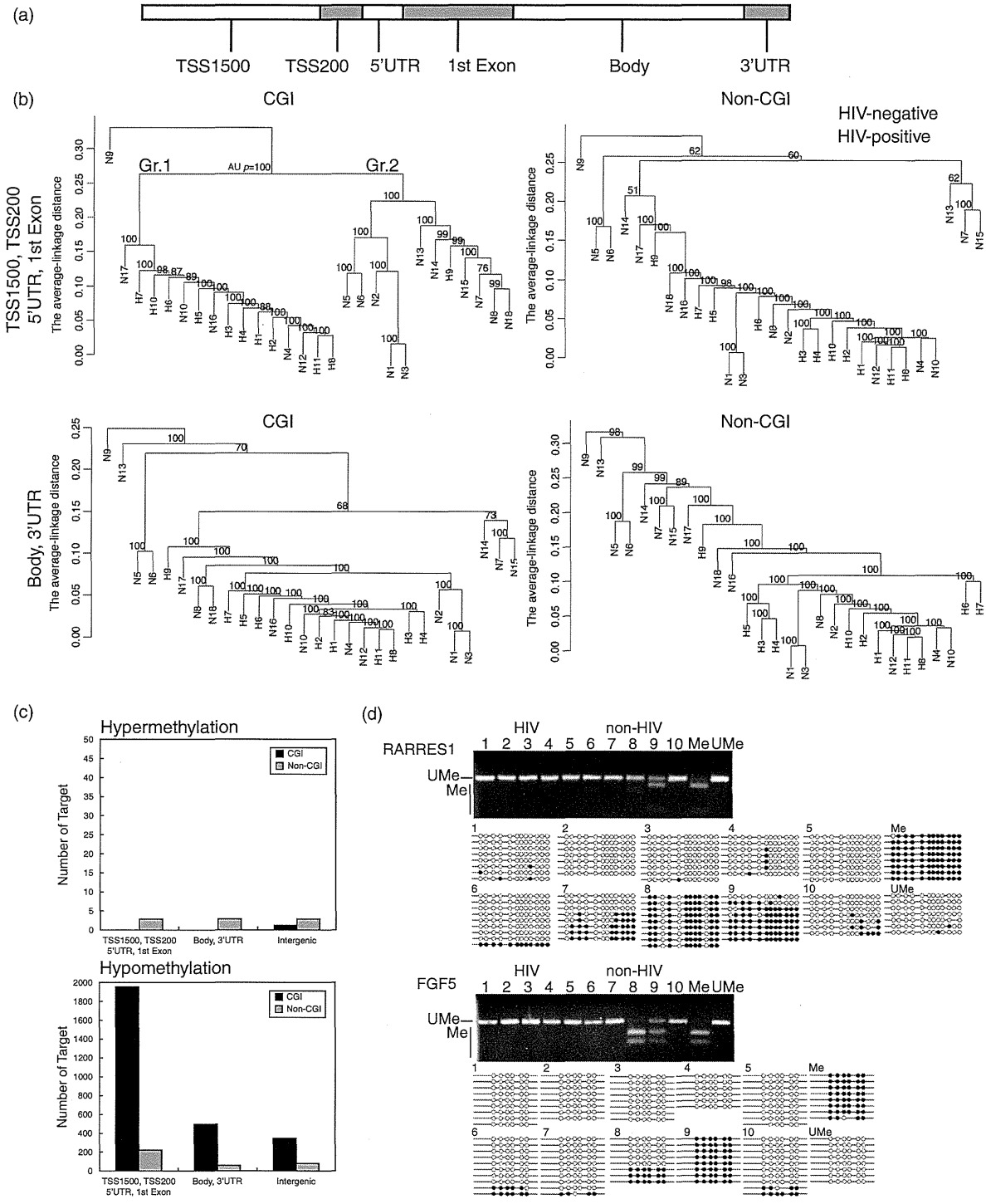

Fig. 1. Methylation profile analysis of HIV-associated and non-HIV lymphoma DNA in Cohort I, using Infinium HumanMethylation450 BeadChip technology. (a) Schematic of the gene regions examined for methylation. (b) Hierarchical clustering analysis of CpG island (CGI) and non-CGI methylation of lymphoma DNA in Cohort I. The analysis of CGI methylation in the promoter regions (TSS1500, TSS200, 5′UTR, and 1stExon) produced two groups that distinguished between HIV-associated lymphomas (Group 1, Gr. 1) and non-HIV lymphomas (Group 2, Gr. 2). TSS, transcription start site; AU *p* value, approximately unbiased *P* value computed using multiscale bootstrap resampling. (c) Numbers of hypermethylation or hypomethylation targets in HIV-associated lymphomas compared with non-HIV-lymphomas. (d) Validation by combined bisulfite restriction analysis (COBRA) and bisulfite DNA sequences. Retinoic acid receptor responder 1 (*RARRES1*) and fibroblast growth factor 5 (*FGF5*) are representative targets in the array analysis. Me, methylated allele or methylated control; UMe, unmethylated allele or unmethylated control; open circle, unmethylated CpG site; solid circle, methylated CpG site; HIV, HIV-associated lymphoma; non-HIV, non-HIV lymphoma.

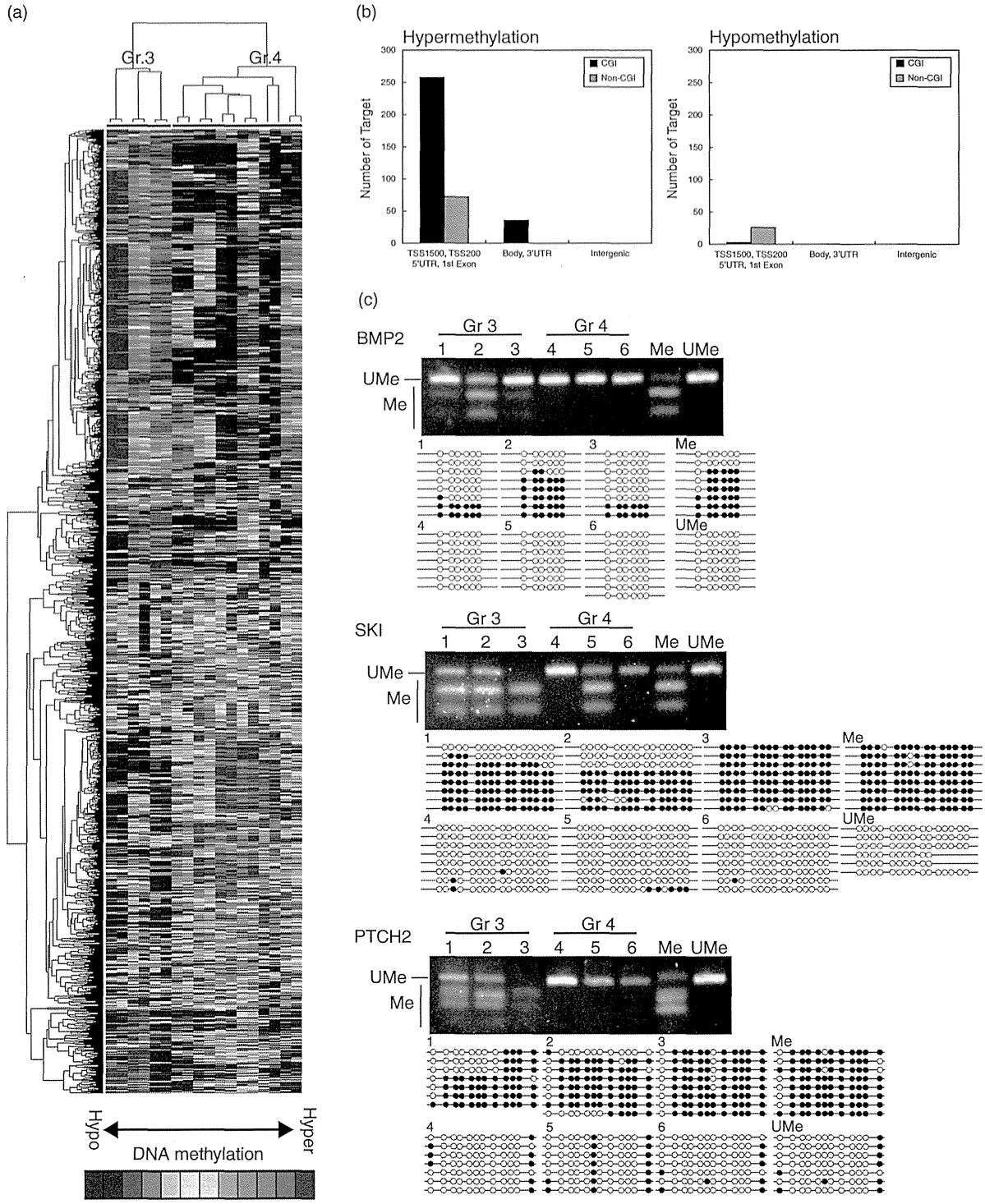

Fig. 2. Methylation profile clustering of HIV-associated lymphoma DNA in Cohort II, using Cancer Panel I. Cancer Panel I microarray analysis was performed for nine HIV-associated lymphomas in Cohort II. The color bar indicates hypermethylation and hypomethylation. Hierarchical clustering analysis of methylation gave two groups: Group 3 (Gr. 3) and Group 4 (Gr. 4). (b) Numbers of hypermethylation or hypomethylation targets in Group 3 compared with Group 4. (c) Validation by combined bisulfite restriction analysis (COBRA) and bisulfite DNA sequences. *BMP2* (bone morphogenetic protein 2), *SKI* (oncogene), and *PTCH2* (patched 2) are representative targets in the array analysis. Me, methylated allele or methylated control; UMe, unmethylated allele or unmethylated control; open circle, unmethylated CpG site; solid circle, methylated CpG site.

methylated, whereas fewer in Group 4 were methylated among those tested (Fig. 2c, upper). Bisulfite DNA sequencing clearly showed that Group 3 was highly methylated (Fig. 2c, lower), confirming the tendency toward hypermethylation in Group 3. Two cases in Group 3 subsequently showed recurrence, representing a significant patient characteristic ($P=0.083$), if 0.1 was considered a significant level (Table 2). In another case in Group 3, a tumor mass appeared in the cervical spinal cord about 17 months later, although recurrence was not confirmed pathologically. Notably, the methylation profile of nonrecurrent HIV-associated lymphomas (Group 4) did not differ significantly from that of non-HIV lymphomas (non-Group 3, Supplementary Fig. 5 and Supplementary Table 1, http://links.lww.com/QAD/A441). These data suggest that recurrent HIV-associated lymphomas have a specific methylation profile.

Discussion

The prognosis of HIV-associated lymphoma has improved with the development of HIV and cancer therapies [15]. Nevertheless, it is important to identify the mechanism responsible for the aggressiveness of HIV-associated lymphomas. Our data suggest that the DNA methylation profile is a molecular indicator of prognosis.

In the methylation analyses, we examined nine or 11 HIV-associated lymphomas. This number was relatively small because of the small HIV-positive population in Japan [13]. Even so, our data clearly suggest that DNA methylation profiles, especially CGI methylation in promoter regions, differ between HIV-associated and non-HIV lymphomas. As the tumor location varies in HIV-associated lymphoma [2], it is essential to know whether tumor location influenced our analyses. Lymph nodes were the most frequent tumor location and were broadly similar in Groups 1 and 2 ($P=0.45$; Supplementary Fig. 6a, http://links.lww.com/QAD/A441), although Group 1 had more extra-node variation, probably due to the high proportion of HIV-associated lymphoma. It is noteworthy that Group 1 had narrower correlation distances than Group 2, indicating that the DNA methylation profiles in Group 1 were quite similar, although Group 1 included various tumor locations (Supplementary Fig. 6b, http://links.lww.com/QAD/A441). Additionally, the lymph node cases in Group 1 were very dissimilar from the lymph node cases in Group 2. The data suggested that the clustered results were not due to tumor location. The differences between the profiles may not be related to antiretroviral therapy either, as only two HIV-positive lymphomas in Cohort I were treated with antiretroviral therapy. Coinfections such as EBV with HIV may influence DNA methylation profiles, but we found no significant difference between HIV-associated and non-HIV lymphomas in terms of EBV infection status in our study. However, we cannot exclude the influence of HIV infection on methylation profiles. One of our validation genes, *RARRES1*, is a cancer methylation target [16] that is differentially expressed in various tumors [17,18], although its clinical relevance to lymphomas remains unknown. *FGF5* is reported to be a bone metastasis-related gene related to angiogenesis [19]. As angiogenic growth factors have been implicated in a

Table 2. Patient characteristics of lymphoma samples in Cohort II for Cancer Panel I.

Items examined		HIV-associated lymphomas		*P* value (Group 3 vs. Group 4)
		Group 3	Group 4	
Sex	Female	1	0	
	Male	2	6	0.33
Age	Mean	36.66	35.00	
	SD	5.77	13.78	1.00
Histology	BL	2	3	
	DLBCL	1	1	
	HD	0	2	1.00
Bcl-2	+	0	1	
	−	3	5	1.00
Stage	I & II	0	2	
	III & IV	3	4	0.50
EBV	+	1	4	
	−	2	2	0.52
Recurrence	+	2	0	
	−	1[a]	6	0.083
IPI score[b]	0 or 1	0	1	
	2 or 3	1	1	
	4 or 5	2	2	1.00

The statistical significance of differences in the categorical variables was calculated by Fisher's exact test or Wilcoxon's rank-sum test. BL, Burkitt lymphoma; DLBCL, diffuse large B-cell lymphoma; EBV, Epstein–Barr virus; HD, Hodgkin's disease.
[a]A tumor mass appeared in the cervical spinal cord about 17 months later, although recurrence was not confirmed pathologically.
[b]IPI, International Prognostic Index for non-HD [stage, lactate dehydrogenase (LDH), performance status, age]. SD, standard deviation.

poor prognosis in non-Hodgkin lymphomas [20], hypomethylated *FGF5* may similarly influence the prognosis in HIV-associated lymphomas. Note that several significant pathways related to cell adhesion were found (Supplementary Table 2, http://links.lww.com/QAD/A441). Of these, those involving laminins, collagens, N-cadherin, and caveolin2 were significantly hypomethylated in HIV-associated lymphomas, suggesting that their increased expression initiates and promotes tumors and results in a poor prognosis [21–23]. These data partly support the poor prognosis seen in HIV-associated lymphomas.

Clustering analysis of the Cohort II data obtained using Cancer Panel I placed recurrent or suspicious and nonrecurrent HIV-associated lymphomas into separate groups, suggesting that recurrence of HIV-associated lymphomas is attributable to specific gene regulation involving DNA methylation. *PTCH2*, which was used for validation, was a significant component of the Hedgehog signaling pathway (Supplementary Table 3, http://links.lww.com/QAD/A441), which is related to relapse rate in carcinomas [24]. The data imply that the DNA methylation profile is a good indicator of prognosis. Recently, specific methylation targets have been reported as candidates for new biomarkers of prognosis or metastasis [25,26]. Careful determinations in more cases will identify biomarkers for recurrence in HIV-associated lymphomas.

To our knowledge, this is the first report using molecular technology to distinguish HIV-associated lymphomas from non-HIV lymphomas. Our findings contribute to the understanding of HIV-associated lymphomagenesis and suggest new prognostic biomarkers.

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Conflicts of interest

There are no conflicts of interest.

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