one of two methods that are currently in clinical use, and one EMN system (inReach System; superDimension, Minneapolis, Minnesota, USA) has been marketed mainly in Europe and in the USA.[14] Bronchoscopists navigate EMN systems using a positional sensor that determines direction based on an electromagnetic field generated around the patient's chest. Reported diagnostic yields of EMN for variously sized peripheral pulmonary lesions range from 69% to 74%.[15–17] A randomised study has shown that EMN-assisted bronchoscopy combined with EBUS is more sensitive than either modality alone (diagnostic yield, 88% vs 69% and 59%, respectively).[18]

Virtual bronchoscopic navigation (VBN) is another method in which virtual bronchoscopic images of the bronchial path to a peripheral lesion are generated and used as a guide to navigate the bronchoscope.[19] Since virtual bronchoscopic (VB) and bronchoscopic images are similar, the bronchoscope can be advanced near a target lesion according to the bronchial pathway displayed on the VB images. A VBN system has been developed that allows the automatic production of virtual images of the bronchial path that are matched with actual images for reliable bronchoscopic navigation to sampling sites.[20 21] However, the value of VBN has not yet been clearly and statistically demonstrated. The present multicentre, prospective, randomised study examines the value of VBN-assisted EBUS for diagnosing small peripheral pulmonary lesions of suspected lung cancer.

METHODS

Participants

We enrolled 199 patients who were referred to three Japanese medical centres between April 2006 and August 2007 with peripheral pulmonary lesions (mean diameter ≤3 cm calculated from axial CT images) suspected to be cancer that were not pathologically confirmed. Peripheral pulmonary lesions were defined as those that are surrounded by normal lung parenchyma and thus unlikely to be visualised by bronchoscopy. Most of these lesions were discovered by plain chest x-rays and/or CT images acquired for reasons other than the symptoms caused by the lesions. Eligible patients were men and women ≥20 years old who could tolerate bronchoscopy. The exclusion criteria comprised evidence of endobronchial disease revealed by chest CT, percutaneous oxygen saturation <90%, a range of known severe co-morbid conditions (unstable angina, acute myocardial infarction within the past 3 months, severe asthma or uncontrolled pulmonary infection), pregnancy and unable to proceed without anticoagulant or antiplatelet medications. We monitored the course of lesions that were <10 mm with ground-glass opacity confirmed by CT and excluded them from the study.

Randomisation and intervention

Eligible patients were randomly assigned to VBN-assisted (VBNA) or non-VBN-assisted (NVBNA) groups. Because others have shown that bronchoscopic diagnostic yield is associated with lesion size[8] and physician skill, randomisation was based on lesion size (mean diameter <2 cm or 2–3 cm) and bronchoscopists used a randomised block design to ensure that these factors were balanced in the study arms. Independent, blinded, trial staff randomly assigned the patients before bronchoscopy.

Scan data from multidetector chest CT (16- or 64-row; slice width, 0.5–2 mm) were acquired from all patients without using contrast medium before bronchoscopy. Individual CT data sets from the VBNA group were transferred to a workstation on which VBN software automatically created virtual bronchoscopic images[21] within 20 min. The consecutive images could be moved forwards and backwards and rotated like a bronchoscope in a monitor positioned beside the video-bronchoscopic screen in the endoscopy suite. As assistant physician controlled the virtual bronchoscopic images[20] during bronchoscopy and a bronchoscopist inserted an endoscope as instructed (figure 1). All patients were locally anaesthetised with lidocaine and examined using a thin video-bronchoscope (type P260F; outer diameter, 4.0 mm; Olympus Medical Systems, Tokyo, Japan). Additional pentazocine hydrochloride or hydroxyzine chloride was administered as required.

Bronchoscopic insertion was assisted using the VBN system in the VBNA group. The bronchoscope was introduced into the bronchus of the NVBNA group without VBN support and with reference only to CT axial images. Lesions were visualised in both groups by inserting a 20 MHz mechanical radial-type EBUS probe (external diameter, 1.4 mm; UM-S20-17S; Olympus Medical Systems) with a guide sheath (K-201; Olympus Medical

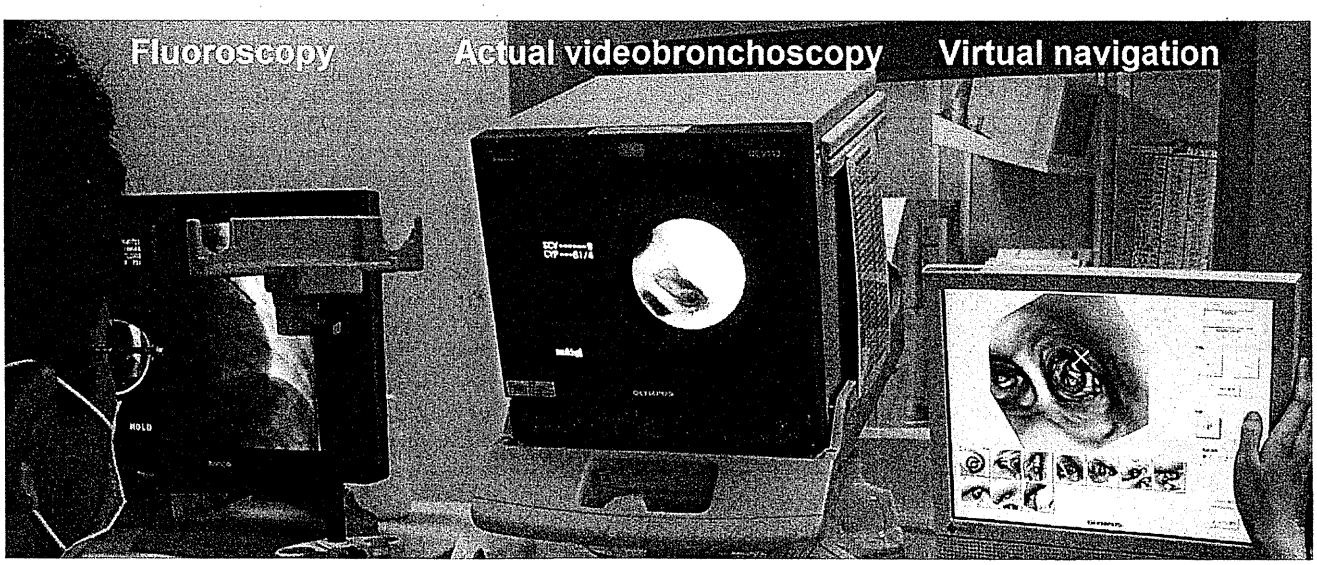

Figure 1 Virtual bronchoscopic navigation. An assistant physician controls virtual bronchoscopic images of the path leading to a peripheral lesion and a bronchoscopist inserts an endoscope as instructed.

Systems) through an endoscopic channel. This probe was withdrawn as soon as the lesion was visualised. Pathological samples were collected using forceps and/or a brush introduced into the guide sheath.[12] If the lesion was not visualised by EBUS, the approach to sampling was decided by the bronchoscopist. The area around the bronchial target was washed as determined by the bronchoscopist with 20 ml of saline as a supplementary procedure. Biopsy samples were immediately fixed in formalin, brush smears on glass slides were immediately fixed in alcohol, and pathologists who were blinded to the results of randomisation processed and evaluated all specimens using standard procedures. The presence of bacteria was assessed in some portions of brush smears and/or lavage. All patients were positioned on an x-ray table, correct device placement was confirmed and sampling was conducted under fluoroscopy.

Outcomes

The primary end point was bronchoscopic diagnostic yield defined as all instances in which the results matched the final diagnosis confirmed by pathological and/or bacterial assessment of bronchoscopic samples. The key secondary end point was total examination duration, which was calculated as the interval between the moment the endoscope passed the vocal cords until its withdrawal from the trachea. Other secondary end points were the interval until the start of sample collection, duration of x-ray fluoroscopy and the generation number of the inserted bronchi. A segmental bronchus was defined as third generation. Safety end points of interest included severe haemorrhage, pneumothorax, hypoxaemia, lidocaine intoxication, arrhythmia, pneumonia and other serious adverse events. Retrieved blood loss >50 ml mixed with or without saline lavage was defined as significant. The safety of all patients was assessed.

Study follow-up

If a lesion was undiagnosed by bronchoscopy, we recommended that the patient consider undergoing other diagnostic procedures, including CT-guided fine needle aspiration (FNA) or surgical intervention. If an undiagnosed patient refused further intervention, follow-up was considered as the second best strategy. Thus, this study continued until the last enrolled patient had been followed up for 2 years. Follow-up information was derived from outpatient clinics, by telephone or by fax contact.

Statistical analysis

Sample size was calculated based on the primary end point. The estimated diagnostic yields in the VBNA and NVBNA groups based on published records were 70% and 50%, respectively.[13 21 22] Thus, at 80% power and α=0.05, we calculated that 190 patients would be required (n=95 in each group) to determine whether diagnostic yield improved with the addition of VBNA. We planned to enrol 200 patients to account for incomplete data or undiagnosed patients.

We analysed the diagnostic yield and safety of the entire intent-to-treat population. Data from the per-protocol population that included all randomised patients with planned bronchoscopic procedures for peripheral lesions were also statistically analysed. Primary and secondary variables were analysed using

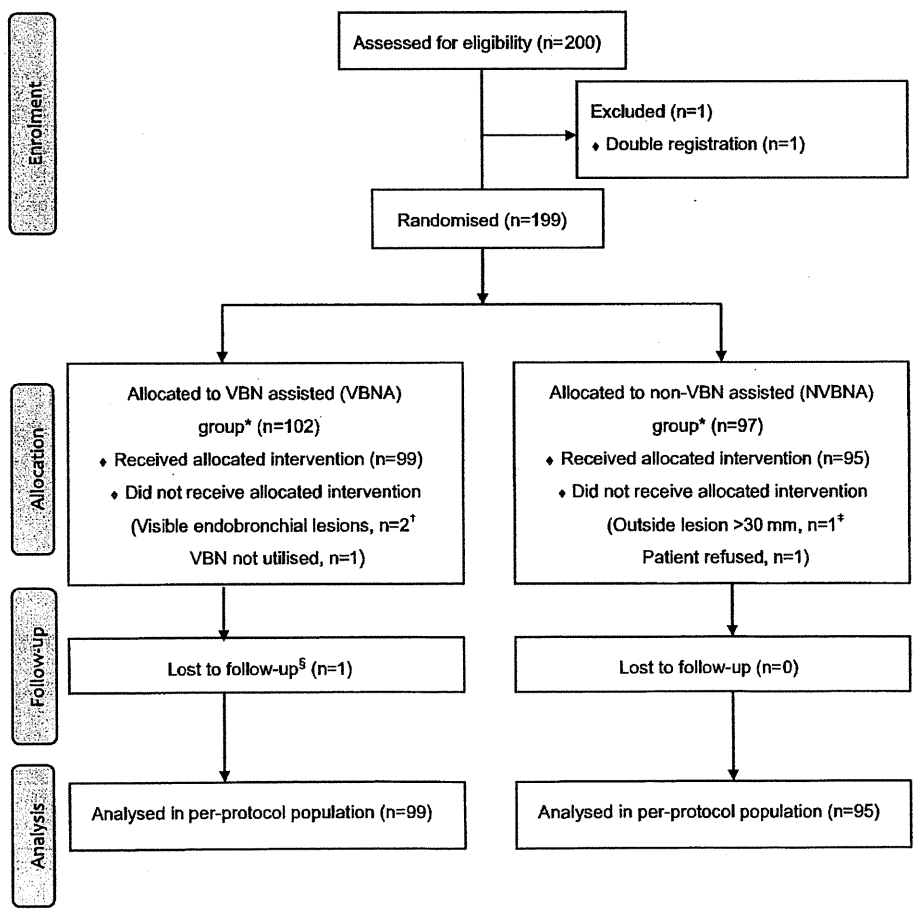

Figure 2 CONSORT flow diagram. *All allocated patients were included in the intention-to-treat population. †Diagnoses established without operating virtual bronchoscopic navigation (VBN). ‡The bronchoscopic procedure was performed according to protocol. §Final diagnosis of this patient was not established; however, this patient was included in the per-protocol analysis.

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the Pearson χ^2 test and the Mann–Whitney U test. Continuous variables were assessed for normality in distribution and the Mann–Whitney test/description as medians was used. All p values were two-sided. A p value of <0.05 indicated statistical significance. All data were statistically analysed using IBM SPSS Statistics, V 18.

RESULTS

We randomly assigned 102 and 97 patients to the VBNA and NVBNA groups. One and two patients in the NVBNA and VBNA groups, respectively, with endobronchially visible malignant or large (>3 cm) lesions were ineligible. We could not use VBN in one patient in the VBNA group and another refused bronchoscopy after assignment to the NVBNA group. We excluded these five patients from the per-protocol populations. The trial profile is shown in figure 2. Age, sex, lesion size and location in patients at baseline were similar between the groups (table 1).

The proportions of primary lung cancer, other malignancies and non-malignant diseases were similar between the groups. Non-malignant diseases comprised tuberculosis, non-tuberculous mycobacterial infection, fungal disease, organising pneumonia, hamartoma and lipoma. Among bronchoscopically undiagnosed patients, 23 (44.2%) of 52 underwent video-assisted thoracoscopy and/or surgery and 8 (15.4%) were diagnosed by CT-guided FNA or repeated bronchoscopy. Twenty of the 21 patients who refused further intervention were followed up for 2 years.

The results indicated a significant difference in diagnostic yield between arms both in the intent-to-treat (all randomly assigned patients, n=199) and the per-protocol populations (finally analysed patients, n=194). Diagnostic yield was significantly higher for the VBNA than for the NVBNA groups in both the intent-to-treat (82 (80.4%) of 102 vs 65 (67.0%) of 97; p=0.032) and per-protocol (80 (80.8%) of 99 vs 64 (67.4%) of 95; p=0.032; table 2) populations.

Diagnostic yield did not differ significantly according to lesion size between the groups in the per-protocol population (table 3).

Table 1 Baseline characteristics and final diagnoses

	VBNA group (n=102)	NVBNA group (n=97)
Age (years, median; range)	69 (21–85)	67 (27–82)
Male, n (%)	64 (62.7)	57 (58.8)
Lesion size (mm, median; range)	18.0 (9.5–30.0)	18.0 (7.0–30.0)
<20 mm, n (%)	59 (57.8)	59 (60.8)
20–30 mm, n (%)	43 (42.2)	38 (39.2)
Lesion location		
Rt. upper lobe, n (%)	32 (31.4)	35 (36.1)
Rt. middle lobe, n (%)	12 (11.8)	6 (6.2)
Rt. lower lobe, n (%)	23 (22.5)	18 (18.6)
Lt. upper lobe, n (%)	25 (24.5)	21 (21.6)
Lt. lower lobe, n (%)	10 (9.8)	17 (17.5)
Final diagnosis		
Malignant disease n (%)		
Primary lung cancer n (%)	69 (67.6)	76 (78.4)
Other malignant disease n (%)	10 (9.8)	4 (4.1)
Non-malignant disease n (%)		
Infectious disease n (%)	17 (16.7)	8 (8.2)
Other benign condition n (%)	5 (4.9)	9 (9.3)
Undetermined n (%)	1 (1.0)	0 (0)

Lt., left; NVBNA, non-virtual bronchoscopic navigation-assisted; Rt., right; VBNA, virtual bronchoscopic navigation-assisted.

Table 2 Diagnostic yields in the intent-to-treat and per-protocol populations

	Bronchoscopic diagnosis		
	VBNA	NVBNA	p Value
Full intent-to-treat	82/102 (80.4)	65/97 (67.0)	0.032
Per-protocol	80/99 (80.8)	64/95 (67.4)	0.032

Data are shown as numbers of lesions/total lesions (%).
NVBNA, non-virtual bronchoscopic navigation-assisted; VBNA, virtual bronchoscopic navigation-assisted.

Of the virtual images constructed based on data from the VBNA group, 98% agreed with actual images of the shape of each bronchial bifurcation on the route. The median generation of virtual bronchial images was six (range, 4–12 bronchi).

The VBN system thus allowed insertion of the endoscope into significantly further generations of bronchi (VBNA vs NVBNA median (range): 4 (2–8) vs 4 (2–7); p<0.001; table 4). The endoscope was also accurately positioned in the VBNA group, with more targets being confirmed by EBUS (VBNA vs NVBNA: 92/99 (92.9%) vs 77/95 (81.1%); p=0.014). Numbers of biopsies and brushings/washes did not differ significantly between the groups. Total examination duration was significantly shorter in the VBNA than in the NVBNA group (median (range): 24.0 (8.7–47.0) vs 26.2 (11.6–58.6) min; p=0.016). The interval to starting sample collection was significantly shorter in the VBNA versus the NVBNA group (8.1 (2.8–39.2) vs (9.8 (2.3–42.3) min; p=0.045). The duration of x-ray fluoroscopy exposure did not differ significantly between the groups. No severe or moderate adverse events were associated with bronchoscopy except for mild pneumothorax that did not require chest drainage in a patient from the NVBNA group.

DISCUSSION

This is the first prospective, multicentre, randomised trial to examine the value of using a VBN system to assist radial EBUS. The findings showed that VBN-assisted EBUS with a guide sheath significantly improved the diagnostic yield of small pulmonary peripheral lesions to 80.4%, which was 13% higher than that in the NVBNA group (67.0%). The diagnostic yield for similar lesions in our previous study using a conventional or thin bronchoscope assisted by VBN under fluoroscopy was 62.5%.[23] The diagnostic yield was 58.3% in another of our studies using EBUS.[13] The high diagnostic yield in the present study was achieved by combining these two procedures. Moreover, although the study cohort was small and diagnostic yields for lesions <20 mm did not differ significantly, the diagnostic yield with VBNA was nevertheless high at 75.9%. Comparison is difficult since x-ray fluoroscopy was used in this study, but the diagnostic yield with EBUS + VBN was comparable with that reported by Eberhardt *et al*.[18] One advantage of VBN compared with EMN is simplicity. Operations such as steering the sensor

Table 3 Diagnostic yield according to lesion size in the per-protocol population

	Bronchoscopic diagnosis		
Lesion size	VBNA	NVBNA	p Value
<20 mm	44/58 (75.9)	35/59 (59.3)	0.056
20–30 mm	36/41 (87.8)	29/36 (80.6)	0.382
Total	80/99 (80.8)	64/95 (67.4)	0.032

Data are presented as numbers of lesions/total lesions (%).
NVBNA, non-virtual bronchoscopic navigation-assisted; VBNA, virtual bronchoscopic navigation-assisted.

Table 4 Bronchoscopic outcomes in the per-protocol population

	VBNA	NVBNA	p Value
Endoscopically inserted bronchial generation (n, median) (range)	4 (2–8)	4 (2–7)	<0.001
EBUS-visualised peripheral lesion, n (%)	92 (92.9)	77 (81.1)	0.014
Sampling by biopsy, (n, median) (range)	5 (0–12)	4 (0–12)	0.113
Sampling by brushing/washing (n, median) (range)	3 (0–6)	3 (0–5)	0.42
Duration			
Total examination (min, median) (range)	24.0 (8.7–47.0)	26.2 (11.6–58.6)	0.016
Initial sampling (min, median) (range)	8.1 (2.8–39.2)	9.8 (2.3–42.3)	0.045
x-ray fluoroscopy exposure (min, median) (range)	9.7 (1.5–22.7)	11.0 (1.3–31.0)	0.058

EBUS, endobronchial ultrasound; NVBNA, non-virtual bronchoscopic navigation-assisted; VBNA, virtual bronchoscopic navigation-assisted.

probe or registration during tests are not required to superimpose the electromagnetic sensor position and CT information as with EMN.[14] Furthermore, the bronchoscope is advanced together with virtual image indications in VBN, which is essentially identical to conventional bronchoscope manipulation.[19] Thus, bronchoscopists with basic skills can easily operate this system after practice on a simulator. Locatable guides attached to the EMN sensor probe are single use, which imposes a cost burden of US$700–1000 per patient depending on market price.[24]

The diagnostic sensitivity of TTNA differs depending on the technique and lesion size, but it is 92% with FNA under CT guidance[6] and 82–90% even for lesions ≤2 cm. However, the main complication with FNA under CT guidance is pneumothorax, which occurs at an incidence of 15–42% [25–27] and is quite frequent with small lesions or emphysema.[27] Reported complications comprise bronchial haemorrhage,[25] needle tract implantation and air embolism.[28] In contrast, the complication rate with transbronchial lung biopsy is 0.2–5% for pneumothorax and 1.2–9% for haemorrhage.[9 29 30] Pneumothorax or other complications have not been found in other studies of EBUS + VBN to date, including the present study.[21 22] The diagnostic sensitivity of EBUS + VBN may not be as high as that with CT-guided FNA, but it is considerably higher than the diagnostic sensitivity of normal bronchoscopy indicated in the ACCP guidelines.[8] Therefore, considering the low rate of complications, VBN combined with EBUS may be a viable option for diagnosing small peripheral lesions.

To our knowledge, this is the first report to describe the duration of navigational bronchoscopy, and that the amount of time required for guidance was decreased in the VBNA group. Moreover, VBN improved diagnostic yield while decreasing the overall duration of the examination by ~2 min. This is thought to be significant in terms of patient comfort, especially considering that patients endured the procedure under local anaesthesia. Although the time required for fluoroscopy tended to decrease in the VBNA group, the difference did not reach statistical significance. Fluoroscopy duration before and after initial sampling was not measured in this study, but almost all of the fluoroscopic exposure was taken up by specimen sampling to confirm proper device use, such as forceps opening and cutting, as well as brushing at adequate sites. Steinfort *et al* reported that radiation exposure from fluoroscopy used together with EBUS does not pose a clinical problem.[31] However, we found that radiation exposure accounted for ~40% of the total duration of the examination. More reliable sampling devices are needed for EBUS with a guide sheath.

We identified the following limitations. The precision of VB decreases when CT data are inadequate during VB; for example, the branch order that can be visualised is lower when slices are too thick.[32] Under such circumstances, repeat CT might be required, which imposes additional cost. Many of the patients in this study were referred from private clinics after abnormalities on plain x-rays had been identified. Therefore, row CT data with a slice thickness of ≤2 mm were collected from the start with multidetector CT instruments at the institution where the present study was conducted and, as a result, virtual images could be created up to sixth generation bronchi. In contrast, the amount of CT data increases with thinner slices and more time is required to create VB images. Most of the time required to create VB images in this study was due to PC processing, but the VB images were created over a period of ~20 min. This depends to some extent on the performance of the software and PC hardware, which could be further enhanced. The VBN system used in the present study (Bf-NAVI; Cybernet Systems, Tokyo, Japan) has been promoted mainly in Japan. The automatic tracking system for the VBN system used herein will allow automatic synchronisation of the virtual to the actual bronchoscopic view.[33] Another VBN system with an automatic tracking system has recently been developed.[24 34] This study showed that diagnostic yield improved with VBN when combined with EBUS which is still not globally applied. We also combined VBN with fluoroscopy, and confirmed lesions in 83% of patients with plain x-rays. Although VBN has been applied without fluoroscopy,[24] when not combined with EBUS it is usually combined with CT[20 35] or fluoroscopy[23] to confirm lesions. Randomised studies with each of these combinations may be necessary to define the diagnostic value of VBN.

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Competing interests FA, HM, KY, TI and Olympus Medical Systems Corporation co-developed the VBN system. FA, HM, KY and TI legally transferred all patent rights to Olympus Corporation without compensation. TI, FA and NS have received speaker fees of less than three hundred thousand yen (~US$3500) per year each from the Olympus Corporation as invited guests to academic medical meetings. All other authors declare that they have no conflict of interest.

Patient consent Obtained.

Ethics approval This study was conducted with the approval of the ethics committee of each of the following institutions: Gifu Prefectural General Medical Center, Gifu, Japan, Hokkaido University School of Medicine, Sapporo, Japan and Fukushima Medical University, Fukushima, Japan.

Provenance and peer review Not commissioned; externally peer reviewed.

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ORIGINAL ARTICLE

A phase III open-label study to assess safety and efficacy of palonosetron for preventing chemotherapy-induced nausea and vomiting (CINV) in repeated cycles of emetogenic chemotherapy

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Abstract
Purpose Prevention of chemotherapy-induced nausea and vomiting (CINV) is of great importance for the completion of multiple cycles of cancer chemotherapy. Palonosetron is a second-generation 5-HT_3 receptor antagonist with proven efficacy for both acute and delayed CINV. This study was designed to assess the safety and efficacy of 0.75 mg palonosetron in repeated cycles of highly emetogenic chemotherapy or anthracycline–cyclophosphamide combination (AC/EC).
Methods We gave 0.75 mg palonosetron to 538 patients 30 min prior to ≥50 mg/m^2 cisplatin or AC/EC on day 1. Prophylactic dexamethasone was administered on days 1–3. The primary endpoint was the incidence rate of adverse events (AEs). The secondary endpoint was complete response rate (CR, defined as no emesis and no rescue medication) throughout the study period.
Results Treatment-related AEs were seen in 44% (237 of 538 patients). Serious AEs were seen in 4% (23 of 538 patients), all considered unrelated or unlikely to be related to palonosetron. Only one patient discontinued the study due to a treatment-related AE. No trend toward worsening of AEs was observed in subsequent cycles of chemotherapy.

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Complete response rates were maintained throughout repeated cycles.
Conclusion The extraordinary safety profile and maintenance of efficacy of 0.75 mg palonosetron combined with dexamethasone were demonstrated throughout repeated chemotherapy cycles.

Keywords Palonosetron · 5-HT_3 receptor antagonist · Antiemetic · Chemotherapy-induced nausea and vomiting · Highly emetogenic chemotherapy

Introduction

Cancer chemotherapy plays a key role in cancer treatment, and it is essential to continue multiple cycles aimed at stabilizing cancer growth and to cure the disease in various clinical settings. Chemotherapy-induced nausea and vomiting (CINV) are among the most problematic adverse events (AEs) in cancer chemotherapy [1–3].

Palonosetron is a second-generation 5-HT_3 receptor antagonist, which has been reported to be effective in the prevention of acute and delayed CINV compared to previous 5-HT_3 receptor antagonists, dolasetron, and ondansetron in moderately emetogenic chemotherapy [4, 5].

Two phase II studies performed in Japan reported a tendency toward better efficacy with the 0.75-mg dose than with 0.25- and 0.075-mg doses of palonosetron, and the excellent safety profile of all these doses suggested that 0.75 mg palonosetron could be the recommended dose for use in a trial [6, 7]. A phase III trial showed non-inferiority of palonosetron to granisetron in the acute phase, superiority of palonosetron to granisetron in the delayed phase in prevention of CINV, and similar safety profiles of palonosetron and granisetron in patients receiving cisplatin or anthracycline–cyclophosphamide combination therapy (AC/EC) [8].

A study has reported the safety and efficacy profile of 0.75 mg palonosetron in repeated cycles of chemotherapy [9].

The goal of this trial was to confirm the safety and efficacy profile of 0.75 mg palonosetron, combined with dexamethasone in patients receiving repeated cycles of highly emetogenic chemotherapy or AC/EC.

Methods

Patients

The patients enrolled in this open-label study on repeated chemotherapy cycles were selected from among patients who had previously completed the randomized phase III trial of palonosetron compared to granisetron [8] and were scheduled to receive the same chemotherapy regimen as in the randomized phase III study (≥50 mg/m^2 cisplatin or AC/EC). All patients provided written informed consent prior to enrollment. Eligible patients were men and women ≥20 years of age with a confirmed diagnosis of malignant disease. Patients were required to have an ECOG performance status of 0–2, adequate bone marrow function (WBC≥3,000/mm^3), hepatic function (AST and ALT <100 U/L or grade ≤3 according to the Common Terminology Criteria for Adverse Events v3.0 (CTCAE) for patients with liver metastasis), and renal function (creatinine clearance ≥60 mL/min).

The exclusion criteria included severe, uncontrolled, concurrent illness other than neoplasia; asymptomatic metastases to the brain; seizure disorders requiring anticonvulsants, unless clinically stable; gastric outlet or intestinal obstruction; any vomiting, retching, or grade ≥2 nausea according to CTCAE v3.0; a known hypersensitivity to palonosetron or other 5-HT_3 receptor antagonists or dexamethasone ingredients; participation in another drug study or receipt of any investigational agents other than palonosetron within a month of enrollment in the study; pregnant or breast-feeding women; and all subjects (men or women) who planned conception during the study period.

Study design

This phase III, multicenter, open-label trial was conducted between July 2006 and August 2007 in Japan. Eligible patients received 0.75 mg palonosetron 30 min before cisplatin or AC/EC initiation on day 1 in each cycle. Administration of 16 mg prophylactic dexamethasone i.v. within 45 min before palonosetron on day 1 was also required. Additionally, 8 mg dexamethasone i.v. for patients receiving cisplatin or 4 mg p.o. for patients receiving AC/EC was administered on day 2 (24–26 h after chemotherapy) and day 3 (48–50 h after chemotherapy). For patients receiving irinotecan on day 8 or after, palonosetron was administered 30 min before the administration of irinotecan (e.g., day 8 and day 15 in combination chemotherapy of cisplatin and irinotecan for lung cancer). The interval between administrations of palonosetron had to be 7 days or more. Administration of dexamethasone was permitted before irinotecan at the discretion of each investigator. More than one factor influenced the choice of dexamethasone dose and schedule in this trial, including international antiemetic guidelines [10–12], the results of Japanese clinical studies on antiemetic agents [13, 14], and the findings of a survey on antiemetic treatments conducted in the trial sites. Patients repeatedly received up to four cycles of the study treatment, including treatment received during the first cycle, described as the treatment administered in the previous randomized phase III trial in which the patients participated before entering this

trial. Patients were confirmed for eligibility to continue study treatment before the start of each cycle according to the following discontinuation criteria: not meeting the eligibility criteria; receiving an antiemetic drug within 24 h before the start of a cycle; or vomiting, retching, or grade 2 or higher nausea within 24 h before the start of a cycle.

Efficacy was assessed every 24 h for 5 days, only after administration of cisplatin or AC/EC. The safety profile of palonosetron was assessed from its first administration, until 8 days after its last administration.

The study was conducted according to the Declaration of Helsinki, and written approval was obtained from the Institutional Review Boards at each site before study commencement.

Study visits and assessment procedures

The 12 lead-ECG and laboratory assessments were conducted within 8 days before the beginning of the first cycle, and once each during days 2–4 and 8–10 of each cycle. In patients receiving irinotecan, these assessments were also carried out 7–9 days after every administration of palonosetron. AEs and concomitant medications were recorded.

The investigators judged the causal relationship between AE and palonosetron according to five categories (none, unlikely, possible, probable, and definite). Any AE judged by the investigator to be possibly, probably, or definitely related to palonosetron was regarded as a treatment-related AE.

Study endpoints

The primary endpoint was the rate of AEs in the study. The secondary endpoints were the type, severity, and causal relationship of the AEs, the proportion of patients with a complete response (CR; defined as no emetic episodes and no rescue medication use), and severity of nausea. Severity of nausea was indicated as none, mild, moderate, or severe, according to a Likert scale, based on subjective evaluation by each patient. Patient diaries were used for recording of emetic episodes, nausea, or rescue anti-emetics at daily (24-h) intervals.

Statistical analysis

The safety analysis cohort included all patients who received the study drug. This safety analysis cohort was divided into three subset cohorts: patients receiving irinotecan combined with cisplatin (irinotecan cohort), patients receiving cisplatin combined with other treatment excluding irinotecan (cisplatin cohort), and patients receiving AC/EC (AC/EC cohort). The modified intent-to-treat (ITT) cohort included all patients who received the study drug and chemotherapy (cisplatin or AC/EC). This modified ITT cohort was used for efficacy analysis.

The data for the patients who received palonosetron in the randomized phase III trial [8] have been considered as both "first cycle" efficacy and safety data; thereafter, the first cycle of this open-label study was counted as the second cycle of chemotherapy.

Safety data were listed and summarized descriptively (data on file). Toxicity grades were generated for hematology and blood chemistry parameters, according to CTCAE v.3.0 adapted toxicity grades, and treatment-related AE were tabulated. New adverse events (NAE) and worsened adverse events (WAE) were listed to identify the safety profile of palonosetron on repeated administration. An NAE was defined as an AE not observed in the first cycle and observed only in the second or subsequent cycles. A WAE was defined as an AE that could be seen in the first cycle but worsened in grade only from the second cycle or later compared to the grade observed in the first cycle.

To evaluate the influence of palonosetron on cardiovascular abnormality, the proportion of patients with QTc prolonged to more than 60 ms from baseline or more than 500 ms was examined in the safety analysis cohort by chemotherapy (cisplatin or AC/EC).

A sample size of 300 patients was needed to find AEs observed in 1% or more of patients after repeating the administration of palonosetron two or more times, including the safety data of palonosetron in the randomized phase III study.

The proportions of patients with CR or no nausea were assessed during the acute phase (0–24 h post-chemotherapy), the delayed phase (24–120 h post-chemotherapy), and the overall phase (0–120 h post-chemotherapy) in each cycle.

All statistical analyses were performed using SAS software (version 8.2; SAS Institute, Cary, NC, USA).

Results

We enrolled 546 patients to receive a single i.v. dose of palonosetron, but eight of these patients did not receive the study treatment since three patients met discontinuation criteria for this study and five patients were withdrawn from this study at the discretion of the investigators. Therefore, 538 patients were evaluated for safety. These 538 patients were also included in the modified intention-to-treat (ITT) cohort for efficacy analysis.

Demographic data for the safety analysis cohort are presented in Table 1. Of the 538 patients in the safety analysis cohort, 304 (57%) women and 358 (67%) patients overall were aged ≥55 years. The most common types of malignant disease were non-small cell lung carcinoma (249 patients [46%]) and breast carcinoma (224 patients [42%]).

Table 1 Patient demographics and baseline characteristics

		N=538	
		N	%
Age categories (years)	Mean, SD	57.8, 10.4	
	≥55	358	66.5
	<55	180	33.5
Height (cm)	Mean, SD	160.00, 8.25	
Weight (kg)	Mean, SD	57.89, 10.07	
Sex	Women	304	56.5
	Men	234	43.5
PS	0	388	72.1
	1	147	27.3
	2	3	0.6
Previous surgery	No	257	47.8
	Yes	281	52.2
Previous radiation	No	486	90.3
	Yes	52	9.7
Alcohol consumption within 180 days of enrollment	No	236	43.9
	Rarely	72	13.4
	Sometimes	60	11.2
	Everyday	170	31.6
Tumor type	Non-small cell lung carcinoma	249	46.3
	Small cell lung carcinoma	45	8.4
	Breast carcinoma	224	41.6
	Others	20	3.7
Chemotherapy	Cisplatin with treatment excluding irinotecan	277	51.5
	Cisplatin with irinotecan	37	6.9
	AC/EC	224	41.6

Regarding chemotherapy regimen, 277 of 538 patients (51%) were given cisplatin combined with other treatment excluding irinotecan, 224 of 538 patients (42%) received AC/EC, and 37 of 538 patients (7%) were given irinotecan. Furthermore, vinorelbine (95 of 277 patients [34%]) and gemcitabine (89 of 277 patients [32%]) were agents commonly combined with cisplatin; fluorouracil (92 of 224 patients [41%]) was associated with AC/EC.

The numbers of patients receiving palonosetron in each cycle are shown in Table 2. Over 50% of the patients received palonosetron through cycle 3. The minimum, median, and maximum numbers of administrations of palonosetron throughout the study period were 1, 3, and 10, respectively.

Of the 538 patients in the safety analysis cohort, 536 patients (99.6%) experienced at least one AE. In the subcohort of the safety analysis, patients reported to have at least one AE, 99% (275 of 277) of patients were in the cisplatin cohort, 100% (224 of 224) in the AC/EC cohort, and 100% (37 of 37) in the irinotecan cohort. Treatment-related AEs judged by the investigators to be possibly, probably, or definitely related to palonosetron were reported in a total of 44% (237 of 538) of the safety analysis cohort, including 35% (97 of 277) of the cisplatin cohort, 55% (123 of 224) of the AC/EC cohort, and 46% (17 of 37) of the irinotecan cohort.

Table 3 shows the main treatment-related AEs that occurred in at least 2% of patients in the safety analysis

Table 2 Number of patients in each cycle in the safety analysis cohort

N = total number of patients in a cohort

n = number of patients for each cycle

Cohort	N	Cycle 1		Cycle 2		Cycle 3		Cycle 4	
		n	%	n	%	n	%	n	%
Cisplatin	277	277	100.0	230	83.0	153	55.2	66	23.8
Irinotecan	37	37	100.0	36	97.3	25	67.6	7	18.9
AC/EC	224	224	100.0	220	98.2	211	94.2	98	43.8

Table 3 Treatment-related adverse events

Cohort	Cisplatin (*N*=277)			AC/EC (*N*=224)			Irinotecan (*N*=37)			Total (*N*=538)		
	G1, *n* (%)	G2, *n* (%)	G3, *n* (%)	G1, *n* (%)	G2, *n* (%)	G3, *n* (%)	G1, *n* (%)	G2, *n* (%)	G3, *n* (%)	G1, *n* (%)	G2, *n* (%)	G3, *n* (%)
Constipation	31 (11.2)	12 (4.3)	1 (0.4)	57 (25.4)	19 (8.5)	2 (0.9)	5 (13.5)	2 (5.4)	0 (0.0)	93 (17.3)	33 (6.1)	3 (0.6)
Electrocardiogram QTc prolonged	6 (2.2)	2 (0.7)	1 (0.4)	14 (6.3)	16 (7.1)	0 (0.0)	0 (0.0)	2 (5.4)	0 (0.0)	20 (3.7)	20 (3.7)	1 (0.2)
Angiopathy	19 (6.9)	1 (0.4)	0 (0.0)	16 (7.1)	0 (0.0)	0 (0.0)	2 (5.4)	0 (0.0)	0 (0.0)	37 (6.9)	1 (0.2)	0 (0.0)
Alanine aminotransferase increased	12 (4.3)	6 (2.2)	3 (1.1)	4 (1.8)	2 (0.9)	0 (0.0)	0 (0.0)	1 (2.7)	1 (2.7)	16 (3.0)	9 (1.7)	4 (0.7)
Aspartate aminotransferase increased	11 (4.0)	5 (1.8)	4 (1.4)	2 (0.9)	2 (0.9)	0 (0.0)	0 (0.0)	2 (5.4)	0 (0.0)	13 (2.4)	9 (1.7)	4 (0.7)
Headache	9 (3.2)	0 (0.0)	0 (0.0)	12 (5.4)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	21 (3.9)	1 (0.2)	0 (0.0)
Gamma-glutamyl-transferase increased	5 (1.8)	2 (0.7)	1 (0.4)	2 (0.9)	0 (0.0)	1 (0.4)	1 (2.7)	0 (0.0)	1 (2.7)	8 (1.5)	2 (0.4)	3 (0.6)

Possibly, probably, or definitely related to study product and over 2% incidence of patients in the safety analysis cohort

N = total number of patients in a cohort

G1, G2, G3 = Grade of adverse event as per CTCAE v.3

n = number of patients with at least one treatment-related AE

cohort. The incidences of constipation and electrocardiographic QTc variation were higher in patients receiving AC/EC than in those receiving cisplatin.

The proportions of patients who experienced an increase in QTc value more than 60 ms (QT1) from baseline or more than 500 ms (QT2) are summarized in Table 4. There was no clinically significant difference in the proportion of patients who experienced increase in QTc value between the patients receiving cisplatin and those receiving AC/EC, and the proportion was low (less than 3%) in both treatments, with no QTc variation reported to be symptomatic.

The incidence of NAE, defined as AEs observed only from the second cycle, was very low (less than 1%). In addition, the incidence of WAE, defined as AEs worsened by grade, starting from the second cycle compared to their grade in the first cycle, was very low (less than 0.5%). Among NAEs and WAEs, only one case of angiopathy was judged to be definitely related to palonosetron. This patient recovered within a day without treatment.

Serious AEs were reported in 4% of patients (23 of 538). All of these events were judged to be unrelated or unlikely to be related to palonosetron by the investigators.

Three patients withdrew from the study. Only one withdrawal, due to atrial fibrillation, was judged to be possibly related to palonosetron. The atrial fibrillation was not serious and resolved in 8 days with medical treatment. Two other patients withdrew from the study due to AEs judged to be related to chemotherapy or treatment for concomitant disease.

The proportion of patients with complete response to each of the four chemotherapy cycles considered in this study ranged from 72% to 77% in the acute phase, from 56% to 63% in the delayed phase, and from 52% to 56% in the overall phase (Fig. 1a). Similarly, the proportion of patients with no nausea in each cycle ranged from 55% to 61% in the acute phase, from 35% to 41% in the delayed phase, and from 33% to 39% in the overall phase (Fig. 1b). There were no major differences in the efficacy parameters

Table 4 Number of patients (percent) with QTc variations in cisplatin and AC/EC cohort in each cycle

Chemotherapy		Cycle 1	%	Cycle 2	%	Cycle 3	%	Cycle 4	%
Cisplatin	Evaluable patients (*N*)	314		266		178		73	
	QT1 (*n*)	3	1.0	5	1.9	1	0.6	2	2.7
	QT2 (*n*)	1	0.3	2	0.8	0	0.0	0	0.0
AC/EC	Evaluable patients (*N*)	223		220		211		98	
	QT1 (*n*)	4	1.8	3	1.4	4	1.9	1	1.0
	QT2 (*n*)	0	0.0	1	0.5	0	0.0	0	0.0

QT1 more than 60 ms from baseline, *QT2* more than 500 ms (absolute QTc value)

N = total number of evaluable patients in a cohort for each cycle

n = number of patients with QT1 or QT2 for each cycle

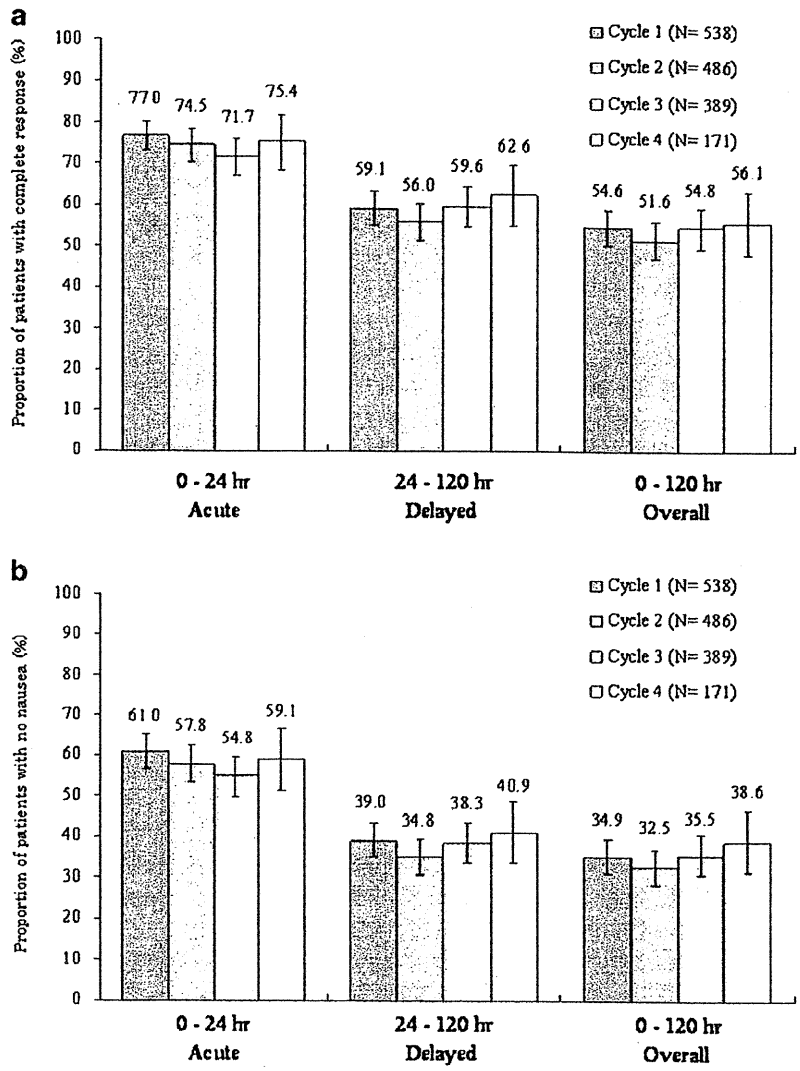

Fig. 1 **a** Proportion of patients with complete response for each study cycle. **b** Proportion of patients with no nausea for each study cycle. *Error bars* indicate 95% confidence intervals

among cycles within the acute (0–24 h), delayed (24–120 h), or overall (0–120 h) phases.

Discussion

In this phase III trial for patients receiving cisplatin or AC/EC in repeated chemotherapy cycles, an excellent palonosetron safety profile was observed.

Many women were enrolled in this trial because AC/EC was the treatment for breast cancer. Although the population of this study consisted of patients receiving highly emetogenic chemotherapy or AC/EC, they did not receive three-drug antiemetic regimens including a 5-HT_3 receptor antagonist, dexamethasone, and aprepitant. This is because aprepitant was not available in Japan at the time when this study was conducted. The dose of palonosetron, approved by the Ministry of Health, Labor and Welfare (MHLW) in Japan, was higher than that recommended by the international guidelines [12]. Both 0.75- and 0.25-mg doses of palonosetron exhibited superiority to ondansetron or dolasetron in the delayed phase in two comparative phase III studies for moderately emetogenic chemotherapy [4, 5]. Additionally, 0.75 mg palonosetron was superior to granisetron in the delayed phase in a phase III study for highly emetogenic chemotherapy [8]. The 0.75-mg i.v. dose of palonosetron is the dose approved in Japan by the MHLW, driven by results of the phase III comparative study [8] and two phase II dose-ranging studies for use in combination with dexamethasone [6, 7]. These dose-ranging phase II and comparative phase III studies showed no difference in safety between the two doses of 0.75 and 0.25 mg palonosetron.

The safety profile of this study showed that AEs related to palonosetron were similar to those identified in the safety profile described in a single chemotherapy cycle studies,

with a single administration of palonosetron [8]. Also, in a small population treated with palonosetron in each irinotecan cycle, almost weekly, the safety profile was similar to that described in single palonosetron dose studies.

NAE and WAE were reported in a very small number of patients and were mainly judged not to be related to palonosetron but to antineoplastic treatment or to the primary disease. No worsening trend in AEs was observed in the subsequent cycles of chemotherapy. Therefore, the results of this study did not arouse any special concern related to the administration of palonosetron in repeated cycles of chemotherapy.

Interactions of some 5-HT_3 receptor antagonists with human cardiac ion channels are known and have been reported [15], and recently, the effect of palonosetron on QTc prolongation has been studied in an European double-blind, randomized, placebo-controlled trial, which showed no significant effect on any ECG interval, including QTc duration, with intravenous palonosetron administered up to 2.25 mg, three times the study dose [16]. In the double-blind, randomized phase III study, the incidences of QTc prolongation in the palonosetron group and in the granisetron group were comparable [8]. In the present study, we carefully evaluated ECG because the effect of palonosetron on ECG interval was not known at the start of this study. The incidence of QTc prolongation was higher in those patients receiving AC/EC than in those receiving cisplatin; however, the proportion of patients with an increase in QTc (more than 500 ms as an absolute value or more than 60 ms difference from the baseline value) was very low, both in the patients receiving cisplatin and those receiving AC/EC. Therefore, the influence of palonosetron on QTc interval was not found to be clinically significant, as reported in previous studies [8, 16].

Maintenance of the efficacy of palonosetron was also shown during its administration throughout repeated chemotherapy cycles. De Wit et al. [17] reported that the antiemetic effect of granisetron plus dexamethasone was not maintained over multiple cycles of highly emetogenic chemotherapy because failure in its protection against delayed emesis negatively influenced the antiemetic effect against acute emesis in the subsequent cycles. We considered that efficacy of palonosetron in the delayed phase might contribute to the maintenance of antiemetic effect throughout repeated chemotherapy cycles. It is of great importance to assure maintenance of efficacy, as well as to provide a very good safety profile to assure patient compliance with chemotherapy, especially when administered in multiple cycle regimens.

In conclusion, in this multiple cycle study conducted with palonosetron, the analysis of AEs did not raise any safety concerns; the type and intensity of treatment-related AEs were consistent with previous reports for palonosetron and for 5-HT_3 receptor antagonists; they did not change after repeated administration of the study drug. Both the excellent safety profile and the sustained efficacy of 0.75 mg palonosetron were shown throughout repeated chemotherapy cycles in this study, and also even when it was administered more frequently (at least 7-day intervals) in patients receiving irinotecan-containing regimens.

Further research is warranted to assess this maintenance of efficacy and the excellent safety profile of palonosetron in multiple cycles of emetogenic chemotherapy as well as in combination with other antiemetic class agents.

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CASE REPORT Open Access

Henoch Schönlein purpura associated with pulmonary adenocarcinoma

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Abstract

Introduction: Henoch-Schönlein purpura is a common immunoglobulin A-mediated vasculitis syndrome in children. Henoch-Schönlein purpura can also affect adults and is probably related to malignancy.

Case presentation: We report the case of a 61-year-old Japanese man who presented for examination after an abnormal shadow was detected by chest radiography. He received a diagnosis of pulmonary adenocarcinoma, stage IV. Purpura on the legs, abdominal pain, diarrhea, hematuria and proteinuria developed at this time. Henoch-Schönlein purpura was diagnosed, base on the clinical symptoms and histological findings of biopsy specimens of the skin, which showed vasculitis with immunoglobulin A deposits. Our patient received chemotherapy with gemcitabine after successful steroid therapy for the Henoch-Schönlein purpura.

Conclusion: Although hematological malignancies are well-known causes of vasculitides, cases of Henoch-Schönlein purpura associated with lung adenocarcinoma are rare. Our patient was treated with corticosteroid therapy, which cleared the purpura and cytotoxic chemotherapy for the non-small cell lung cancer. However, he died from heart failure due to cardiac tamponade.

Introduction

Henoch-Schönlein Purpura (HSP) is a systemic vasculitis that involves the small vessels, most notably those in the skin, gastrointestinal tract and glomeruli, and is accompanied by arthralgia or arthritis [1]. HSP occurs commonly in children but can also affect adults [2]. Although the exact cause of HSP remains unknown, malignancy has been reported as a causative factor [3-5]. This report illustrates a rare case of HSP associated with pulmonary adenocarcinoma.

Case Presentation

A 61-year-old Japanese man presented for examination after an abnormal lung shadow was detected during population-based radiological screening. He was a current smoker with a 41 pack-year smoking history. Chest computed tomography (CT) showed a mass measuring 60 × 50 mm in the left upper lobe, with small nodules in both lung fields and left pleura, and mediastinal and subclavicular lymphadenopathy (Figure 1). Bronchoscopy revealed stenosis of the left B3 bronchus with mucosal abnormalities at points from which biopsy specimens were obtained. Cerebral magnetic resonance imaging (MRI) demonstrated multiple brain metastases. The final oncological diagnosis was a stage IV adenocarcinoma (T4N3M1b) without epidermal growth factor receptor gene mutation. After stereotactic radiosurgery for brain metastases, carried out in another hospital, our patient was referred to us to receive chemotherapy.

On admission, our patient had progressive abdominal pain, diarrhea and purpuric rash on the legs, which had developed over the previous month. He was not taking any medication. The abdomen was soft and flat with minor tenderness. Palpable purpura was present on both legs. His Eastern Cooperative Oncology Group performance status was 1. His hematologic and blood chemical values were: white blood cell count of 9530/mm^3, a hemoglobin concentration of 11.2 g/dL, an erythrocyte sedimentation rate of 83 mm/hour, 6.52 mg/dL serum C-reactive protein, >55U/mL CH50, 132 mg/dL C3, 37.7 mg/dL C4, 10.7 μg/dL D-dimer and 694 mg/dL fibrinogen (normal 160-400). Urine analysis showed proteinuria

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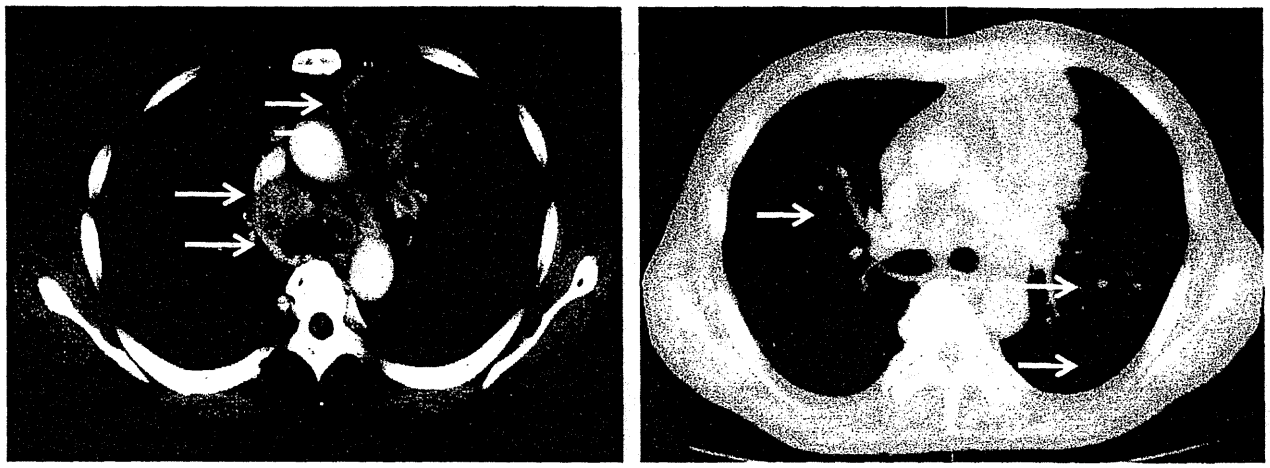

Figure 1 Computed tomography (CT) scan of the chest on admission to our hospital. Tumor in the left upper lobe, swelling of mediastinal lymph nodes, and small nodules in both lung fields and pleura (arrows).

of 1.28 g/day and hematuria of >100 erythrocytes per high-power field. Stool examination for occult blood was positive. The findings were normal or negative for platelet count, prothrombin time, activated partial thromboplastin time, transaminases, creatinine, antistreptolysin O, rheumatoid factor (RF), antinuclear antibody (ANA), IgA, antineutrophil cytoplasmic antibody (ANCA), cryoglobulin, rapid plasma regain, hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV) antibody and human immunodeficiency virus antibody. Blood, urine and sputum cultures were negative. Abdominal CT revealed diffuse swelling and thickening of the small intestine walls and an absence of metastases (Figure 2). Enteroscopy demonstrated multiple erosions and ulcers of the intestine. A biopsy taken from the intestinal mucus showed non-specific chronic inflammation without evidence of vasculitis. On histopathologic examination of the palpable purpura, vasculitis was seen, with infiltration of polymorphonuclear cells into the vessel walls of the subpapillar plexus under the epidermis (Figure 3AB). Immunofluorescent studies of the biopsy specimen revealed deposition of IgA without C3 in superficial blood vessels (Figure 3C).

Our patient was diagnosed with HSP. Treatment with prednisolone 0.8 mg/kg/day was initiated following deterioration of renal function, as evidenced by a drop in creatinine clearance from 82 mL/min at the time of admission to 27 mL/min before starting prednisolone. The clinical features of HSP, including abdominal pain and skin rash, resolved within a week. Creatinine clearance improved to 46 mL/min four weeks after the start of steroid treatment. Prednisolone was tapered and maintained at 0.6 mg/kg/day. Our patient received two cycles of chemotherapy with gemcitabine 1000 mg/m^2

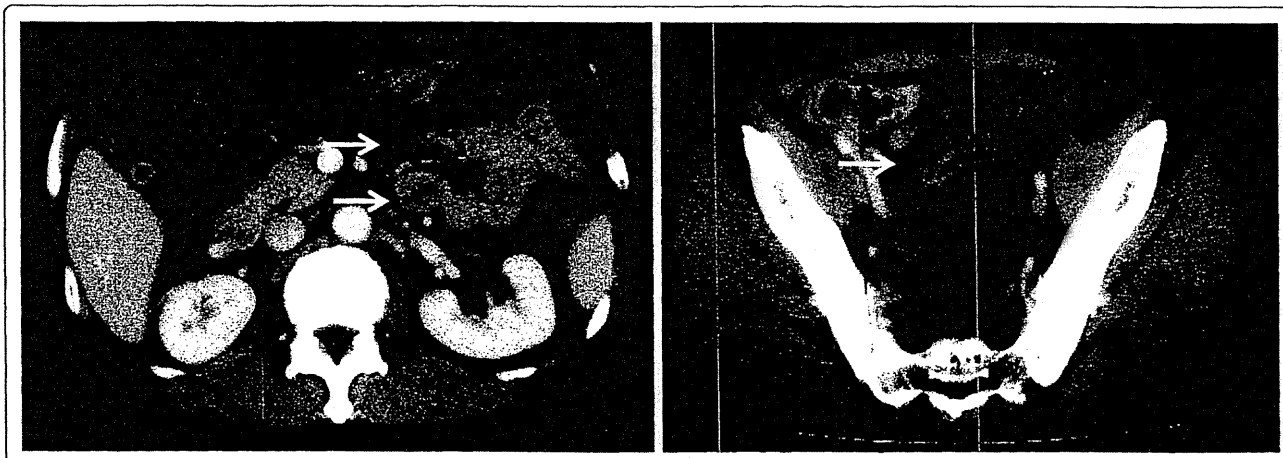

Figure 2 Computed tomography (CT) scan of the abdomen and pelvis on admission to our hospital. No evidence of metastasis but did identify diffuse swelling and thickening of the small intestine walls (arrows).

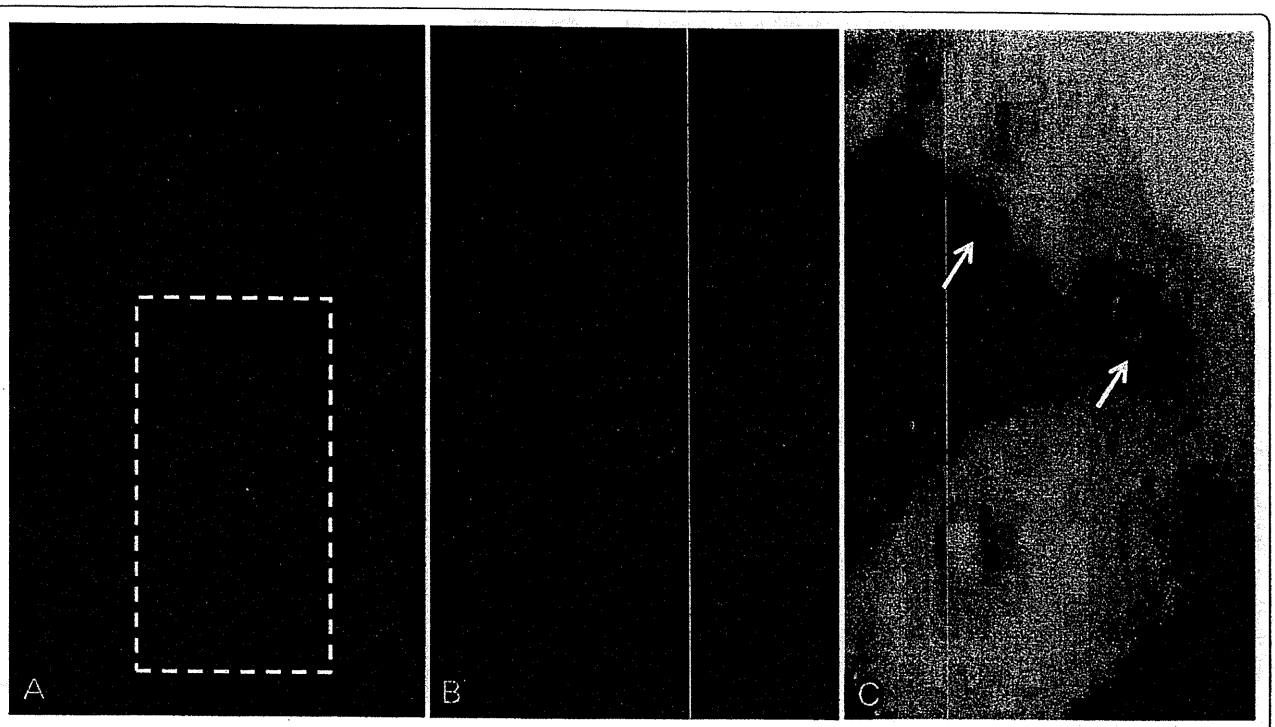

Figure 3 Histopathology of the palpable purpura. (A) Cellular infiltration into the obscured small vessel walls of the subpapillar plexus under the epidermis (hematoxylin and eosin, original magnification × 100). **(B)** Infiltration of polymorphonuclear cells (haematoxylin and eosin, original magnification × 150) **(C)** Fluorescent immunoglobulin A deposits (arrows) (original magnification × 100).

administered on days one and eight of each three-weeks cycle. HSP was well controlled, and no other adverse events were observed during chemotherapy. Two months after of the start of chemotherapy, the patient developed rapidly progressing dyspnea and hypoxia. Chest CT and cardiac ultrasonography identified tumor invasion of the pericardium and massive pericardial effusion. Cytological examination of the pericardial effusion revealed adenocarcinoma cells. Our patient was diagnosed with heart failure due to cardiac tamponade, and twice underwent surgical drainage of the pericardial effusion. His general condition gradually deteriorated with disease progression, and he died three months after the onset of HSP.

Discussion

HSP is a systemic vasculitis with IgA-dominant immune deposits affecting small vessels, especially those in the skin, gastrointestinal tract and kidney, and is accompanied by arthralgia or arthritis [1]. HSP occurs more commonly in children. The clinical presentation of HSP is more severe in adults than in children, and the outcome in adults is relatively poor [2].

The diagnostic criteria for HSP by the American College of Rheumatology are: palpable purpura, patient aged ≤20 years; bowel angina, and granulocytes in the walls of small arterioles or venules seen on biopsy. The presence of two or more of these criteria has a sensitivity and specificity of 87.1% and 87.7%, respectively [6]. Our case met three of the four criteria, with the exception of age. Other vasculitides were highly unlikely because markers for other systemic vasculitides (antistreptolysin O, RF, ANA, ANCA, cryoglobulin, rapid plasma regain, HBsAg, HCV) were all negative. Moreover, immunofluorescent studies of the biopsied skin specimen showed IgA deposition in blood vessels. These findings confirmed HSP. Novák *et al.* reported that vasculitic endoscopic lesions had been documented in only some patients with HSP with abdominal symptoms [7]. Their findings may explain the finding in our patient's intestinal specimen of non-specific chronic inflammation without vasculitis.

The occurrence of vasculitis in patients with malignancy has been estimated at one in 1800 for hematological malignancies and one in 80,800 for solid tumors [3]. HSP represents only 5% of vasculitis cases associated with malignancy [4]. Although hematological malignancies were more common than solid tumors in all types of vasculitis, solid tumors were more common in association with HSP [5]. Mitsui *et al.* reported that 23 of 53 patients with HSP (43.4%) aged >40 years had underlying malignancies [8]. The etiology of HSP remains obscure, and various triggers, including bacterial or viral infections, foods and drugs, have been hypothesized. Adult

Table 1 Patients with Henoch-Schönlein purpura and lung cancer

Author	Histology	Occurrence of vasculitis in relation to tumor	HSP therapy	Malignancy therapy
Cairns [9]	Squamous	eight months before tumor	Not done	None
	Squamous	Synchronous[a]	Not done	Surgical resection
Maurice [10]	Squamous	Synchronous	Not done	Surgical resection
Mitchell [11]	Squamous	22 months before tumor	Prednisone + azathioprine	Surgical resection
Pfitzenmeyer [12]	Squamous	six months before tumor	Not done	None
Gutiérrez [13]	Squamous	Synchronous	Not available	Not available
Frigui [14]	Squamous	six months before tumor	Methylprednisolone pulses, prednisone	Chemotherapy, radiotherapy
Blanco R [15]	Small cell	Synchronous	Not done	Chemotherapy
Ponge T [16]	Small cell	eight months before tumor	Not done	Chemotherapy
Weiler-Bisig [17]	Adenocarcinoma	five months before tumor	Prednisolone	Chemotherapy
Salon [18]	Adenocarcinoma	Synchronous	Prednisone + intravenous gammaglobulins	Surgical resection
Present case	Adenocarcinoma	Synchronous	Prednisolone	Chemotherapy

[a]Synchronous: within 1 month of cancer diagnosis.

patients with HSP should be investigated for malignancies, especially in the absence of these triggers [8].

Lung cancer is the most common solid malignancy associated with HSP. Twelve cases of lung cancer presenting with HSP have been reported to date, including this one (Table 1) [9-18]. Mean patient age was 64.9 years (range 50-79 years) and all patients were men. The most common lung-cancer histological diagnoses were squamous cell (n = 7), adenocarcinoma (n = 3) and small cell (n = 2). In six cases, the diagnosis of both processes was simultaneous, and in four cases, HSP antedated lung cancer by an average of 9.2 months (range five-22 months). Lung cancer did not antedate HSP in any of the cases. All patients had palpable purpura and renal involvement, 55% of patients had gastrointestinal symptoms and 64% had joint involvement. In the case reported here, there were no identifiable triggers for the onset of HSP other than the malignancy itself. The diagnosis of malignancy and the onset of HSP occurred at the same time, therefore we believe that the HSP was associated with the malignancy.

Treatment for patients with HSP and lung cancer is also summarized in Table 1. Therapies for lung cancer induced remission of HSP in some cases [9,10,15,16], whereas other case reports indicated that corticosteroid therapy was required for improvement of HSP [11,14,17,18], as with our case. Our patient had an Eastern Cooperative Oncology Group performance status of 1, and after treatment of the HSP to correct the progressive renal insufficiency, there was no contraindication for chemotherapy. Corticosteroid therapy obviously contributed to the improvement of the HSP and safe chemotherapy. Unfortunately, the patient did not respond to chemotherapy and died from causes associated with the underlying cancer. Mitsui *et al.* found a correlation between the development of HSP and poor prognosis of malignancies [8]. They reported that seven of nine patients with HSP and malignancies exhibited new metastatic lesions or died of cancer within one-32 months from the onset of HSP.

Conclusion

We report a rare case of HSP associated with non-small cell lung cancer. To the best of our knowledge, this is only the third case of HSP presenting with pulmonary adenocarcinoma. Although the optimum treatment of HSP with malignancy remains unclear, corticosteroid therapy dramatically improved our patient's clinical symptoms, and contributed to the management of his lung cancer.

Consent

Written informed consent was obtained from the patient's spouse for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

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Authors' contributions

DM wrote the manuscript. SW was responsible for the manuscript concept and final corrections to the manuscript. YW and IN analyzed and interpreted our patient data regarding the renal disease. AM and KI performed the

histological examination of the skin. RK, HM, HK, HY and TT participated in patient care and collected data. All authors have read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Vaccination with CD133$^+$ melanoma induces specific Th17 and Th1 cell–mediated antitumor reactivity against parental tumor

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Abstract Accumulating evidence suggests that cancer cells possess a small subpopulation that survives during potentially lethal stresses, including chemotherapy, radiation treatment, and molecular-targeting therapy. CD133 is a putative marker that distinguishes a minor subpopulation from normal differentiated tumor cells in many cancers. Although it is necessary to eradicate all cancer cells to obtain a cure, effective treatment to eliminate the CD133$^+$ treatment–tolerant cells has not been elucidated. In this study, we demonstrated that a CD133$^+$ subpopulation in murine melanoma is immunogenic and that effector T cells specific for the CD133$^+$ melanoma cells mediated potent antitumor reactivity, curing the mice of the parental melanoma. CD133$^+$ melanoma antigens preferentially induced type 17 T helper (Th17) cells and Th1 cells but not Th2 cells. CD133$^+$ melanoma cell–specific CD4$^+$ T-cell treatment eradicated not only CD133$^+$ tumor cells but also CD133$^-$ tumor cells while inducing long-lasting accumulation of lymphocytes and dendritic cells with upregulated MHC class II in tumor tissues. Further, the treatment prevented regulatory T-cell induction. These results indicate that T-cell immunotherapy is a promising treatment option to eradicate CD133$^+$ drug-tolerant cells to obtain a cure for cancer.

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Keywords Cancer stem cells · Melanoma · CD133 · Th17 · Antitumor immunotherapy

Introduction

The cancer stem cells (CSCs) theory states that a minor subpopulation can initiate differentiated cancer cells and tumor tissues via self-renewal and asymmetrical cell division and that this plays a critical role in metastasis and recurrence [1–7]. It is controversial whether the classical CSC theory is applicable for all solid tumors. However, accumulating evidence suggests that a small subpopulation with unique features plays an important role in cancer recurrence after classical anticancer treatment and molecular-targeting therapy [8–12]. An excess of multidrug efflux transporters, antiapoptotic factors, DNA repair gene products, stem cell–specific growth signaling, and relative dormancy contribute to the ability of these cells to resist treatment. CD133 is a stem cell marker and putative CSC marker [13, 14]. It was demonstrated that all of examined cancer cells surviving after potentially lethal drug treatments uniformly express CD133 [15]. These drug-tolerant cancer cell populations use an altered chromatin state to induce a reversible drug-tolerant state and give rise to a permanent drug-tolerant cell population with genetic mutations. Unless these CD133$^+$ cancer cells are eradicated, it is impossible to achieve a lasting cure.

T-cell-mediated immunotherapy can mediate antitumor reactivity. We previously reported that effector T cells primed in tumor-draining lymph nodes (LNs) possessed antitumor therapeutic efficacy in brain, pulmonary, and skin metastasis models [16–18]. In this study, we found that LN T cells primed with the CD133$^+$ tumor vaccine

mediated potent antitumor therapeutic efficacy by eradicating $CD133^+$ tumor cells in tumors, thereby curing parental melanomas that comprised <1% $CD133^+$ tumor cells. Interestingly, $CD133^+$ melanoma antigens tended to prime type 17 helper T (Th17) cells and Th1 cells but not Th2 cells. These results indicate that T-cell immunotherapy may be a promising strategy to eradicate treatment-tolerant $CD133^+$ cancer cells.

Materials and methods

Mice

Female C57BL/6J (B6) mice were purchased from the CLEA Laboratory (Tokyo, Japan). They were maintained in a specific pathogen-free environment and used for experiments at the age of 8–10 weeks. All animal experiments were conducted with the permission of the Niigata University ethics committee for animal experiments.

Tumor cells

B16F10 melanomas, which originate from B6 mice, were maintained in vitro. Parental tumor cells were labeled with phycoerythrin (PE)-conjugated anti-CD133 monoclonal antibody (mAb; 13A4) and anti-PE microbeads (Miltenyi Biotec, Auburn, CA). $CD133^+$ and $CD133^-$ tumor cells were isolated with autoMACS™ (Miltenyi Biotec) according to the manufacturer's instructions. Cell purity was >90%.

mAbs and flow cytometry

Hybridomas producing mAbs against murine CD4 (GK1.5, L3T4), CD8 (2.43, Lyt-2), CD3 (2C11), and CD62L (MEL14) were obtained from the American Type Culture Collection (Rocksville, MD). Anti-CD4, anti-CD8, and anti-CD62L mAbs were obtained from ascitic fluid of sublethally irradiated (500 cGy) DBA/2 mice. PE-conjugated anti-CD80 (16-10A), anti-CD86 (GL1), anti-CD62L (MEL14), anti-CD8 (2.43), and anti-CD25 (PC61) mAbs; fluorescein isothiocyanate (FITC)-conjugated anti-Thy1.2 (30-H12); and anti-CD4 (GK1.5) mAbs were purchased from BD PharMingen (San Diego, CA). Analyses of cell surface phenotypes were carried out by direct immunofluorescence staining of $0.5–1 \times 10^6$ cells with conjugated mAbs. In each sample, 10,000 cells were analyzed using a FACScan™ flow microfluorometer (Becton–Dickinson, Sunnyvale, CA). PE-conjugated subclass-matched antibodies used as isotype controls were also purchased from BD PharMingen. Samples were analyzed using the CellQuest™ software (BD PharMingen).

Fractionation of T cells

T cells in the LN cell suspension were concentrated by passing through nylon wool columns (Wako Pure Chemical Industries, Osaka, Japan). To yield highly purified (>90%) cells with downregulated CD62L expression ($CD62L^{low}$), LN T cells were further isolated by a panning technique using T-25 flasks pre-coated with goat anti-rat immunoglobulin antibody (Ig Ab) (Jackson ImmunoResearch Laboratories, West Grove, PA)/anti-CD62L mAb (MEL14) and sheep anti-rat-Ig Ab/anti-CD62L mAb-coated DynaBeads M-450 (Dynal, Oslo, Norway). In some experiments, cells were further separated into $CD4^+$ and $CD8^+$ cells by depletion using magnetic beads, as described previously [18]. For in vitro experiments, highly purified $CD4^+$ cells were obtained using anti-CD4 mAb-coated Dynabeads and Detachabeads (Invitrogen) according to the manufacturer's instructions.

Bone marrow–derived dendritic cells

Dendritic cells (DCs) were generated from bone marrow cells (BMs), as described previously. In brief, BMs obtained from femurs and tibias of treatment-naïve mice were placed in T-75 flasks for 2 h at 37°C in complete medium (CM) containing 10 ng/ml of recombinant murine granulocyte–macrophage colony-stimulating factor (rmGM-CSF; a gift from KIRIN, Tokyo, Japan). Non-adherent cells were collected by aspirating the medium and transferred into fresh flasks. On day 6, non-adherent cells were harvested by gentle pipetting. CM consisted of RPMI 1640 medium supplemented with 10% heat-inactivated lipopolysaccharide (LPS)-qualified fetal calf serum (FCS), 0.1 mM nonessential amino acids, 1 μM sodium pyruvate, 100 U/ml of penicillin, 100 μg/ml of streptomycin sulfate (all from Life Technologies Inc.), and 5×10^{-5} M 2-ME (Sigma Chemical Co., St. Louis, MO).

DC/tumor-draining LN cells

BMs and DCs were co-cultured with the same number of irradiated tumor cells (5,000 cGy) in CM overnight. B6 mice were inoculated subcutaneously (s.c.) with 10×10^6 BM–DC and tumor cells in both flanks. Inguinal LNs draining BM–DC and tumor cells were harvested. Single-cell suspensions were prepared mechanically as described previously [19].

Adoptive immunotherapy

B6 mice were injected s.c. with parental B16-F10 tumor cells in 100 μl of Hank's balanced salt solution (HBSS) to establish subcutaneous tumors. Two or three days after the