(cisplatin, doxorubicin, cyclophosphamide), and VIP (etoposide, ifosfamide, cisplatin), and CODE (cisplatin, vincristine, doxorubicin, and etoposide) were conducted for thymoma and TC collectively [6–13]. Although these studies included a small number of TC in each and are inherently evaluated for thymoma, anthracycline-based regimens such as ADOC are now chosen as the current standard for TC based on the extrapolation of the results of the studies (Table 1).

In advanced non-small-cell carcinoma (NSCLC), a platinum-based regimen with a new-generation agent has been established to be the standard care [20, 21]. Of those, carboplatin plus paclitaxel (CbP) is one of the most widely used combinations regardless of histological subtype. A retrospective study reported an efficacy and tolerability using cisplatin plus irinotecan treatment, which was a one of the standard treatment for advanced NSCLC, for advanced TC [17]. Thus, the standard chemotherapy of advanced NCSLC might be an effective treatment for advanced TC. Several case series have observed that CbP yielded a response in patients with advanced TC [15, 22], while a recent prospective study against thymoma and TC have suggested insufficient efficacy of CbP for 23 TC patients [16]. With this background, we conducted the largest clinical trial, limiting to patients with TC, to evaluate the efficacy and safety of CbP. This study was registered with UMIN Clinical Trials Registry (ID: UMIN000001358).

patients and methods

patients

Patients were eligible for inclusion in the study if they were chemo-naive and more than 20 years of age, and had a histologically or cytologically confirmed Masaoka's stage III (which was not amenable to curative surgery or radiotherapy), IVa, and IVb [23] tumor, which was in the anterior mediastinum. Other inclusion criteria were: patients having measurable lesions as defined by Response Evaluation Criteria in Solid Tumors (RECIST) 1.1, an ECOG performance status (PS) of 0 or 1, and adequate bone marrow reserve (with leukocyte count $\geq$4000 cells/μl, neutrocyte count $\geq$2000 cells/μl, hemoglobin $\geq$9.5 g/dl, and platelet count $\geq$100 000 cells/μl), hepatic function [aspartate aminotransferase (AST)/alanine aminotransferase (ALT) $\leq$100 IU/l and serum bilirubin $\leq$1.5 mg/dl], renal function (creatinine $\leq$1.2 mg/dl) and PaO_2 $\geq$70 torr (or SpO_2 $\geq$92%). Exclusion criteria included the presence of uncontrolled pleural or pericardial effusion, brain tumor with symptoms, superior vena cava syndrome, interstitial pneumonitis, other active malignancy, serious allergy to medical drugs, and myasthenia gravis. All patients signed a written informed consent before entering the study.

treatment. The patients received paclitaxel [at a dose of 200 mg/m^2, given intravenously (i.v.) over a period of 3 h] followed by carboplatin [at a dose equivalent to an area under the curve (AUC) of 6, given i.v. over a period of 1 h], both administered on the first day of every 3 weeks. All the patients were premeditated with anti-histamine and H2-receptor-antagonists (e.g. famotidine), diphenhydramine, and dexamethasone before the paclitaxel infusion. Patients without unacceptable toxicity or progressive disease repeated the therapy for a maximum of six cycles.

evaluation. Progression-free survival (PFS) was assessed from the date of enrollment to the earliest sign of disease progression as determined by chest computed tomography (CT) or magnetic resonance imaging (MRI) using RECIST criteria, or death from any cause. Overall survival (OS) was assessed from the date of enrollment until death from any cause. Safety was assessed according to National Cancer Institute Common Terminology Criteria (NCI-CTC) for Adverse Events, Version 3.0. Disease was assessed with a chest CT or MRI after the second, fourth, and sixth cycles. After six cycles,

Table 1. Chemotherapy for advanced thymic carcinoma in previous studies

Authors	P or R	Regimen	No. of patients	Response rate (%)	PFS (month)	MST (month)
Loehrer et al. [11]	P (Phase II)	CAP	30 (T = 29, TC-1)	50	18.4	37.7
Koizumi et al. [7]	R (Case series)	ADOC	8 (TC = 8)	75	—	19
Agatsuma et al. [12]	R	ADOC	34 (TC = 34)	50	—	21.3
Fornasiero et al. [13][a]	R (Case series)	ADOC	37 (T = 37, TC = 0)	91.8	12	15
Loehrer et al. [8]	P (Phase II)	VIP	28 (T = 20, TC = 8)	32	11.9	31.6
Grassin et al. [9]	P (Phase II)	VIP	16 (T = 12, TC = 4)	25	—	Not reached
Igawa et al. [14]	R	CbP	11 (TC = 11)	36	7.9	22.7
Furugen et al. [15]	R	CbP	16 (TC = 16)	37.5	8.6	49.4
Lemma et al. [16]	P (Phase II)	CbP	46 (T = 23, TC = 23)	21.7 (TC) 41.9 (T)	5 (TC) 16.7 (T)	20 (TC) Not reached (T)
Okuma et al. [17]	R	Cisplatin irinotecan	9 (TC = 9)	55.6	7.9	33.8
Palmieri et al. [18]	P (Phase II) [a]second-line	Carboplatin gemcitabine	15 (T = 12, TC = 3)	40	11	Not reached
Oshita et al. [19]	P	PACE	14 (T = 7, TC = 7)	42.9	—	14.7 (no prior Tx; 8.9)
Yoh et al. [10]	R	CODE	12 (TC = 12)	42	5.6	46

[a]Not included thymic carcinoma.

P, prospective; R, retrospective; T, thymoma; TC, thymic carcinoma; CAP, cisplatin, doxorubicin, cyclophosphamide; ADOC, doxorubicin, cisplatin, vincristine, cyclophosphamide; VIP, etoposide, ifosfamide, cisplatin; PACE, cisplatin, doxorubicin, cyclophosphamide, etoposide; CODE, cisplatin, vincristine, doxorubicin, etoposide; CbP, carboplatin, paclitaxel; PFS, progression-free survival; MST, median survival time; Tx, treatment.

patients had a chest CT or MRI every 1–2 months for 2 years until disease progression.

Tumor response was evaluated by extramural central review committee according to RECIST.

pathological diagnosis. This study also provided other extramural review committee of independent pathologists for the definitive diagnosis of TC. The histological type was determined based on the agreement by two of three pathologists. In independent pathological review, the pathologist have diagnosed by using hematoxylin–eosin (H&E) stain and immunostained. Formalin-fixed, paraffin-embedded sections were immunostained for CD5, CD99, c-KIT, AE1/3, chromogranin A, synaptophysin, and TTF-1. The antibodies used were the monoclonal rabbit anti-human-CD5 (BSB5159, Bio SB), the monoclonal mouse anti-human-CD99 (M3601, DAKO), the polyclonal rabbit anti-human-c-Kit (CD117, an epitope of KIT) (A4502, Dako), the monoclonal mouse anti-human Cytokeratin (AE1/AE3) (M3515, DAKO), the monoclonal mouse anti-human synaptophysin (A0010, DAKO), and the monoclonal mouse anti-human TTF-1 (NCL-TTF-1, Novocastra). If consensus between the pathologists was not reachable, that case was classified as 'not otherwise specified (NOS)'.

statistical analysis. The primary end point was objective response rate (ORR). Based on the SWOG two-stage design, the planned sample size of 40 patients was determined to reject a null ORR of 20% at one-sided significance level of 0.10 under an expected ORR of 40% with a power of 0.85. The results of this one-sample test are reported with a two-sided *P*-value. The 95% confidence interval (CI) of ORR was obtained by exact binomial method.

The secondary end points included OS, PFS, and safety. All patients were followed-up until 24 months after last patient enrollment. Survival curves for time-to-end points were estimated by the Kaplan–Meier method and the 95% CIs of the curves were calculated based on Greenwood's variance. Statistical analyses were conducted by SAS Version 9.3.1.

results

patient characteristics

Forty patients were enrolled from 21 centers in Japan for this study from May 2008 to November 2010. The patient demographics and disease characteristics are summarized in Table 2. All patients were eligible, but only one patient withdrew consent and received no protocol treatment (Figure 1). Of the 39 patients evaluable for analysis, 23 (59%) were male with a median age of 62 years (range: 36–84). There were 3 patients in Masaoka's stage III, 10 in stage IVa, and 26 in stage IVb. Twenty-three patients (59%) had an ECOG PS of 0.

histology

The independent review for histological subtype determined that there were 9 squamous cell carcinomas, 11 poorly differentiated neuroendocrine carcinomas, 1 basaloid carcinomas, and 14 NOS. One patient had other organ malignancy (possibly NSCLC). There were no tissue samples for the review in three patients.

In pathological review for histology at each center, there were 21 squamous cell carcinomas, 2 poorly differentiated neuroendocrine carcinomas, 5 undifferentiated carcinomas, 1 basaloid carcinoma, 1 lymphoepithelioma-like carcinoma, and 9 NOS. Among 36 patients who were evaluable for pathological diagnosis by independent review, the diagnosis of TC was consistent at 97% (35/36 patients). However, 39% (14/36) patients could not be determined to diagnosis of histological subtype (Table 3).

Table 2. Patient characteristics

Characteristics	No. of patients ($n = 39$)
Gender	
Male	23 (59.0%)
Female	16 (41.0%)
Age	
Median (range)	62.0 (36–84)
ECOG PS	
0	23 (59.0%)
1	16 (41.0%)
Method of confirmed diagnosis	
Histology	32 (82.1%)
Cytology	4 (10.3%)
Both	3 (7.7%)
Histological subtype	
Squamous cell carcinoma	9 (23.1%)
Basaloid carcinoma	1 (2.6%)
Poorly differentiated neuroendocrine carcinoma	11 (28.2%)
NOS	14 (35.9%)
Tumor of other organ	1 (2.6%)
Not confirmed	3 (7.7%)
Masaoka's stage	
III	3 (7.7%)
Iva	10 (25.6%)
IVb	26 (66.7%)
Smoking history	
Never smoker	16 (41.0%)
Current smoker	23 (59.0%)
Prior therapy	
Radiotherapy	3 (7.7%)
Surgery	8 (20.5%)

NOS, not otherwise specified; ECOG, Eastern Cooperative Oncology Group; PS, performance status.

treatment delivery and efficacy

Of the 39 patients evaluable, the median treatment cycle was six (range: 2–6). Relative dose intensity was 77.8% and 80.1% for carboplatin and paclitaxel, respectively. Nineteen patients discontinued protocol treatment. The reason for discontinuation was disease progression ($n = 6$), withdrew a protocol treatment ($n = 5$), adverse event ($n = 3$), and other ($n = 5$).

Of the 39 patients evaluable, 1 (2.6%) achieved complete response (CR) and 13 (33.3%) achieved partial response (PR) with an ORR of 36% (95% CI 21%–53%; $P = 0.031$; Table 4). Twenty-three patients (59%) had stable disease (SD) with a disease control rate of 95% (95% CI 83%–99%). The median PFS was 7.5 (95% CI, 6.2–12.3) months, while OS did not reach the median value (Figure 2). The OS rates at 1- and 2-year were 85% (95% CI 69%–93%) and 71% (54%–83%), respectively.

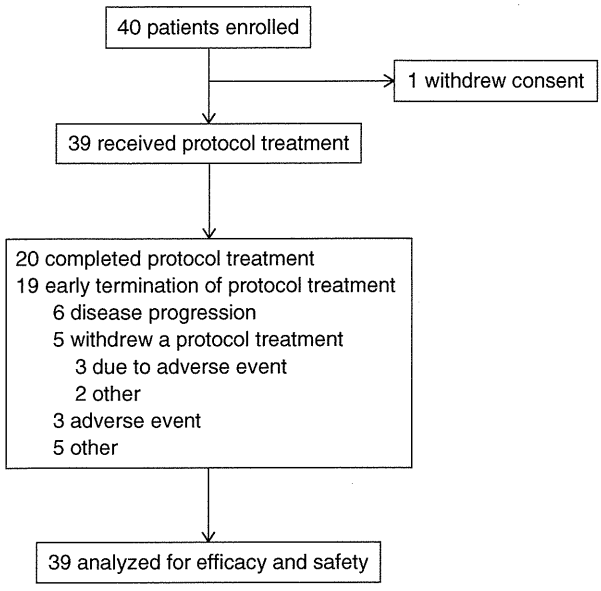

Figure 1. Trial profile in this study.

Table 3. Histological subtype by independent or investigator review

Histological subtype	Independent review	Investigator review
	$n = 39$	
Squamous cell carcinoma	9 (23.1%)	21 (53.8%)
Basaloid carcinoma	1 (2.6%)	1 (2.6%)
Lymphoepithelioma-like carcinoma	—	1 (2.6%)
Poorly differentiated neuroendocrine carcinoma	11 (28.2%)	2 (5.1%)
Undifferentiated carcinoma	—	5 (12.8%)
NOS	14 (35.9%)	9 (23.1%)
Tumor of other organ	1 (2.6%)	—
Not confirmed	3 (7.7%)	—

NOS, not otherwise specified.

Table 4. Treatment responses

	No. of pts. [$n = 39$ (%)]
CR	1 (2.6)
PR	13 (33.3)
SD	23 (59.0)
PD	2 (5.1)
ORR	36% (95% CI, 21–53%; $P = 0.031$)

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; 95% CI, 95% confidence interval.

adverse event

The hematological and non-hematological toxicities of all patients are summarized in Table 5. The major adverse event was grade 3–4 neutropenia in 34 (87%) patients. The grade 3–4 anemia was observed in six (15%) patients. Two cases (5%) of grade 3 febrile neutropenia, sensory neuropathy, and arthralgia (and/or muscle pain) were observed. There was no treatment-related death.

discussion

Here, we reported the results of our multicenter phase II study of CbP for advanced TC. To the best of our knowledge, this is the largest clinical trial focusing on and limiting to TC. The strength of our study is that it was designed to have enough statistical power as a single-arm study and entailed independent expert review boards for pathological classification, as well as tumor response evaluation.

Anthracycline-based regimens of ADOC and CAP are now widely used as the standard for the treatment of TC. ADOC and CAP were, however, introduced into clinical practice based on past studies in which most of the subjects consisted of thymoma; the number of patients with TC were small in all studies. The response rate and MST of ADOC, CAP, and CODE for thymic neoplasms including TC were 22%–75% and 15–50 months [2, 3, 6–12, 14, 16, 18, 19, 22]. However, the response rate and MST of ADOC for TC remained at 50%–75% and 19–21.3 months, respectively. The response rate and MST of CODE were 42% and 46 months ($n = 18$), respectively [7, 10, 12]. Therefore, with regard to TC, CbP seems to be non-inferior in efficacy and superior in safety. In terms of safety, anthracycline-based regimens are well known to be associated with congestive heart failure. The most frequent adverse events of CbP in our study were myelosuppression and neuropathy. Although all grade sensory neuropathy was 87.2%, grade 3–4 of that was only 5.1%. Moreover, severe cardiovascular problems were not observed.

In this trial, CbP showed a higher efficacy with an ORR of 36% and 1-year survival rate of 85% for advanced TC when compared with those from previous studies [15, 16, 22]. A recent report of phase II study with the efficacy and safety of CbP combination for advanced thymoma and TC was relatively limited and concluded that CbP seems inferior to anthracycle-based chemotherapy [16]. Of the 44 thymus-related neoplasms enrolled, the ORRs of 21 and 23 patients with thymoma and TC were 42.9% (90% CI 24.5%–62.8%) and 21.7% (9.0%–40.4%), respectively [16]. This is in contrast to the result of our study, where CbP showed a promising efficacy in advanced TC. The difference of the result of the two studies might be involved in the previous radiation therapy. Whereas previously reported study has included 17.7% patients, our study included only 7.7% patients with radiation therapy.

Our trial was unique because we carried out a central histological review by three independent pathologists. It was revealed from this consideration that there are various neoplasms in anterior mediastinum, including thymus-related neoplasms, lymphomas, and other epithelial neoplasms, such as NSCLC, and the clinical features and patient prognosis were associated with the pathological subtype (data not shown). Determination of TC was relatively accurate, but the classification of subtypes was difficult.

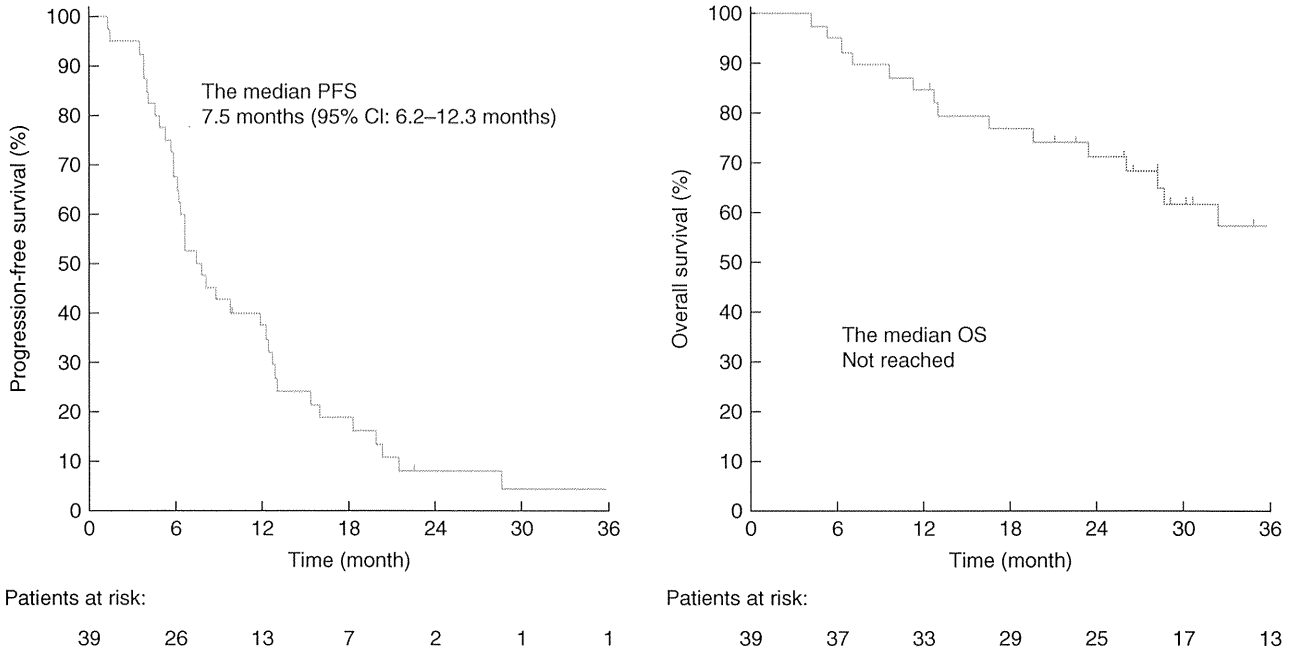

Figure 2. The Kaplan–Meier curves in patients with advanced thymic carcinoma. (A) Progression-free survival and (B) overall survival.

Table 5. Toxicities

	Total ($n = 39$)			
	All grades (%)	Grade 3 (%)	Grade 4 (%)	Grade 3 or more (%)
Hematological				
Leukocytes	94.9	43.6	0.0	43.6
Neutrophils	97.4	35.9	51.3	87.2
Platelets	43.6	5.1	0.0	5.1
Hemoglobin	84.6	12.8	2.6	15.4
Non-hematological				
Febrile neutropenia	5.1	5.1	0.0	5.1
Infection	0	0.0	0.0	0.0
Nausea	53.8	0.0	0.0	0.0
Vomiting	15.4	0.0	0.0	0.0
Anorexia	61.5	2.6	0.0	2.6
Fatigue	61.5	0.0	0.0	0.0
Neuropathy: motor	5.1	0.0	0.0	0.0
Neuropathy: sensory	87.2	5.1	0.0	5.1
Constipation	61.5	0.0	0.0	0.0
AST	38.5	0.0	0.0	0.0
ALT	38.5	2.6	0.0	2.6
Pneumonitis	2.6	0.0	0.0	0.0

AST, aspartate aminotransferase; ALT, alanine aminotranferase.

In conclusion, we conducted the largest study for untreated advanced TC, a rare type of tumor within the thymus gland, and revealed that CbP showed a promising efficacy and tolerable safety than an anthracycline-based one. Our results will contribute to the establishment of the hypothesis that CbP, one of the standards for non-small-cell lung cancer, might be an option as a chemotherapy regimen for TC.

acknowledgements

We thank all the patients and investigators who participated in this study.

disclosure

TY have received honoraria (Takeda, Chugai, Bristol, Taiho), and TY is a consultant/has an advisory role for Yakult, Taiho, Chugai. TS have received research funding from Chugai, Eli Lilly, Pfizer, and Ono. TS (Chugai, Pfizer, Astellas Pharma, AstraZeneca, Novartis Pharma, Eisai, MSD), KN (Bristol Myers Squibb Company, Nippon Kagaku Co, LTD), and JF (Chugai) received research funding. JF has received Patents and Royalties from Toyama University. All remaining authors have declared no conflicts of interest.

references

1. Engels EA, Pfeiffer RM. Malignant thymoma in the United States: demographic patterns in incidence and associations with subsequent malignancies. Int J Cancer 2003; 105: 546–551.
2. de Jong WK, Blaauwgeers JL, Schaapveld M et al. Thymic epithelial tumours: a population-based study of the incidence, diagnostic procedures and therapy. Eur J Cancer 2008; 44: 123–130.
3. Liu HC, Hsu WH, Chen YJ et al. Primary thymic carcinoma. Ann Thorac Surg 2002; 73: 1076–1081.
4. Kondo K, Monden Y. Therapy for thymic epithelial tumors: a clinical study of 1,320 patients from Japan. Ann Thorac Surg 2003; 76: 878–884; discussion 884–875.

5. Travis W, Brambilla E, Muller-Hermelink H, Harris C. Tumours of the Lung, Pleura, Thymus and Heart. Pathology and Genetics. Lyon: World Health Organization Classification of Tumours, IARC Press 2004.
6. Berruti A, Borasio P, Gerbino A et al. Primary chemotherapy with adriamycin, cisplatin, vincristine and cyclophosphamide in locally advanced thymomas: a single institution experience. Br J Cancer 1999; 81: 841–845.
7. Koizumi T, Takabayashi Y, Yamagishi S et al. Chemotherapy for advanced thymic carcinoma: clinical response to cisplatin, doxorubicin, vincristine, and cyclophosphamide (ADOC chemotherapy). Am J Clin Oncol 2002; 25: 266–268.
8. Loehrer PJ, Sr, Jiroutek M, Aisner S et al. Combined etoposide, ifosfamide, and cisplatin in the treatment of patients with advanced thymoma and thymic carcinoma: an intergroup trial. Cancer 2001; 91: 2010–2015.
9. Grassin F, Paleiron N, Andre M et al. Combined etoposide, ifosfamide, and cisplatin in the treatment of patients with advanced thymoma and thymic carcinoma. A French experience. J Thorac Oncol 2010; 5: 893–897.
10. Yoh K, Goto K, Ishii G et al. Weekly chemotherapy with cisplatin, vincristine, doxorubicin, and etoposide is an effective treatment for advanced thymic carcinoma. Cancer 2003; 98: 926–931.
11. Loehrer PJ, Sr, Kim K, Aisner SC et al. Cisplatin plus doxorubicin plus cyclophosphamide in metastatic or recurrent thymoma: final results of an intergroup trial. The Eastern Cooperative Oncology Group, Southwest Oncology Group, and Southeastern Cancer Study Group. J Clin Oncol 1994; 12: 1164–1168.
12. Agatsuma T, Koizumi T, Kanda S et al. Combination chemotherapy with doxorubicin, vincristine, cyclophosphamide, and platinum compounds for advanced thymic carcinoma. J Thorac Oncol 2011; 6: 2130–2134.
13. Fornasiero A, Daniele O, Ghiotto C et al. Chemotherapy for invasive thymoma. A 13-year experience. Cancer 1991; 68: 30–33.
14. Igawa S, Murakami H, Takahashi T et al. Efficacy of chemotherapy with carboplatin and paclitaxel for unresectable thymic carcinoma. Lung Cancer 2010; 67: 194–197.
15. Furugen M, Sekine I, Tsuta K et al. Combination chemotherapy with carboplatin and paclitaxel for advanced thymic cancer. Jpn J Clin Oncol 2011; 41: 1013–1016.
16. Lemma GL, Lee JW, Aisner SC et al. Phase II study of carboplatin and paclitaxel in advanced thymoma and thymic carcinoma. J Clin Oncol 2011; 29: 2060–2065.
17. Okuma Y, Hosomi Y, Takagi Y et al. Cisplatin and irinotecan combination chemotherapy for advanced thymic carcinoma: evaluation of efficacy and toxicity. Lung Cancer 2011; 74: 492–496.
18. Palmieri G, Merola G, Federico P et al. Preliminary results of phase II study of capecitabine and gemcitabine (CAP-GEM) in patients with metastatic pretreated thymic epithelial tumors (TETs). Ann Oncol 2010; 21: 1168–1172.
19. Oshita F, Kasai T, Kurata T et al. Intensive chemotherapy with cisplatin, doxorubicin, cyclophosphamide, etoposide and granulocyte colony-stimulating factor for advanced thymoma or thymic cancer: preliminary results. Jpn J Clin Oncol 1995; 25: 208–212.
20. Schiller JH, Harrington D, Belani CP et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 2002; 346: 92–98.
21. Ohe Y, Ohashi Y, Kubota K et al. Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan. Ann Oncol 2007; 18: 317–323.
22. Maruyama R, Suemitsu R, Okamoto T et al. Persistent and aggressive treatment for thymic carcinoma. Results of a single-institute experience with 25 patients. Oncology 2006; 70: 325–329.
23. Masaoka A, Monden Y, Nakahara K, Tanioka T. Follow-up study of thymomas with special reference to their clinical stages. Cancer 1981; 48: 2485–2492.

Annals of Oncology 26: 368–374, 2015
doi:10.1093/annonc/mdu519
Published online 25 November 2014

Denosumab for the prevention of skeletal complications in metastatic castration-resistant prostate cancer: comparison of skeletal-related events and symptomatic skeletal events

M. R. Smith[1]*, R. E. Coleman[2], L. Klotz[3], K. Pittman[4], P. Milecki[5,6], S. Ng[7], K. N. Chi[8], A. Balakumaran[9], R. Wei[10], H. Wang[10], A. Braun[9] & K. Fizazi[11]

[1]Department of Hematology/Oncology, Massachusetts General Hospital Cancer Center, Boston, USA; [2]Academic Unit of Clinical Oncology, Sheffield Cancer Research Centre, Sheffield, UK; [3]Division of Urology, Sunnybrook and Women's College Health Sciences Centre, Toronto, Canada; [4]Department of Medical Oncology, The Queen Elizabeth Hospital, Woodville, Australia; [5]Department of Electroradiology, Poznan University of Medical Sciences, Poznan; [6]Department of Radiotherapy, Wielkopolskie Centrum Onkologii, Poznan, Poland; [7]St John of God Hospital, Subiaco, Australia; [8]Department of Medical Oncology, BC Cancer Agency, Vancouver, Canada; [9]Department of Hematology/Oncology; [10]Global Biostatistical Science, Amgen Inc., Thousand Oaks, USA; [11]Department of Medical Oncology, Institut Gustave Roussy, University of Paris Sud, Paris, France

Received 14 August 2014; revised 1 October 2014; accepted 2 October 2014

Background: In a phase III trial in patients with castration-resistant prostate cancer (CRPC) and bone metastases, denosumab was superior to zoledronic acid in reducing skeletal-related events (SREs; radiation to bone, pathologic

*Correspondence to: Dr Matthew R. Smith, Department of Hematology/Oncology, Massachusetts General Hospital Cancer Center, Yawkey 7030, 55 Fruit St, Boston, MA 02114, USA. Tel: +1-617-724-5257; Fax: +1-617-724-3166; E-mail: smith.matthew@mgh.harvard.edu

Hazama et al. Journal of Translational Medicine 2014, 12:108
http://www.translational-medicine.com/content/12/1/108

JOURNAL OF TRANSLATIONAL MEDICINE

RESEARCH Open Access

A phase II study of five peptides combination with oxaliplatin-based chemotherapy as a first-line therapy for advanced colorectal cancer (FXV study)

Shoichi Hazama[1*], Yusuke Nakamura[2], Hiroaki Tanaka[3], Kosei Hirakawa[3], Ko Tahara[4], Ryoichi Shimizu[5], Hiroaki Ozasa[5], Ryuichi Etoh[5], Fumiaki Sugiura[6], Kiyotaka Okuno[6], Takumi Furuya[7], Taku Nishimura[8], Koichiro Sakata[8], Kazuhiko Yoshimatsu[9], Hiroko Takenouchi[1], Ryouichi Tsunedomi[1], Yuka Inoue[1], Shinsuke Kanekiyo[1], Yoshitaro Shindo[1], Nobuaki Suzuki[1], Shigefumi Yoshino[1], Hirokazu Shinozaki[10], Akira Kamiya[10], Hiroyuki Furukawa[10], Takeharu Yamanaka[11], Tomonobu Fujita[12], Yutaka Kawakami[12] and Masaaki Oka[1]

Abstract

Background: We previously conducted a phase I trial for advanced colorectal cancer (CRC) using five HLA-A*2402-restricted peptides, three derived from oncoantigens and two from vascular endothelial growth factor (VEGF) receptors, and confirmed safety and immunological responses. To evaluate clinical benefits of cancer vaccination treatment, we conducted a phase II trial using the same peptides in combination with oxaliplatin-based chemotherapy as a first-line therapy.

Methods: The primary objective of the study was the response rates (RR). Progression free survival (PFS), overall survival (OS), and immunological parameters were evaluated as secondary objective. The planned sample size was more than 40 patients for both HLA2402-matched and -unmatched groups. All patients received a cocktail of five peptides (3 mg each) mixed with 1.5 ml of IFA which was subcutaneously administered weekly for the first 12 weeks followed by biweekly administration. Presence or absence of the HLA-A*2402 genotype were used for classification of patients into two groups.

Results: Between February 2009 and November 2012, ninety-six chemotherapy naïve CRC patients were enrolled under the masking of their HLA-A status. Ninety-three patients received mFOLFOX6 and three received XELOX. Bevacizumab was added in five patients. RR was 62.0% and 60.9% in the HLA-A*2402-matched and -unmatched groups, respectively ($p = 0.910$). The median OS was 20.7 months in the HLA-A*2402-matched group and 24.0 months in the unmatched group (log-rank, $p = 0.489$). In subgroup with a neutrophil/lymphocyte ratio (NLR) of < 3.0, patients in the HLA-matched group did not survive significantly longer than those in the unmatched group (log-rank, $p = 0.289$) but showed a delayed response.

Conclusions: Although no significance was observed for planned statistical efficacy endpoints, a delayed response was observed in subgroup with a NLR of < 3.0. Biomarkers such as NLR might be useful for selecting patients with a better treatment outcome by the vaccination.

Trial registration: Trial registration: UMIN000001791.

Keywords: Peptide vaccine, Peptide cocktail, Colorectal cancer, Phase II study, FOLFOX, Chemotherapy

* Correspondence: hazama@yamaguchi-u.ac.jp
[1]Department of Digestive Surgery and Surgical Oncology, Yamaguchi University Graduate School of Medicine, Ube, Japan
Full list of author information is available at the end of the article

Background

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related death in industrialized countries [1]. In the past decade, a combination treatment of fluorinated-pyrimidine with irinotecan (FOLFIRI) or oxaliplatin (FOLFOX, XELOX), with or without monoclonal antibodies such as anti-vascular endothelial growth factor (VEGF) antibody or anti-epidermal growth factor receptor (EGFR) antibody, has markedly improved the prognosis of patients with metastatic CRC (mCRC) [2-6]. However, most of the patients reveal progression of the disease due to chemo-resistance and lose their lives.

As an attempt to validate a new treatment modality to overcome the limited disease control status of mCRC, we conducted a combination treatment of five therapeutic epitope-peptides with chemotherapy. Recent developments in genome-based technologies have enabled us to obtain comprehensive gene expression profiles of malignant cells and compare them with normal cells [7]. We had previously identified three oncoantigens, RNF43 (ring finger protein 43) [8], 34 kDa translocase of the outer mitochondrial membrane (TOMM34) [9], and KOC1 (IMP-3; IGF-II mRNA binding protein 3) [10], as targets for the development of cancer peptide vaccines for CRC.

Although immunotherapy using tumor infiltrating cells (TIL) or vaccine treatment are promising modalities for the treatment of cancer, recent reports have indicated several mechanisms in tumor tissues which make cancer cells escape from immune system attacks [11]. For example, the limited antitumor effects of cytotoxic T lymphocytes (CTL) were explained by tumor heterogeneity; a subset of tumor cells revealed the down-regulation or absence of human leukocyte antigen (HLA) or targeted antigen proteins [12,13]. Since the growth of solid neoplasms is almost always accompanied with neovascularization [14], which is associated with the expression of vascular endothelial growth factor receptor 1 (VEGFR1) [15] and/or VEGFR2 [16], our vaccine treatment also included the peptides derived from VEGFR1 and VEGFR2 that target neovascular endothelial cells. We selected five HLA-A*2402-restricted peptides derived from RNF43, TOMM34, KOC1, VEGFR1, and VEGFR2 for the clinical trial due to the abundance of the HLA-A*2402 allele in the Japanese population (an allelic frequency of approximately 60%) [17]. We previously performed a phase I study of a combination vaccine treatment for mCRC, and confirmed the safety and the promising potential of our five-peptide-cocktail treatment to improve the prognosis of advanced CRC [18].

FOLFOX (or XELOX) with/without bevacizumab is a widely-used chemotherapy [4] and has been reported to possibly reduce the number of Tregs [19]. We therefore conducted a phase II study of a cancer vaccine consisting of five peptides in combination with oxaliplatin-based chemotherapy as a first-line therapy for advanced CRC.

The purpose of this study was to evaluate the clinical benefit of this cancer vaccine treatment by adding to oxaliplatin-based chemotherapy. Furthermore, we explored a predictive biomarker for its response and for the selection of patients who are likely to exhibit better treatment outcomes following the vaccine treatment. We here demonstrate a promising result of our combination immuno-chemotherapy and predictive biomarkers for immunotherapy.

Patients and methods

Patients and eligibility criteria

Patients were eligible for enrollment when they were ≥ 20 years old with a histologically confirmed advanced CRC, had one or more measurable lesions according to the Response Evaluation Criteria in Solid Tumors version 1.0 (RECIST), were naïve for chemotherapy, had adequate functions of critical organs, had an ECOG performance status (PS) of 0 or 1, and had a life expectancy of ≥ 3 months. The exclusion criteria were CNS involvement, second primary tumors, active infectious disease, any steroid treatment, or any prior peptide vaccination therapies. Written informed consent was obtained from each patient at the time of enrollment. The study was carried out in accordance with the Helsinki declaration on experimentation on human subjects, was approved by the Institutional Ethics Review Boards of Yamaguchi University (H20-102) and each study site, and was registered in the UMIN Clinical Trials Registry as UMIN000001791.

Peptides

The RNF43-721 (NSQPVWLCL) [20], TOMM34-299 (KLRQEVKQNL) [9], KOC1(IMP-3)-508 (KTVNELQNL) [21], VEGFR1-1084 (SYGVLLWEI) [22] and VEGFR2-169 (RFVPDGNRI) [23] peptides restricted with HLA-A*2402 were synthesized by American Peptide Company Inc. (Sunnyvale, CA, USA) according to a standard solid-phase synthesis method, and were purified by reverse-phase high performance liquid chromatography (HPLC). The purity (> 95%) and the identity of the peptides were determined by analytical HPLC and mass spectrometry analysis, respectively. Endotoxin levels and the bio-burden of these peptides were tested and determined to be within acceptable levels as Good Manufacturing Practice grade for vaccines.

Study design

This phase II, single arm, non-randomized, HLA-A status double-blind study was conducted to assess the efficacy of this combination therapy for first-line treatment for advanced CRC. The therapy consisted of a cocktail of five

therapeutic epitope-peptides in addition to oxaliplatin-containing chemotherapy. Although the peptides used in this study were HLA-A*2402 restricted peptides, all enrolled patients whose HLA-A status were double-blinded were administrated the same regime of peptide cocktail and oxaliplatin-containing chemotherapy.

The cocktail of 3 mg each of five peptides derived from RNF43-721, TOMM34-299, KOC1-508, VEGFR1-1084 and VEGFR2-169, was mixed with 1.5 ml of incomplete Freund's adjuvant (IFA) (Montanide ISA51; Seppic, Paris, France) and administered subcutaneously into the thigh or axilla regions on day 1 of each week for 13 weeks, then the vaccination schedule was reduced to once every 2 weeks. Vaccination was continued even if the disease progressed when the patient wished and a primary doctor who provided additional chemotherapies agreed.

Oxaliplatin-containing regimens were administrated concurrently with the vaccination. Detailed informations of the chemotherapies were described in Additional file 1. Briefly, mFOLFOX6 [24,25] consisted of oxaliplatin (85 mg/m^2) with leucovorin (400 mg/m^2), followed by a FU (400 mg/m^2) bolus, and then 2,400 mg/m^2 continuous infusion with/without bevacizumab (5 mg/kg) [4]. This treatment was repeated every 14 days. XELOX [4] consisted of oxaliplatin (130 mg/m^2) on day 1 followed by oral capecitabine (1,000 mg/m^2) twice daily on days 1 through 14 of a 21-day cycle with/without bevacizumab at a dose of 7.5 mg/kg.

Study objectives

The primary objective was the comparison of the efficacy of the peptide-cocktail plus oxaliplatin-containing regimen on patients with HLA-A*2402 compared with those without HLA-A*2402 by assessing the objective response rate (ORR; complete response (CR) and partial response (PR)). Secondary objectives included comparisons between the two groups for progression free survival (PFS), overall survival (OS), safety, and tolerability. Exploratory end points included the assessments of tumor and blood-based immunological biomarkers.

Assessments

Medical history, physical examination, chest X-ray, ECG, and carcinoembryonic antigen (CEA) measurements were performed within 21 days before starting the treatment. Assessments of vital signs, ECOG performance status, height, weight, and routine blood analysis (hematology and chemistry) were performed within 7 days of starting the treatment. During treatment, physical examination, hematology, and biochemistry analyses were repeated on day 1 of every treatment cycle. Tumor assessments (computed tomography scan, magnetic resonance imaging) were made before starting the study treatment and were repeated every 4 to 8 weeks after the treatment. The RECIST guidelines were used to define all responses. Signs of hematological toxicity and non-hematological toxicity were assessed according to CTCAE during therapy and for 28 days after the last study drug dose.

Immunological biomarkers

We investigated the neutrophil/lymphocyte ratio (NLR) and the peripheral blood lymphocyte counts per the entire white blood cells (lymphocyte-%) before the treatment as predictive markers of the efficacy of the vaccination. NLR and lymphocyte-% were determined immediately at each study site.

Statistical analysis

This study was designed to test the hypothesis that a regime consisting of vaccination plus oxaliplatin-containing

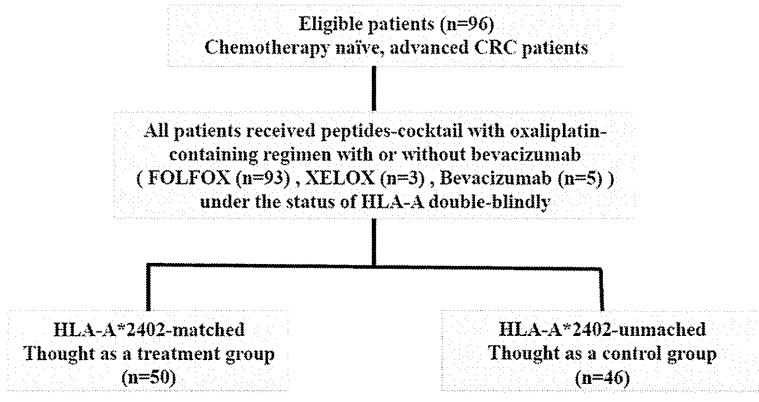

Figure 1 CONSORT diagram. Scheme showing an HLA-A-status double-blind, biologically-randomized phase II study of five therapeutic epitope-peptides combined with oxaliplatin-based chemotherapy as a first-line therapy for advanced colorectal cancer (FXV study). CRC, colorectal cancer; FOLFOX, infusional fluorouracil, leucovorin, and oxaliplatin; XELOX, capecitabine and oxaliplatin; HLA, human leukocyte antigen.

Table 1 Baseline Patient Characteristics

Characteristics		HLA-A*2402 Matched (n = 50)	HLA-A*2402 Unmatched (n = 46)	*p value*
Sex				
	Male	25	24	NS
	Female	25	22	
Age				
	Mean	64.3	63.4	NS
	Standard error	10.9	8	
	Range	36-82	38-77	
Unresectable site				
	Liver	27	35	
	Lung	18	12	
	Dissemination	5	4	NS
	Bone	1	2	
	Lymphnode	13	13	
	Other	5	1	
Number of unresectable sites				
	1	36	30	
	2	9	11	
	3	5	5	
Resection of primary lesion				
	yes	41	43	
	no	9	3	NS
Chemotherapy				
	FOLFOX	48	45	
	XELOX	2	1	NS
	(Bevacizumab)	0	(5)	
Primary minor site				
	Colon	29	36	0.057
	Rectal	21	10	

FOLFOX. infusional fluorouracil. leucovorin. and oxaliplatin: XELOX. capecitabine and oxaliplatin; HLA, human leukocyte antigen; NS. not significant.

chemotherapy is more effective for patients with HLA-A*2402 positive aCRC when compared to those without HLA-A*2402, defining the HLA-A*2402 matched group as the study group and the unmatched group as the control group. Because the response rate of colorectal cancer patients to first line-treatment is generally about 50%, we estimated that a minimum of 40 patients for both arms would be required, assuming a response rate of 50% in the HLA-unmatched control group and 65% in the HLA-matched study group. A two-sided Alpha level of 0.2 and a beta level of 0.5 were assumed.

Response rates were compared by chi-squared test. OS and PFS rates were analyzed by the Kaplan-Meier method and log rank test. For the evaluation of delayed response, we also performed a supplemental analysis of the weighted log-rank tests with the Harrington-Fleming class of weights test for 3 parameter settings ($\rho = 0$ and $\gamma = 0.5$; $\rho = 0$ and $\gamma = 1$; $\rho = 0$ and $\gamma = 2$) [26].

Statistical analyses were performed using SPSS statistics version 20 (SPSS, Chicago, IL, USA) and SAS v9.2. A p value < 0.05 was considered statistically significant.

Results

Patients

Between January 2009 and November 2012, ninety-six patients were enrolled in this trial applying the peptide cocktail treatment in combination with an oxaliplatin-based regimen in 13 hospitals. Fifty patients had at least one allele of HLA-A*2402 and forty-six patients had no HLA-A*2402 allele. The peptide vaccination was administered to all patients. Among the 96 patients enrolled to this trial, 93 patients received mFOLFOX6 and three received XELOX. Five patients were additionally treated with bevacizumab (Figure 1). The baseline characteristics were generally well balanced between the HLA-matched and HLA-unmatched groups, although the proportion of rectal cancer was slightly higher in the HLA-matched group (Table 1). On the cut-off date (25 December, 2013), 87 patients (91%) revealed the progression of the disease with the median OS follow-up period of 38.2 months.

Objective response rate

The ORR was 62.0% and 60.9% in the HLA-matched and HLA-unmatched groups (p = 0.910), respectively (Table 2). The proportions of CR, PR, and SD as well as the disease control rate were 2.0% (1/50), 60.0% (30/50), 32.0% (16/50), and 94.0% (47/50) in the HLA-matched group, respectively, and 0% (0/46), 60.9% (28/46), 37.0% (17/46), 97.8% (45/46) in the HLA-unmatched group, respectively.

Progression free survival

The median PFS was 7.2 months for the HLA-matched group and 8.7 months for the HLA-unmatched group. There was no significant difference between two groups (Figure 2A, P = 0.971). We also performed sub-group analyses using

Table 2 Objective Response rate

HLA-status	HLA-A*2402-matched			(n = 50)	HLA-A*2402-unmatched			(n = 46)
Response	CR	PR	SD	PD	CR	PR	SD	PD
Number	1	30	16	3	0	28	17	1
Response rate		31/50	(62.0%)			28/46	(60.9%)	

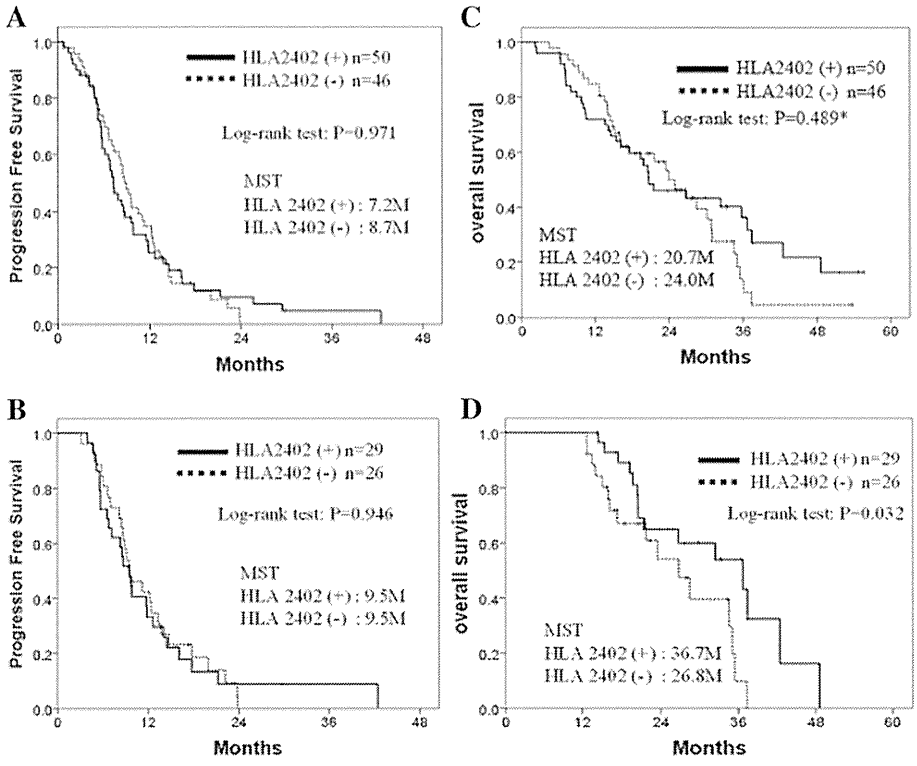

Figure 2 Progression free survival and overall survival. A and **B**, comparison of progression free survival between HLA-A*2402-mached and -unmatched groups; **A**, all patients; **B**, the patients who received the vaccination for more than 12 months. **C** and **D**, comparison of overall survival between HLA-A*2402-matched and -unmatched groups; **C**, all patients; **D**, patients who received the vaccination for more than 12 months. MST, median survival time; HLA, human leukocyte antigen; M, months; *the weighted log-rank tests with the Harrington-Fleming class of weights were performed and resulted in, $\rho = 0$, and $\gamma = 0.5$, $p = 0.186$; $\rho = 0$, and $\gamma = 1$, $\rho = 0.080$; $\rho = 0$, and $\gamma = 2$, $\rho = 0.101$.

the patients who received the vaccination for more than 12 months, but there was also no difference between these two groups (Figure 2B, P = 0.946).

Overall survival

The median OS was calculated to be 20.7 months in the HLA-A*2402-matched group and 24.0 months in the unmatched group. There was no significant difference between the two groups (Figure 2C; log-rank test, p = 0.489; Harrington-Fleming method, $\rho = 0$ and $\gamma = 0.5$, $p = 0.186$; $\rho = 0$ and $\gamma = 1$, $p = 0.080$; $\rho = 0$ and $\gamma = 2$, $p = 0.101$). Interestingly, when the patients were able to receive the vaccination for more than 12 months, the OS of the HLA-A*2402-matched group was significantly better than that of the unmatched group (Figure 2D; log-rank test, p = 0.032).

Safety

The most common adverse events (AEs) observed in this trial were neurologic toxicity and hematologic toxicities (Table 3). There was no significant difference in the incidence of AEs including injection site reaction in the two groups. Although the incidences of serious adverse events (SAEs) were almost similar in the two groups, that of neutropenia was relatively higher in the HLA-A*2402-matched group than the unmatched group. Interstitial pneumonia that led to the death was observed in two cases in the HLA-matched group and in one case in the HLA-unmatched group (Table 4).

Immunological biomarkers

NLR is defined as the neutrophil to lymphocyte ratio, and in this study we categorized the patients into two groups (< 3 and ≥ 3) according to the papers reported previously [27]. In this study, NLR of <3.0 was a prognostic marker for the longer survival with peptide cocktail and oxaliplatin-containing chemotherapy (Figure 3A; log-rank test, p = 0.043). The Lymphocyte-% of ≧ 15% was also associated with a long survival (Figure 3B; log-rank test, p = 0.034). Hence, we examined the combined effect of each of these two markers and the HLA types on the clinical efficacy of the vaccination. In patients with a NLR of < 3.0, a significantly longer overall survival was observed in the HLA-A*2402-matched group than the HLA-A*2402-unmatched group (Figure 3C; log-rank test, P = 0.289; Harrington-Fleming method, $\rho = 0$ and $\gamma =$

Table 3 Frequent and Severe Adverse Events (CTCAE version 3.0)

	FOLFOX (n = 89), FOLFOX + Bev (n = 4), XELOX + Bev (n = 1)																			
	HLA-A*2402-matched (n = 50)										HLA-A*2402-unmatched (n = 46)									
	FOLFOX (n = 48), XELOX (n = 2)										FOLFOX (n = 41) + Bev (n = 4), XELOX + Bev (n = 1)									
Adverse Event	1		2		3		4		5		1		2		3		4		5	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Hand-foot syndrome	0	0	0	0	1	2	0	0	0	0	1	2	0	0	1	2	0	0	0	0
Allergy	4	8	3	6	2	4	0	0	0	0	3	7	4	9	0	0	0	0	0	0
Mucositis	2	4	1	2	1	2	0	0	0	0	2	4	0	0	0	0	0	0	0	0
Nausea/vomiting	5	10	1	2	2	4	0	0	0	0	6	13	2	4	1	2	0	0	0	0
Neurologic toxicity	15	30	10	20	4	8	0		0	0	17	37	10	22	5	11	1	2	0	0
Anorexia	10	20	3	6	4	8	0	0	0	0	10	22	4	9	2	4	0	0	0	0
Diarrhea	3	6	6	12	2	4	0	0	0	0	3	7	0	0	1	2	0	0	0	0
Fatigue/Asthenia	5	10	1	2	2	4	0	0	0	0	5	11	1	2	1	2	0	0	0	0
Fever	2	4	0	0	0	0	0	0	0	0	3	7	2	4	0	0	0	0	0	0
Injection site reaction	18	36	18	36	9	18	0	0	0	0	20	43	17	37	3	13	0	0	0	0
Interstitial pneumonia	0	0	0	0	4	8	0	0	2	4	0	0	0	0	4	9	0	0	1	2
Neutropenia	5	10	10	20	10	20	1	2	0	0	8	17	14	30	2	4	1	2	0	0
Leukopenia	10	20	12	24	1	2	0	0	0	0	12	26	9	20	2	4	0	0	0	0
Thrombocytopenia	17	34	3	6	0	0	0	0	0	0	20	43	2	4	0	0	0	0	0	0
Bilirubin	2	4	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AL-P	11	22	1	2	1	2	0	0	0	0	10	22	1	2	0	0	0	0	0	0
Creatinine	3	6	1	2	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0
Hemoglobin	11	22	5	10	0	0	0	0	0	0	13	28	7	15	0	0	0	0	0	0
Embolism	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0
AST/ALT	12	24	0	0	1	2	0	0	0	0	6	13	1	2	0	0	0	0	0	0

No gastrointestinal perforation nor bleeding wound healing complication was observed. FOLFOX, infusional fluorouracil, leucovorin, and oxaliplatin; XELOX, capecitabine and oxaliplatin; Bev, bevacizumab; AL-P, alkaline phosphatese; AST, aspartete aminotransfarase; ALT, alanine aminotransferase; CTCAE, the Common Terminology Criteria for Adverse Event version 3.0; HLA, Human leukocyte antigen.

0.5, p = 0.152; ρ = 0 and γ = 1, p = 0.064; ρ = 0 and γ = 2, p = 0.035) while this difference was not observed in patients with NLR of ≧ 3.0 (log-lank test, p = 0.962; Harrington-Fleming method, ρ = 0 and γ = 0.5, p = 0.495; ρ = 0 and γ = 1, p = 0.346; ρ = 0 and γ = 2, p = 0.251). Similarly, in a patient group with a lymphocyte% of > 15%, a longer overall survival was observed in the HLA-A*2402-matched group (Figure 3D; log-lank test, p = 0.340; Harrington-Fleming method, ρ = 0 and γ = 0.5, p = 0.114; ρ = 0 and γ = 1, p = 0.051; ρ = 0 and γ = 2, p = 0.029).

Discussion

We performed a phase II study using a cocktail of five epitope peptides, which we previously confirmed its safety, together with oxaliplatin-based chemotherapy. The cocktail contained three peptides derived from three oncoantigens and two peptides targeting VEGFR1 and VEGFR2. This study was an HLA-A-status double-blind, phase II study of five therapeutic epitope-peptides with oxaliplatin-based chemotherapy as a first-line therapy for advanced

Table 4 Interstitial Pneumonia

HLA genotype	CTCAE grade	Result of DLLT
2402/2402	3	5FU
2402/1101	3	negative
2402/1101	5	negative
2402/0206	3	negative
2402/2603	3	5FU
2402/2602	5	negative
1101/2601	3	5FU
2601/3101	3	5FU
1101/3101	3	5FU
3004/3303	5	not examined
1101/3101	3	not examined

CTCAE, the Common Terminology Criteria for Adverse Event version 3.0; HLA, Human leukocyte antigen; DLTT, drug-induced lymphocyte transformation test; 5FU, 5-fluorouracil.

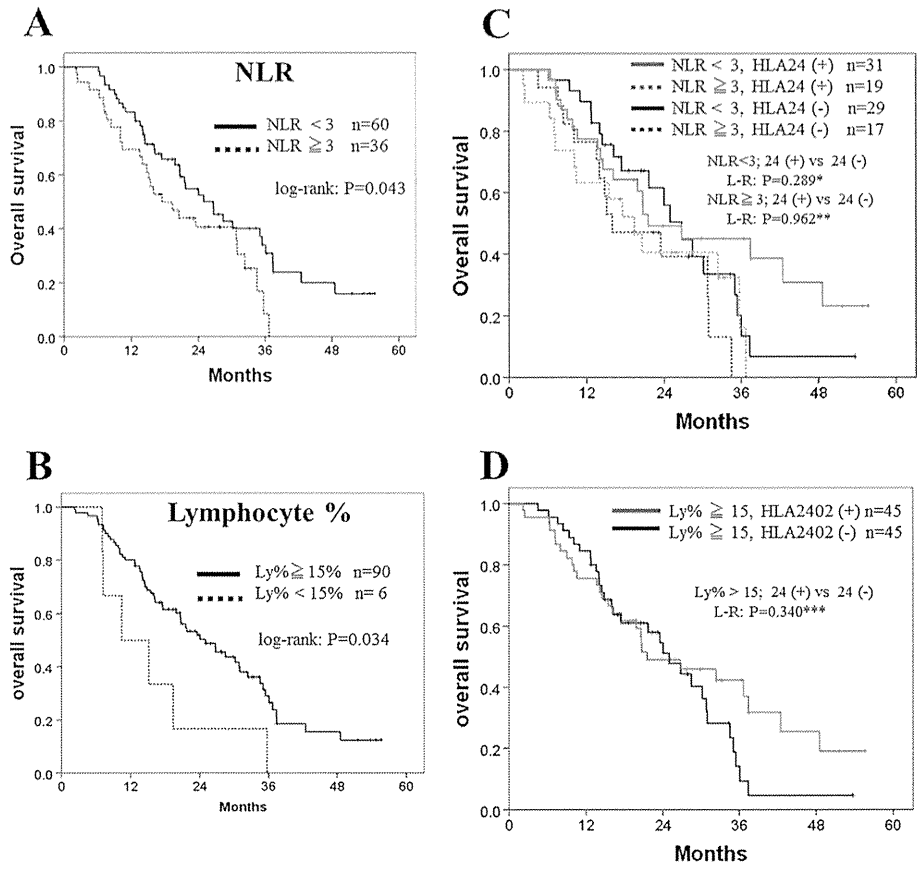

Figure 3 Biomarkers for the survival and the clinical efficacy of vaccination. Neutrophil/lymphocyte ratio (NLR) < 3.0 and Lymphocyte-% ≧ 15% were considered as indicative factors. **A** and **B**, comparison between the favorite group and others. **C**, comparison of the patients with a NLR of ≧ 3 or a NLR of <3 between the HLA-A*2402-matched and -unmatched groups. **D**, comparison of the patients with Lymphocyte-% ≧ 15% between the HLA-A*2402 positive and negative groups. Lymphocyte (Ly)-%, the percentage of lymphocytes among the peripheral leukocytes; NLR, neutrophil/lymphocyte ratio; HLA, human leukocyte antigen; L-R, log-rank test; *the weighted log-rank tests with the Harrington-Fleming class of weights were performed and resulted in, $\rho = 0$, and $\gamma = 0.5$, $p = 0.152$; $\rho = 0$, and $\gamma = 1$, $p = 0.064$; $\rho = 0$, and $\gamma = 2$, $p = 0.035$; **the Harrington-Fleming tests were resulted in, $\rho = 0$, and $\gamma = 0.5$, $p = 0.495$; $\rho = 0$, and $\gamma = 1$, $\rho = 0.346$; $\rho = 0$, and $\gamma = 2$, $\rho = 0.251$; *** the Harrington-Fleming tests were resulted in, $\rho = 0$, and $\gamma = 0.5$, $p = 0.114$; $\rho = 0$, and $\gamma = 1$, $\rho = 0.051$; $\rho = 0$, and $\gamma = 2$, $\rho = 0.029$.

colorectal cancer (FXV study). In this study, we observed many interesting results.

Firstly, the OS of the HLA-A*2402-matched group was significantly higher compared to that of the unmatched group (log-rank test, $p = 0.032$) when patients who received the vaccination for more than 12 months (Figure 2D) although no difference in PFS was observed between the two groups (Figures 2B). These results indicated that the additional effect of vaccination on the standard chemotherapy was likely to be slow-acting as this kind of delayed response by the vaccine treatment was indicated in the guidance for therapeutic cancer vaccines released from the US Food and Drug Administration in October, 2011 [28].

Secondly, neutrophil/lymphocyte ratio (NLR) might become a prognostic marker for patients who received the peptide vaccine in combination with standard chemotherapy (Figure 3A, log-rank; $p = 0.043$), and there was an obvious tail effect for extremely long survival. Then we examined the efficacy of vaccination by comparing HLA-matched group and -unmatched group. In patients with an NLR of < 3.0, a significantly longer survival in the HLA-matched group than the HLA-unmatched group was observed (Figure 3B; log-rank, $p = 0.289$; Harrington-Fleming, $p = 0.035$), while this difference was not observed in the two groups with NLR of ≧ 3.0 (log-rank, $p = 0.962$; Harrington-Fleming, $p = 0.251$). This result also support the idea that it may be critically important to apply vaccine treatment to patients with better immune status, and NLR might be a one of good predictive markers to select the appropriate patient populations for this type of treatment. A similar result was observed when we analyzed patients with lymphocyte% of ≧ 15%; HLA-matched patients with lymphocyte% of ≧ 15 showed significantly better prognosis than HLA-unmatched patients (Figure 3D; log-rank, $p = 0.340$; Harrington-Fleming,

p = 0.029). The selection of patients with lower NLR and higher lymphocyte percentage might be useful to the selection of patients who are likely to respond well to vaccine treatment and improve clinical outcomes.

Vaccinations with a cocktail of five peptides together with oxaliplatin-based chemotherapy in metastatic CRC patients were well tolerated, except for relatively frequent cases (11 cases; 11.4%) of pneumonitis (Tables 3 and 4), whose incidence seemed to be higher than previously reported for oxaliplatin-based chemotherapies although no difference was observed between HLA-matched and -unmatched group. Correale et al. reported two cases (5.5%) in 36 patients with advanced gastric cancer treated with gemcitabine plus oxaliplatin, folinic acid, and 5-fluorouracil (FOLFOX-4) [29]. Usui et al. reported that four cases (3.9%) of pneumonitis among 104 Japanese patients treated with oxaliplatin-containing regimes for advanced colorectal cancer [30]. In addition, there have been many case reports of oxaliplatin-related pneumonitis [31-35]. In this study, eleven (11.4%) of 96 patients suffered from severe pneumonitis including three cases with grade 5 pneumonitis. To investigate the possible cause of pneumonitiswe performed drug-induced lymphocyte transformation test (DLTT) for nine patients whose samples were available. Among them, five patients (55.6%) were judged to be positive to fluorouracil alone, and the remaining four patients were negative for all of the antigens tested. Although the size of this study is not large enough to make any conclusion and there is no difference between the two groups, this adverse event should be carefully monitored when we will perform the next-step clinical trial.

Although the efficacy of our peptide vaccine was not clearly demonstrated in this phase II study, the timing of and combination treatment with vaccination might not be optimized, and the sample size was limited. Recently, regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) are reported as potent immunosuppressive cells to protect cancer cells from the host immune system [36,37]. Over expression of PD-L1and PD-1 as well as up-regulation of indoleamine-2,3-dioxygenase (IDO) in the tumor microenvironment also inhibit the CTL functions [38]. Hence, to overcome these immune-escape mechanisms, various approaches have been taken in the last decade [39,40]. For example, anti-PD1antibody [41], anti-PD-L1antibody [42], and anti-CTL4 antibody [43] were applied in clinical trials to overcome the suppressive immuno checkpoints, and surprisingly high objective response rates were observed in many types of malignant neoplasm. Small-molecule inhibitors [44] that block IDO enzymatic activity or cyclophosphamide to reduce the number of Tregs [45] were also applied in clinical trials to dissolve the suppressive immunity. For the successful next generation immunotherapy, peptide vaccine should be combined with some agents to modify the immune-suppressive tumor microenvironments.

In conclusion, our cocktail of five therapeutic epitope peptides appears to be effective in a subset of patients, and warrants a randomized phase III study. In the phase III study, biomarkers such as NLR and lymphocyte-% might be useful for assessing the response to the peptide vaccine and for selecting patients likely to have a better treatment outcome with the vaccination.

Conclusions

This phase II cancer vaccine therapy demonstrated that our therapeutic peptides cocktail was likely to be effective in a subset of patients and warrants a randomized phase III study. In the phase III study, predictive biomarkers such as NLR and lymphocyte-% should be used for its response and for selecting patients to have a better treatment outcome with the vaccination.

Additional file

Additional file 1: Summary of the protocol.

Abbreviations

RNF43: Ring finger protein 43; TOMM34: 34 kDa-translocase of the outer mitochondrial membrane; KOC1: insulin-like growth factor–II mRNA binding protein 3; VEGFR: Vascular endothelial growth factor receptor; HPLC: High performance liquid chromatography; CRC: Colorectal cancer; ELISPOT: Enzyme-linked immunospot; PBMC: Peripheral blood mononuclear cells; CTL: Cytotoxic T lymphocytes; RR: Response rates; CR: Complete clinical response; SD: Stable disease; PD: Progressive disease; PFS: Progression free survival; OS: Overall survival; HLA: Human leukocyte antigen; MST: Median overall survival time; ECOG: Eastern cooperative oncology group; RECIST: Response evaluation criteria in solid tumors; TIL: Tumor infiltrating cells; CTCAE: Common terminology criteria for adverse events version3.0; AEs: Adverse events; SAEs: Serious adverse events; DLTT: Drug-induced lymphocyte transformation test; PS: Performance status; IFA: Incomplete freund's adjuvant; CT: Computed tomography; MRI: Magnetic resonance imaging; NLR: Neutrophil/lymphocyte ratio; FOLFOX: Infusional fluorouracil, leucovorin, and oxaliplatin; XELOX: Capecitabine and oxaliplatin, Tregs, regulatory T cells; MDSCs: Myeloid-derived suppressor cells; IDO: Indoleamine-2,3-dioxygenase.

Competing interests

Yusuke Nakamura is a stock holder and a scientific advisor of OncoTherapy Science, Inc. The other authors have no potential conflicts of interest to disclose.

Authors' contributions

SH designed, performed and evaluated clinical study, and wrote the manuscript. YN and MO participated in the design, review and revision of the manuscript. HT, KH, KT, RS, HO, RE, FS, KO, TF, TN, KS, KY, YI, SK, YS, NS, SY, HS, AK, TF, YK and HF assisted to perform clinical study. RT, HT, and TY contributed in the data collection and statistical analysis. All authors participated in the data acquisition and discussion of the manuscript and approved the final manuscript.

Acknowledgements

This study was supported partially by the Project for Development of Innovative Research on Cancer Therapeutics (P-DIRECT) of the Ministry of Education, Culture, Sports, Science and Technology of Japan. The authors would like to thank Prof. Yusuke Nakamura, Dr. Takuya Tsunoda, Dr. Koji Yoshida, Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, for their excellent advice and

cooperation and providing all the peptides. The authors also thank Prof. Koji Kono, Department of Surgery, National University of Singapore, for his excellent managements as The Data and Safety Monitoring Committee of this study.

Author details
[1]Department of Digestive Surgery and Surgical Oncology, Yamaguchi University Graduate School of Medicine, Ube, Japan. [2]Department of Medicine and Surgery, The University of Chicago, Chicago, IL, USA. [3]Department of Surgical Oncology, Osaka City University Graduate School of Medicine, Osaka, Japan. [4]Department of Surgery, Kure-Kyosai Hospital, Kure, Japan. [5]Department of Surgery, Ogori Daiichi General Hospital, Yamaguchi, Japan. [6]Department of Surgery, Kinki University Faculty of Medicine, Osaka-Sayama, Japan. [7]Department of Surgery, Kanmon-Medical Center, Shimonoseki, Japan. [8]Department of Surgery, Shimonoseki-Kosei Hospital, Shimonoseki, Japan. [9]Tokyo Women's Medical University Medical Center East, Tokyo, Japan. [10]Department of Pharmacy, Yamaguchi University Hospital, Ube, Japan. [11]Department of Biostatistics, National Cancer Center, Chiba, Japan. [12]Division of Cellular Signaling, Institute for Advanced Medical Research, Keio University School of Medicine, Tokyo, Japan.

Received: 10 March 2014 Accepted: 16 April 2014
Published: 30 April 2014

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM: **Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008.** *Int J Cancer* 2010, **127**:2893–2917.
2. Tournigand C, Andre T, Achille E, Lledo G, Flesh M, Mery-Mignard D, Quinaux E, Couteau C, Buyse M, Ganem G, Landi B, Colin P, Louvet C, de Gramont A: **FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study.** *J Clin Oncol* 2004, **22**:229–237.
3. Cassidy J, Clarke S, Diaz-Rubio E, Scheithauer W, Figer A, Wong R, Koski S, Lichinitser M, Yang TS, Rivera F, Couture F, Sirzen F, Saltz L: **Randomized phase III study of capecitabine plus oxaliplatin compared with fluorouracil/folinic acid plus oxaliplatin as first-line therapy for metastatic colorectal cancer.** *J Clin Oncol* 2008, **26**:2006–2012.
4. Saltz LB, Clarke S, Diaz-Rubio E, Scheithauer W, Figer A, Wong R, Koski S, Lichinitser M, Yang TS, Rivera F, Couture F, Sirzen F, Cassidy J: **Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study.** *J Clin Oncol* 2008, **26**:2013–2019.
5. Van Cutsem E, Kohne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, D'Haens G, Pinter T, Lim R, Bodoky G, Roh JK, Folprecht G, Ruff P, Stroh C, Tejpar S, Schlichting M, Nippgen J, Rougier P: **Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer.** *N Engl J Med* 2009, **360**:1408–1417.
6. Douillard JY, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocakova I, Ruff P, Blasinska-Morawiec M, Smakal M, Canon JL, Rother M, Oliner KS, Wolf M, Gansert J: **Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study.** *J Clin Oncol* 2010, **28**:4697–4705.
7. Okabe H, Satoh S, Kato T, Kitahara O, Yanagawa R, Yamaoka Y, Tsunoda T, Furukawa Y, Nakamura Y: **Genome-wide analysis of gene expression in human hepatocellular carcinomas using cDNA microarray: identification of genes involved in viral carcinogenesis and tumor progression.** *Cancer Res* 2001, **61**:2129–2137.
8. Yagyu R, Furukawa Y, Lin YM, Shimokawa T, Yamamura T, Nakamura Y: **A novel oncoprotein RNF43 functions in an autocrine manner in colorectal cancer.** *Int J Oncol* 2004, **25**:1343–1348.
9. Shimokawa T, Matsushima S, Tsunoda T, Tahara H, Nakamura Y, Furukawa Y: **Identification of TOMM34, which shows elevated expression in the majority of human colon cancers, as a novel drug target.** *Int J Oncol* 2006, **29**:381–386.
10. Kikuchi T, Daigo Y, Katagiri T, Tsunoda T, Okada K, Kakiuchi S, Zembutsu H, Furukawa Y, Kawamura M, Kobayashi K, Imai K, Nakamura Y: **Expression profiles of non-small cell lung cancers on cDNA microarrays: identification of genes for prediction of lymph-node metastasis and sensitivity to anti-cancer drugs.** *Oncogene* 2003, **22**:2192–2205.
11. Rosenberg SA, Yang JC, Restifo NP: **Cancer immunotherapy: moving beyond current vaccines.** *Nat Med* 2004, **10**:909–915.
12. Khong HT, Restifo NP: **Natural selection of tumor variants in the generation of tumor "escape" phenotypes.** *Nat Immunol* 2002, **3**:999–1005.
13. Ryschich E, Notzel T, Hinz U, Autschbach F, Ferguson J, Simon I, Weitz J, Frohlich B, Klar E, Buchler MW, Schmidt J: **Control of T-cell-mediated immune response by HLA class I in human pancreatic carcinoma.** *Clin Cancer Res* 2005, **11**:498–504.
14. Folkman J: **Tumor angiogenesis: therapeutic implications.** *N Engl J Med* 1971, **285**:1182–1186.
15. Olofsson B, Korpelainen E, Pepper MS, Mandriota SJ, Aase K, Kumar V, Gunji Y, Jeltsch MM, Shibuya M, Alitalo K, Eriksson U: **Vascular endothelial growth factor B (VEGF-B) binds to VEGF receptor-1 and regulates plasminogen activator activity in endothelial cells.** *Proc Natl Acad Sci U S A* 1998, **95**:11709–11714.
16. Millauer B, Wizigmann-Voos S, Schnurch H, Martinez R, Moller NP, Risau W, Ullrich A: **High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis.** *Cell* 1993, **72**:835–846.
17. Date Y, Kimura A, Kato H, Sasazuki T: **DNA typing of the HLA-A gene: population study and identification of four new alleles in Japanese.** *Tissue Antigens* 1996, **47**:93–101.
18. Hazama S, Nakamura Y, Takenouchi H, Suzuki N, Tsunedomi R, Inoue Y, Tokuhisa Y, Iizuka N, Yoshino S, Takeda K, Shinozaki H, Kamiya A, Furukawa H, Oka M: **A phase I study of combination vaccine treatment of five therapeutic epitope-peptides for metastatic colorectal cancer; safety, immunological response, and clinical outcome.** *J Transl Med* 2014, **12**:63.
19. Maeda K, Hazama S, Tokuno K, Kan S, Maeda Y, Watanabe Y, Kamei R, Shindo Y, Maeda N, Yoshimura K, Yoshino S, Oka M: **Impact of chemotherapy for colorectal cancer on regulatory T-cells and tumor immunity.** *Anticancer Res* 2011, **31**:4569–4574.
20. Uchida N, Tsunoda T, Wada S, Furukawa Y, Nakamura Y, Tahara H: **Ring finger protein 43 as a new target for cancer immunotherapy.** *Clin Cancer Res* 2004, **10**:8577–8586.
21. Suda T, Tsunoda T, Daigo Y, Nakamura Y, Tahara H: **Identification of human leukocyte antigen-A24-restricted epitope peptides derived from gene products upregulated in lung and esophageal cancers as novel targets for immunotherapy.** *Cancer Sci* 2007, **98**:1803–1808.
22. Ishizaki H, Tsunoda T, Wada S, Yamauchi M, Shibuya M, Tahara H: **Inhibition of tumor growth with antiangiogenic cancer vaccine using epitope peptides derived from human vascular endothelial growth factor receptor 1.** *Clin Cancer Res* 2006, **12**:5841–5849.
23. Wada S, Tsunoda T, Baba T, Primus FJ, Kuwano H, Shibuya M, Tahara H: **Rationale for antiangiogenic cancer therapy with vaccination using epitope peptides derived from human vascular endothelial growth factor receptor 2.** *Cancer Res* 2005, **65**:4939–4946.
24. Kato T, Muro K, Yamaguchi K, Bando H, Hazama S, Amagai K, Baba H, Denda T, Shi X, Fukase K, Skamoto J, Mishima H: **Cediranib in combination with mFOLFOX6 in Japanese patients with metastatic colorectal cancer: results from the randomised phase II part of a phase I/II study.** *Ann Oncol* 2012, **23**:933–941.
25. Schmoll HJ, Cunningham D, Sobrero A, Karapetis CS, Rougier P, Koski SL, Kocakova I, Bondarenko I, Bodoky G, Mainwaring P, Salazar R, Barker P, Mookerjee B, Robertson J, Van Cutsem E: **Cediranib with mFOLFOX6 versus bevacizumab with mFOLFOX6 as first-line treatment for patients with advanced colorectal cancer: a double-blind, randomized phase III study (HORIZON III).** *J Clin Oncol* 2012, **30**:3588–3595.
26. Fleming T, Harrington D: *Counting Processes and Survival Analysis.* New York: John Wiley & Sons; 1991.
27. He W, Yin C, Guo G, Jiang C, Wang F, Qiu H, Chen X, Rong R, Zhang B, Xia L: **Initial neutrophil lymphocyte ratio is superior to platelet lymphocyte ratio as an adverse prognostic and predictive factor in metastatic colorectal cancer.** *Med Oncol* 2013, **30**:439.
28. U.S. Department of Health and Human Services, Food and Drug Administration: **Guidance for Industry. Clinical Considerations for Therapeutic Cancer Vaccines.** 2011, http://www.fda.gov/downloads/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/vaccines/ucm278673.pdf.
29. Correale P, Fulfaro F, Marsili S, Cicero G, Bajardi E, Intrivici C, Vuolo G, Carli AF, Caraglia M, Del Prete S, Greco E, Gebbia N, Francini G: **Gemcitabine (GEM) plus oxaliplatin, folinic acid, and 5-fluorouracil (FOLFOX-4) in**

patients with advanced gastric cancer. *Cancer Chemother Pharmacol* 2005, **56**:563–568.
30. Usui K, Katou Y, Furushima K, Tanaka Y, Tanai C, Ishihara T: **Interstitial lung disease during chemotherapy combined with oxaliplatin and/or bevacizumab in advanced colorectal cancer patients.** *Jpn J Clin Oncol* 2011, **41**:498–502.
31. Gagnadoux F, Roiron C, Carrie E, Monnier-Cholley L, Lebeau B: **Eosinophilic lung disease under chemotherapy with oxaliplatin for colorectal cancer.** *Am J Clin Oncol* 2002, **25**:388–390.
32. Fuse N, Doi T, Ohtsu A, Takeuchi S, Kojima T, Taku K, Tahara M, Muto M, Asaka M, Yoshida S: **Feasibility of oxaliplatin and infusional fluorouracil/leucovorin (FOLFOX4) for Japanese patients with unresectable metastatic colorectal cancer.** *Jpn J Clin Oncol* 2007, **37**:434–439.
33. Arevalo Lobera S, Sagastibeltza Marinelarena N, Elejoste Echeberria I, Mele Olive M, Egana Otano L, Basterretxea Badiola L, La Casta Munoa A, Azkue Gabilondo M: **Fatal pneumonitis induced by oxaliplatin.** *Clin Transl Oncol* 2008, **10**:764–767.
34. Pontes LB, Armentano DP, Soares A, Gansl RC: **Fatal pneumonitis induced by oxaliplatin: description of three cases.** *Case Rep Oncol* 2012, **5**:104–109.
35. Muneoka K, Shirai Y, Sasaki M, Wakai T, Sakata J, Hatakeyama K: **Interstitial pneumonia arising in a patient treated with oxaliplatin, 5-fluorouracil, and, leucovorin (FOLFOX).** *Int J Clin Oncol* 2009, **14**:457–459.
36. Facciabene A, Motz GT, Coukos G: **T-regulatory cells: key players in tumor immune escape and angiogenesis.** *Cancer Res* 2012, **72**:2162–2171.
37. Lindau D, Gielen P, Kroesen M, Wesseling P, Adema GJ: **The immunosuppressive tumour network: myeloid-derived suppressor cells, regulatory T cells and natural killer T cells.** *Immunology* 2013, **138**:105–115.
38. Gajewski TF, Schreiber H, Fu YX: **Innate and adaptive immune cells in the tumor microenvironment.** *Nat Immunol* 2013, **14**:1014–1022.
39. Okazaki T, Tanaka Y, Nishio R, Mitsuiye T, Mizoguchi A, Wang J, Ishida M, Hiai H, Matsumori A, Minato N, Honjo T: **Autoantibodies against cardiac troponin I are responsible for dilated cardiomyopathy in PD-1-deficient mice.** *Nat Med* 2003, **9**:1477–1483.
40. Pardoll DM: **The blockade of immune checkpoints in cancer immunotherapy.** *Nat Rev Cancer* 2012, **12**:252–264.
41. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M: **Safety, activity, and immune correlates of anti-PD-1 antibody in cancer.** *N Engl J Med* 2012, **366**:2443–2454.
42. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, Martins R, Eaton K, Chen S, Salay TM, Alaparthy S, Grosso JF, Korman AJ, Parker SM, Agrawal S, Goldberg SM, Pardoll DM, Gupta A, Wigginton JM: **Safety and activity of anti-PD-L1 antibody in patients with advanced cancer.** *N Engl J Med* 2012, **366**:2455–2465.
43. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, Segal NH, Ariyan CE, Gordon RA, Reed K, Burke MM, Caldwell A, Kronenberg SA, Agunwamba BU, Zhang X, Lowy I, Inzunza HD, Feely W, Horak CE, Hong Q, Korman AJ, Wigginton JM, Gupta A, Sznol M: **Nivolumab plus ipilimumab in advanced melanoma.** *N Engl J Med* 2013, **369**:122–133.
44. Liu X, Shin N, Koblish HK, Yang G, Wang Q, Wang K, Leffet L, Hansbury MJ, Thomas B, Rupar M, Waeltz P, Bowman KJ, Polam P, Sparks RB, Yue EW, Li Y, Wynn R, Fridman JS, Burn TC, Combs AP, Newton RC, Scherle PA: **Selective inhibition of IDO1 effectively regulates mediators of antitumor immunity.** *Blood* 2010, **115**:3520–3530.
45. Walter S, Weinschenk T, Stenzl A, Zdrojowy R, Pluzanska A, Szczylik C, Staehler M, Brugger W, Dietrich PY, Mendrzyk R, Hilf N, Schoor O, Fritsche J, Mahr A, Maurer D, Vass V, Trautwein C, Lewandrowski P, Flohr C, Pohla H, Stanczak JJ, Bronte V, Mandruzzato S, Biedermann T, Pawelec G, Derhovanessian E, Yamagishi H, Miki T, Hongo F, Takaha N: **Multipeptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival.** *Nat Med* 2012, **18**:1254–1261.

doi:10.1186/1479-5876-12-108
Cite this article as: Hazama *et al.*: A phase II study of five peptides combination with oxaliplatin-based chemotherapy as a first-line therapy for advanced colorectal cancer (FXV study). *Journal of Translational Medicine* 2014 **12**:108.

World J Surg
DOI 10.1007/s00268-015-2967-y

ORIGINAL SCIENTIFIC REPORT

Impact of Energy Devices During Liver Parenchymal Transection: A Multicenter Randomized Controlled Trial

Naoto Gotohda · Takeharu Yamanaka · Akio Saiura · Katsuhiko Uesaka · Masaji Hashimoto · Masaru Konishi · Kazuaki Shimada

Abstract

Objective To clarify the benefit of energy devices such as ultrasonically activated device and bipolar vessel sealing device in liver surgeries.

Background Several studies have suggested the benefit of energy devices in liver transection, while a randomized trial has found no association between their use and surgical outcomes.

Patients and methods Patients scheduled to undergo open liver resection were eligible for this multicenter non-blinded randomized study. They were randomized to receive an energy device (experimental group) or not (control group) during liver transection. The primary endpoint was the proportion of patients with intraoperative blood loss >1,000 mL. The primary aim was to show non-inferiority of hepatectomy with energy device to that without energy device.

Results A total of 212 patients were randomized and 211 (105 and 106 in the respective groups) were analyzed. Intraoperative blood loss >1,000 mL occurred in 15.0 % patients with energy device and 20.2 % patients without energy device. The experimental minus control group difference was −5.2 % (95 % confidence interval −13.8 to 3.3 %; non-inferiority test, $p = 0.0248$). Hepatectomy with energy device resulted in a shorter median liver transection time (63 vs. 84 min; $p < 0.001$) and a lower rate of postoperative bile leakage (4 vs. 16 %; $p = 0.002$).

Conclusions The hypothesis that hepatectomy with energy device is not inferior to that without energy device in terms of blood loss has been demonstrated. The use of energy devices during liver surgery is clinically meaningful as it shortens the liver transection time and reduces the incidence of postoperative bile leakage.

UMIN Clinical Trials Registry (http://www.umin.ac.jp/ctr/index.htm): UMIN000006044.

N. Gotohda (✉) · M. Konishi
Department of Hepatobiliary and Pancreatic Surgery, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan
e-mail: ngotohda@east.ncc.go.jp

T. Yamanaka
Department of Biostatistics, Yokohama City University, Yokohama, Japan

A. Saiura
Department of Gastrointestinal Surgery, Cancer Institute Hospital, Japanese Foundation for Cancer Research, Tokyo, Japan

K. Uesaka
Department of Hepato-Biliary-Pancreatic Surgery, Sizuoka Cancer Center Hospital, Shizuoka, Japan

M. Hashimoto
Department of Digestive Surgery, Toranomon Hospital, Tokyo, Japan

K. Shimada
Department of Hepatobiliary and Pancreatic Surgery, National Cancer Center Hospital, Tokyo, Japan

Published online: 31 January 2015

Introduction

In the last decade, various energy devices have been developed for reducing blood loss in liver surgeries. Particularly, ultrasonically activated devices(UAD) and bipolar vessel sealing devices have widely been used for thoracic, gastrointestinal, urologic, and gynecologic surgeries. UADs, used for tissue coagulation and cutting, are surgical instruments that utilize ultrasonic vibrations [1, 2]. Bipolar vessel sealing devices can reliably seal and permanently occlude blood vessels by denaturing collagen and elastin present in the vessel wall [3].

Previous studies, including a meta-analysis of the clamp-crushing technique [4], gave conflicting results about the benefit of energy devices in liver surgeries. Several studies, including a small randomized trial comparing a bipolar vessel sealing system with no energy device during liver transection, have suggested the benefit of energy devices [5–8]. In contrast, a recent randomized trial has found no association between the use of a bipolar blood sealing system and blood loss volume, operation time, or incidence of postoperative complications [9]. A well-designed, adequately powered confirmatory study is clearly needed to draw definitive conclusions about this issue.

Methods

Patients

Patients scheduled to undergo open liver resection for liver tumor were eligible for this prospective multicenter randomized study. Five centers were involved in randomization. Other inclusion criteria were age between 20 and 85 years, ECOG performance status of 0–2, and sufficient organ function (white blood cell count 2,000–10,000 mm^{-3}, platelet count $\geq$50,000 μL^{-1}, hemoglobin $\geq$8 g/dL, total bilirubin $\leq$2 mg/dL, prothrombin time activity $\geq$50 %, and creatinine $\leq$1.5 mg/dL). Patients with any of the following conditions were excluded from the study: a schedule of laparoscopic liver resection; need for bilioenteric reconstruction; and a solitary liver surface tumor of <2 cm in diameter (as they were easily removable). In addition, patients who required other resections (except hepatectomy and cholecystectomy) for any simultaneous primary lesion, metastatic lesion, or non-malignant disease were also excluded.

This study was conducted in accordance with the Declaration of Helsinki. Before enrollment, all participating patients gave written informed consent to the study.

Randomization and endpoints

Before laparotomy, patients were randomly assigned to receive an energy device (group E, investigational arm) or not (group C, control arm) at a 1:1 ratio. Randomization was done centrally using the minimization method with stratification with regard to center, liver function (Child-Pugh Classification, A vs. B/C), and extent of hepatectomy (major vs. minor). In each cell defined by three strata, patients were allocated to either group E or group C in a 1:1 ratio. For allocation of the participants, a computer-generated list of random numbers was created. Each patient enrolled was assigned an order number at the coordinating center and the treatment group communicated by FAX on request to the participating centers.

Major hepatectomy was defined as the liver resection of $\geq$2 Couinaud segments, excluding the resection of Couinaud segments II and III. Neither investigators nor patients were blinded to the allocated surgical procedure.

The primary endpoint was the proportion of patients with intraoperative blood loss >1,000 mL. The secondary endpoints included liver transection time, liver transection speed, number of ties, laboratory data [total bilirubin, aspartate aminotransferase (AST), and C-reactive protein (CRP)] on postoperative day 1, postoperative complications [postoperative bleeding, bile leakage, and surgical site infection (SSI)], and postoperative hospital stay. The liver transection speed (cm^2/min) was calculated by dividing the transection area by transection time. Postoperative bleeding was defined as the situation requiring surgical, endoscopic, or radiological intervention in the criteria of Clavien–Dindo classification [10]. Bile leakage was defined as the total bilirubin level of >5 mg/dL in the drained fluid on the fifth postoperative day [11]. The definition of surgical site infection was based on the guideline of SSI [12]. The study protocol was approved by the ethical committees of all participating institutions.

Surgical procedure

For both treatment arms, the liver parenchyma was fractured by the cavitron ultrasonic surgical aspirator (CUSA) or forceps (clamp-crush method). Investigators could choose between these two common methods to divide the liver parenchyma [10] at their discretion. Pringle's maneuver (15 min clamping and 5 min release) was mostly applied as for the intermittent inflow occlusion during the liver transection except when severe adhesion was recognized in repeat hepatectomy case.

Once the parenchyma was crushed, the exposed vessels were ligated with a silk string in group C, while the exposed vessels were sealed with an energy device, if they were <5 mm (but ligated with a silk string if >5 mm), in group E. Either an UAD (Harmonic FOCUS^{TM}, Ethicon Endo-Surgery) or a bipolar vessel sealing device (Ligasure^{TM} Small Jaw, COVIDIEN) was allowed to be used as an energy device. Investigators chose the device according to their preference. Drains are placed routinely.

Statistical methods

In a preliminary survey, the proportion of patients with intraoperative blood loss >1,000 mL was 35.7 % (100/280) in hepatectomy without any energy devices in one of our participating institutes from January 2004 to December 2006, and 25.1 % (63/251) in hepatectomy with the energy device in another one of our participating institutes from September 2007 to December 2009. Then, we hypothesized that use of an energy device in hepatectomy reduced the proportion of patients with intraoperative blood loss >1,000 mL (primary endpoint) from 30 % with no energy device to 20 %. The primary aim of this study was to show non-inferiority (margin 5 %) of hepatectomy with energy device to that without energy device in terms of this proportion. To have a one-sided type 1 error of 5 % and a power of 80 %, the target accrual was 105 patients per group, 210 patients in total.

The Dunnett–Gent method was used for the non-inferiority test of the primary endpoint with strata of center, liver function, and extent of hepatectomy. If non-inferiority was shown, a sequential testing of superiority was performed by the χ^2 test with a one-sided type 1 error of 5 %. Secondary endpoints were tested by the Wilcoxon test for continuous variables and the χ^2 test for categorical variables. Multivariate analysis was tested by logistic regression analysis. All analyses were performed on a modified intention-to-treat (ITT) population which did not include patients who proved to be ineligible after randomization. *P* values of the non-inferiority and superiority tests for the primary endpoint were reported as one-sided, while all other *p* values as two-sided. All statistical analyses were performed with SAS version 9.2 and PASW version 18.

This study was registered at UMIN Clinical Trials Registry (UMIN-CTR) (http://www.umin.ac.jp/ctr/index.htm) as UMIN000006044.

Results

Patient population

Between May 2011 and December 2012, 107 and 105 patients were randomly assigned to groups E and C, respectively. One patient in group C did not undergo hepatectomy because of peritoneal dissemination. This patient was excluded from the modified ITT population, leaving 211 patients evaluable for all endpoints (Fig. 1). Table 1 shows baseline and perioperative patient characteristics. In general, the two groups were well balanced with respect to all demographics, preoperative laboratory data, tumor factors, and perioperative factors. An energy device was used in two patients in group C after intraoperative blood loss exceeded 1,000 mL. In group E, the number case of use of Harmonic FOCUS^{TM} was 42 (39 %) and Ligasure^{TM} was 65 (61 %).

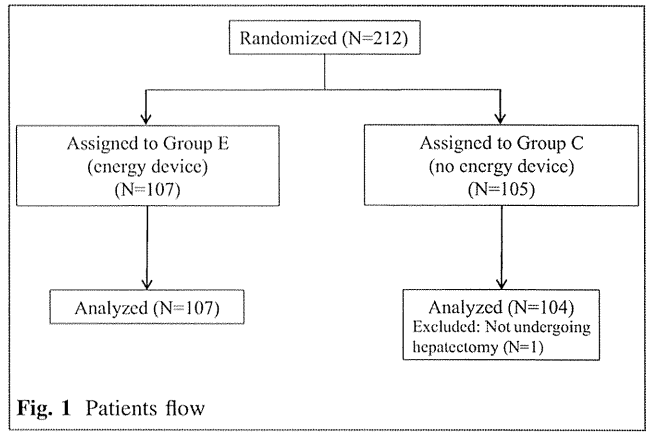

Fig. 1 Patients flow

Blood loss

The proportion of patients with intraoperative blood loss >1,000 mL was 15.0 % in group E and 20.2 % in group C. The difference (E − C) was −5.2 % [95 % confidence interval (CI) −13.8 to 3.3 %; $p = 0.0248$], meeting the criteria for non-inferiority of group E versus group C in terms of the primary endpoint (Table 2). However, the superiority test was not statistically significant ($p = 0.16$).

The median amount of blood loss in group E was 491 mL (range 30–2,760), which was not significantly different from group C [median 615 (range 25–9,300)] mL; $p = 0.169$).

Other surgical outcomes

Table 3 presents the results for secondary endpoints. For operative factors, the median (range) liver transection time in group E was shorter than that in group C [63 (16–196) vs. 84 (12–221) min; $p < 0.001$]. The median (range) transection speed in group E was 1.18 (0.24–7.5) cm^2/min, which was significantly faster than that in group C [0.91 (0.18–3.04) cm^2/min; $p = 0.002$]. The median (range) total numbers of ligations during liver transection were 17 (1–111) and 66 (4–178) in groups E and C, respectively; this difference was statistically significant ($p < 0.001$). Regarding laboratory data (total bilirubin, AST, and CRP) on postoperative day 1, there was no significant difference between the two groups.

For postoperative outcomes, the incidence of bile leakage was significantly lower in group E than in group C (4 vs. 16 %; $p = 0.002$). Postoperative bleeding occurred in two patients in group E (1.9 %) and two in group C (1.9 %). Of these two patients in group C, one patient died due to postoperative bleeding. Surgical site infection occurred in eight patients in group E (7.4 %) and seven in group C (6.7 %).

Table 1 Patients characteristics

	Group E ($n = 107$)	Group C ($n = 104$)	p
Demographic data			
Age (median, range)	63 (27–83)	64 (30–84)	0.299
Gender, male	79 (74 %)	78 (75 %)	0.876
Body mass index[a] (median, range)	22.5 (16.3–33.2)	22.0 (14.9–32.6)	0.684
Disease: HCC[c]/meta[b]/others	45/56/6	42/55/7	0.929
HBs antigen positive	18 (17 %)	9 (9 %)	0.099
HCV antibody positive	14 (13 %)	14 (13 %)	1.000
Preoperative use of anticoagulant drug	6 (6 %)	1 (1 %)	0.119
Preoperative chemotherapy	27 (25 %)	27 (26 %)	1.000
Background liver: cirrhosis or chronic hepatitis	35 (33 %)	36 (35 %)	0.756
Child–Pugh score (A/B,C)	107/0	104/0	ND
Preoperative laboratory data (median, range)			
Hemoglobin (g/dL)	13.2 (9.4–17.7)	13.2 (8.5–16.7)	0.492
Platelet ($\times 10^4/\mu L$)	18.6 (7.1–46.6)	19.5 (7.7–39.0)	0.744
Total bilirubin (mg/dL)	0.7 (0.3–1.8)	0.7 (0.3–1.7)	0.405
AST (U/L)	28.0 (13–178)	28.5 (13–201)	0.859
Indocyanine green retention rate at 15 min(%)	9.4 (1.4–32.6)	10.9 (0.3–24.0)	0.045
Tumor factors			
No. of tumors (median, range)	1 (1–24)	1 (1–12)	0.272
Diameter (mm) (median, range)	30 (10–210)	35 (8–115)	0.221
Vascular invasion	20 (19 %)	17 (16 %)	0.719
Portal vein invasion	15 (14 %)	11 (11 %)	0.532
Hepatic vein invasion	6 (6 %)	8 (8 %)	0.590
Perioperative factors			
Major hepatectomy[d]	40 (37 %)	43 (41 %)	0.485
Repeat hepatectomy	19 (18 %)	20 (19 %)	0.860
Multiple resections	33 (31 %)	29 (28 %)	0.653
Use of Pringle's maneuver	97 (91 %)	96 (92 %)	0.806
Use of IVC (half) clamp	12 (11 %)	12 (12 %)	1.000
Thoracotomy added	5 (5 %)	6 (6 %)	0.766
Use of CUSA[e]	14 (13 %)	13 (13 %)	1.000
Surgeon (consultant)	48 (45 %)	43 (41 %)	0.677
Intraoperative RBC[f] transfusion	6 (6 %)	7 (7 %)	0.781
Mortality	0	1	ND

All p values were calculated by the Wilcoxon test for continuous variables and by the χ^2 test for categorical variables

[a] Body weight (kg)/height $(m)^2$

[b] Metastatic liver carcinoma

[c] Hepatocellular carcinoma

[d] More than one section hepatectomy excluding left lateral sectionectomy

[e] Cavitron ultrasound surgical aspirator

[f] Red blood cell

The duration of postoperative hospital stay was similar for the two groups [median (range) 10 (3–68) days in group E vs. 11 (3–57) days in group C; $p = 0.17$].

Potential risk factors associated with blood loss >1,000 mL were analyzed by logistic regression model (Table 4). Major hepatectomy was significantly identified to be the only risk factor for blood loss >1,000 mL.

Subgroup analysis

All results of subgroup analysis for the primary endpoint were consistent with the overall results, except the patients undergoing major hepatectomy and those with poor liver reserve function (indocyanine green retention time at 15 min [ICG R15] ≥10 %) (Fig. 2). In patients undergoing

Table 2 Primary endpoint

Primary endpoint	Group E ($n = 107$)	Group C ($n = 104$)	Difference (E − C) (95 % CI)	Non-inferiority p^a (one-sided)	Superiority p^b (one-sided)
Patients with intraoperative blood loss >1,000 mL	16 (15.0 %)	21 (20.2 %)	−5.2 % (−13.8 to 3.3)	**0.0248**	0.16

Bold values are statistically significant ($p < 0.05$)

[a] Dunnett–Gent test

[b] χ^2 test

Table 3 Secondary endpoints

Operative factors (median, range)	Group E ($n = 107$)	Group C ($n = 104$)	p
Liver transection time (min)	63 (16–196)	84 (12–290)	**0.000**
Liver transection speed (cm^2/min)	1.18 (0.24–7.5)	0.91 (0.18–3.04)	**0.002**
No. of ligations	17 (1–111)	66 (4–178)	**0.000**
Laboratory data on POD 1 (median, range)			
Total Bilirubin (mg/dL)	1.1 (0.3–3.5)	1.1 (0.3–4.7)	0.91
AST (U/L)	269 (41–1656)	304 (34–3600)	0.50
CRP (mg/dL)	3.5 (0.5–13.9)	3.4 (0.4–20.2)	0.80
Postoperative outcomes			
Postoperative bleeding	2 (1.9 %)	2 (1.9 %)	0.98
Bile leakage	4 (3.7 %)	17 (16.3 %)	**0.002**
Surgical site infection	8 (7.4 %)	7 (6.7 %)	0.83
Postoperative hospital stay (day)	10 (3–68)	11 (3–57)	0.17

All p values were calculated by the Wilcoxon test for continuous variables and by the χ^2 test for categorical variables

Bold values are statistically significant ($p < 0.05$)

Table 4 Multivariate analysis

Variables	Odds ratio	95 % CI	p
Use of energy device	0.727	0.342–1.546	0.407
Age	0.993	0.961–1.027	0.693
ICG R15[a]	0.992	0.917–1.072	0.835
Tumor size	1.010	0.998–1.023	0.114
Tumor vascular invasion (yes)	1.775	0.723–4.360	0.210
Type of hepatectomy (major)	2.294	1.026–5.128	**0.043**
Surgeon (trainee)	1.072	0.494–2.324	0.861

All p value were calculated by the logistic regression analysis

Bold values are statistically significant ($p < 0.05$)

[a] Indocyanine green retention rate at 15 min

major hepatectomy, the proportion of patients with intraoperative blood loss >1,000 mL was 27.5 % in group E and 25.6 % in group C. The difference (E − C) was 1.9 % (95 % CI, −1.4–17.9 %). In case of minor hepatectomy, on the other hand, the proportion of patients with intraoperative blood loss >1,000 mL was 7.5 % in group E and 16.4 % in group C. The difference (E − C) was −8.9 % (95 % CI, −18.2 to 0.3 %). In patients with good liver reserve function (ICG R15 <10 %), 13.1 % patients in group E had intraoperative blood loss >1,000 mL, while group C had 23.3 % such patients. The difference (E − C) was −10.1 % (95 % CI, −25.4 to 5.1 %). In poor liver reserve function (ICG R15 ≥10 %) cases, on the other hand, 17.8 % patients in group E and 16.7 % patients in group C had intraoperative blood loss >1,000 mL. The difference (E − C) was 1.1 % (95 % CI −11.3 to 3.5 %).

Discussion

To the best of our knowledge, this randomized trial is the largest to evaluate benefit of energy devices during liver parenchymal transection. We adopted a randomized trial design for assessing non-inferiority under the expectation of superiority in the energy device arm. This hybrid trial-design approach allows a smaller sample size compared to the standard non-inferiority design that expects equal efficacies in both treatment arms [13]. Planning to conduct this study, our opinions were inclined to use the energy device during the liver transection if group E was not inferior to group C in the point of the main surgical outcome,