Iatron, Inc., Tokyo, Japan). This kit employs a polyclonal antibody against NH_2-terminal fragments that contain n-octanoylated serine at position 3. These assay kits were designed for rats, mice, and humans; however, findings from a recent study show that canine ghrelin is accurately measured using this kit [15].

Motilin concentrations in the plasma were measured using the dog motilin ELISA kit (Mybiosource, Inc., San Diego, California, USA).

Statistical Analysis

The results are expressed as the mean ± SE. Fisher's protected least significant difference test was used to test for the significance of the differences between the groups. Values of $p < 0.05$ were considered statistically significant.

Results

Effect of Nesfatin-1 on Gastrointestinal Motility During Fasting

During fasting, the control solution and each dose of nesfatin-1 (10, 30, or 100 μg/body) were administered intravenously 1 h after the spontaneous gastric phase III contractions had terminated. Nesfatin-1 administration suppressed contractions in the gastric body and antrum and disrupted the cyclic IMCs (Fig. 1). The same experiments were repeated in each dog, and the results are summarized as the MI (Fig. 2). Nesfatin-1 administration reduced the MI in the gastric body and antrum. Notably, nesfatin-1 at a dose of 30 μg/body significantly suppressed gastric body contractions, and at a dose of 100 μg/body, it significantly suppressed antral contractions. In contrast, nesfatin-1 had no effect on duodenal and jejunal contractions.

Effect of Nesfatin-1 on Gastrointestinal Motility After Feeding

To study the effect of nesfatin-1 on gastrointestinal motility after feeding, each dose of nesfatin-1 was administered intravenously 1 h after feeding. Compared with administration of the control solution, nesfatin-1 administration did not induce changes in gastrointestinal motility (Fig. 3).

Plasma Concentrations of Nesfatin-1, Ghrelin, and Motilin While Fasting

IMCs were observed cyclically every 100–120 min while the dogs were fasting. Figure 4 shows the changes in the plasma concentrations of nesfatin-1, ghrelin, and motilin in each dog. Ghrelin concentrations peaked in early phase I, and motilin concentrations peaked in phase III, which is consistent with previous reports. Plasma nesfatin-1 levels peaked in late phase I and plasma nesfatin-1 concentrations tended to mark inverse kinetics for ghrelin. The average hormone concentrations for each dog are shown in Fig. 5,

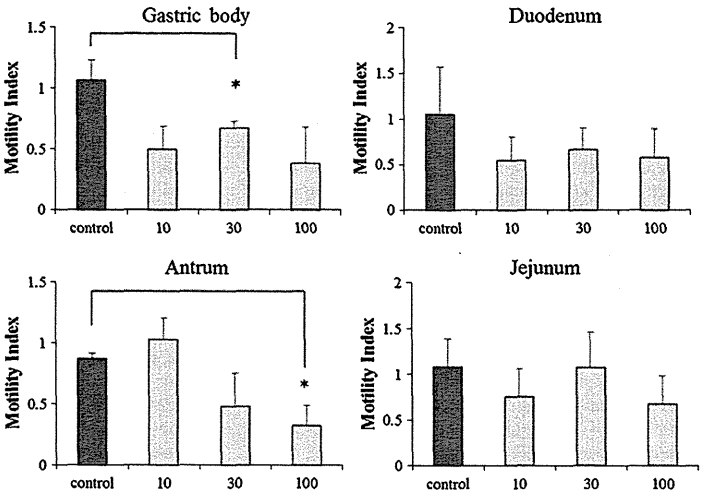


Fig. 2 Comparison of the motility indices after intravenous injections of either nesfatin-1/nucleobindin-2 or saline in the fasted state. Data are presented as the mean ± SE of the mean, $n = 5$. $^*p < 0.05$

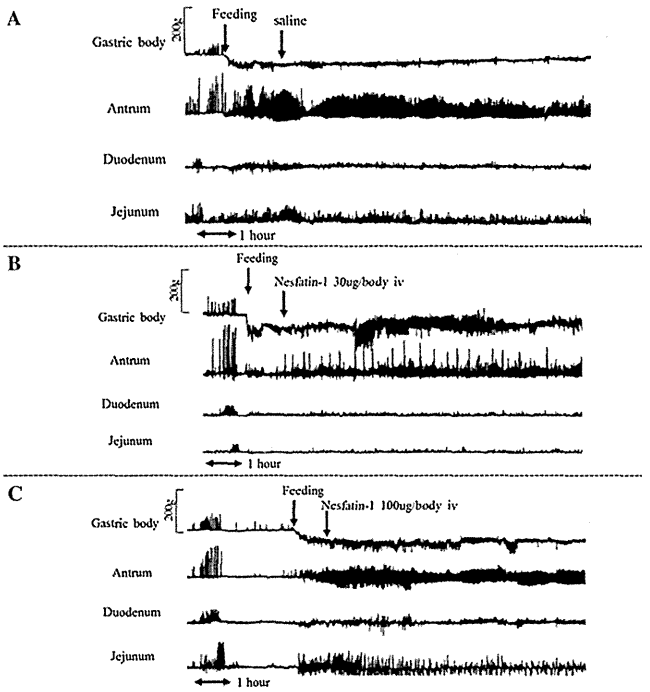


Fig. 3 Effects of intravenous injections of nesfatin-1/nucleobindin-2 on myoelectrical activity measured in the gastric bodies, antra, duodena, and jejuna of conscious dogs in the fed state. **a** The control solution (saline) did not alter the intervals and amplitudes of the gastrointestinal contractions. **b** Nesfatin-1 30 μg/body did not alter the intervals and amplitudes of the gastrointestinal contractions. **c** Nesfatin-1 100 μg/body did not alter the intervals and amplitudes of the gastrointestinal contractions

which illustrates that nesfatin-1 concentrations peaked in late phase I, ghrelin concentrations peaked in early phase I, and motilin concentrations peaked in phase III.

Plasma Concentrations of Nesfatin-1 Before and After Feeding

Plasma concentrations of nesfatin-1 before and after feeding are shown in Fig. 6. Concentrations of nesfatin-1 in the plasma did not show any obvious changes during monitoring from 0 min when the dogs were fed to 90 min after the dogs were fed.

Discussion

In this study, we analyzed the effects of nesfatin-1 on gastrointestinal motility in conscious dogs. The intravenous administration of nesfatin-1 suppressed gastric contractions in the fasted state and inhibited IMCs. Furthermore, plasma nesfatin-1 levels peaked in late phase I and tended to mark inverse kinetics for ghrelin.

Nesfatin-1 has been reported to have anorexic effects in several previously published studies. Oh et al. [1] reported that the intraventricular administration of nesfatin-1 reduced food intakes in rats during the dark phase. Shimizu et al. [4] also reported that the intraperitoneal administration of nesfatin-1 reduced food intakes in rats, and they showed that nesfatin-1 functioned within the peripheral tissues. Other reports have also demonstrated the anorexic effects of nesfatin-1 [16–19], the administration of which has also been reported to reduce gastric acid secretion [20]. Stengel et al. [2] reported that the intracerebroventricular administration of nesfatin-1 reduced gastric emptying in rats. However, few reports have described the relationship between nesfatin-1 and gastrointestinal motility. Atsuchi et al. [11] reported that the central administration of nesfatin-1 suppressed food intakes and gastroduodenal contractions in mice. Li et al. [12] reported that centrally administered nesfatin-1 inhibited gastric contractions in rats while they were anesthetized. Our study used original experimental methods in conscious dogs. In the fasted state, the intravenous administration of nesfatin-1/NUCB2 suppressed gastric contractions within the gastric body and

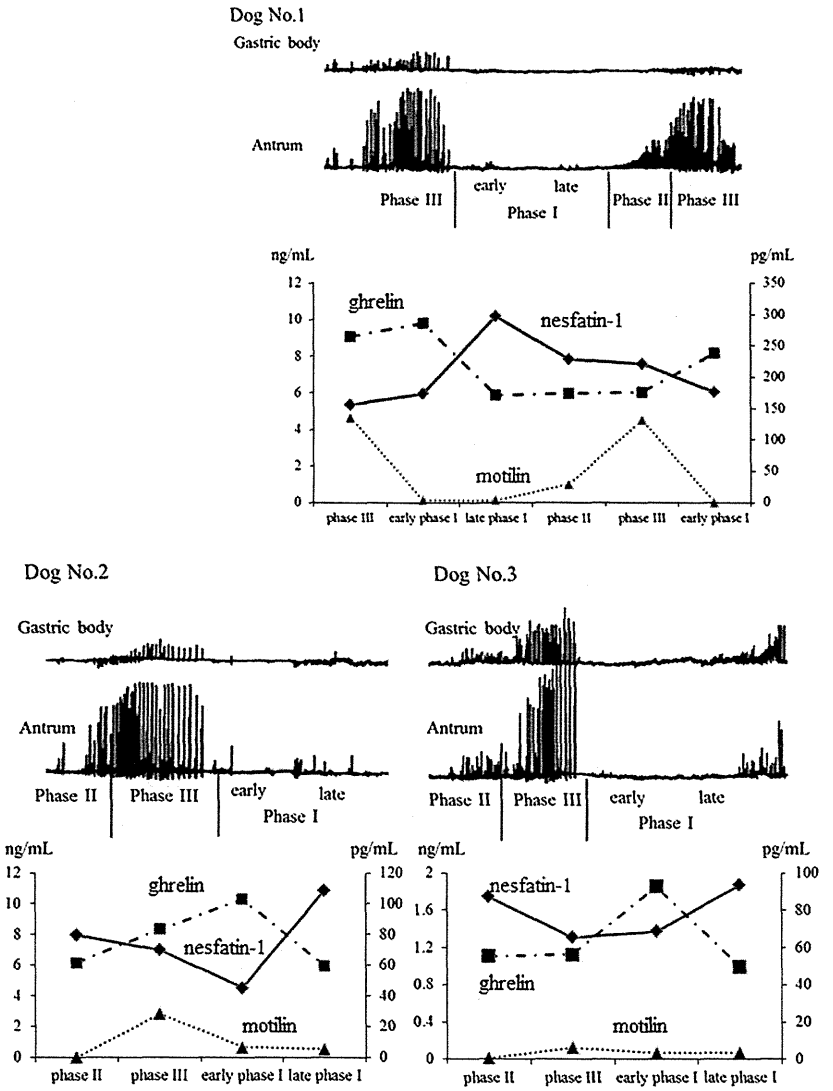


Fig. 4 Data from three dogs which illustrate the associations between plasma nesfatin-1 (*diamond*), ghrelin (*square*), and motilin (*triangle*) levels in the fasted state. Nesfatin-1 and motilin levels are indicated on the *left vertical scale* (ng/mL). Ghrelin levels are indicated on the *right vertical scale* (pg/mL). Blood levels of nesfatin-1, ghrelin, and motilin showed similar patterns in each dog with nesfatin-1 concentrations peaking in late phase I, ghrelin concentrations peaking in early phase I, and motilin concentrations peaking in phase III

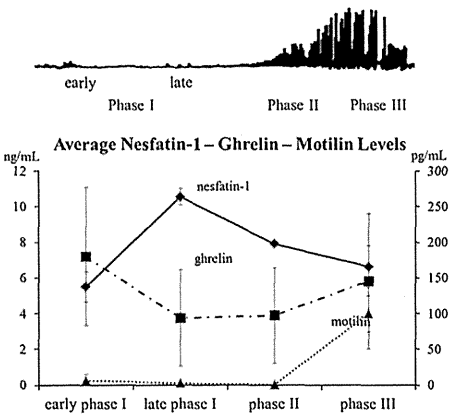
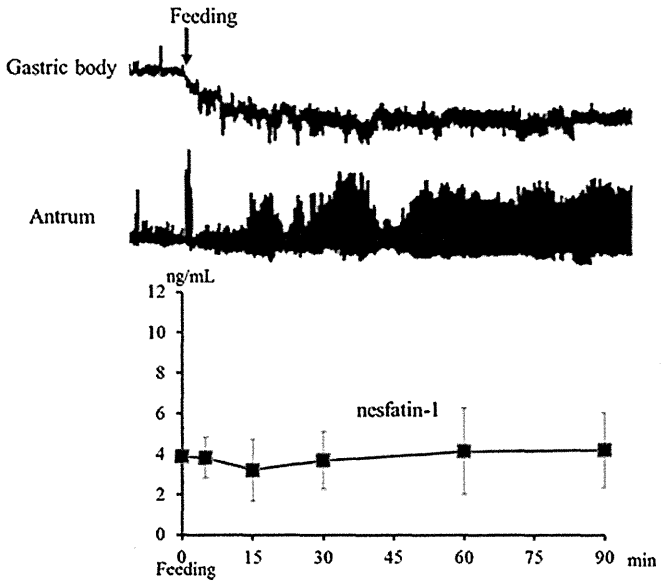


Fig. 5 Associations between plasma nesfatin-1 (*diamond*), ghrelin (*square*), and motilin (*triangle*) concentrations in conscious dogs in the fasted state. Nesfatin-1, ghrelin, and motilin concentrations were averaged and assigned to each phase in three dogs. Nesfatin-1 concentrations peaked in late phase I, ghrelin concentrations peaked in early phase I, and motilin concentrations peaked in phase III

antrum, which concurs with our finding that nesfatin-1 concentrations increased after nesfatin-1/NUCB2 administration (data not shown). These results correspond with previously published reports, and we showed that nesfatin-1 suppressed gastric contractions in the fasted state.

The mechanisms underlying IMCs have been described in several papers. Itoh et al. [21] discovered that motilin induced the phase III migrating motor complex in conscious dogs in the fasted state. Moreover, Bormans et al. [22] reported that motilin peaked at the accrued gastric migrating motor complex in humans. Ogawa et al. [15] reported that IMCs were co-regulated by ghrelin and motilin in the fasted state in conscious dogs. This study revealed that ghrelin injections suppressed plasma motilin levels and that together they terminated the phase III migrating motor complex. This study additionally found that ghrelin concentrations peaked at early phase I. In our study, ghrelin and motilin behaved similarly to that reported previously. Concentrations of nesfatin-1 in the plasma peaked in late phase I after the plasma concentrations of ghrelin had peaked in early phase I. Gastric contractions remain stationary in late phase I, which is consistent with our finding that nesfatin-1 administration suppresses gastric contractions in the fasted state. Although some of the details about the regulation of IMCs remain unclear, our study's findings indicate that nesfatin-1, ghrelin, and motilin could co-regulate IMCs. Furthermore, nesfatin-1 and ghrelin coexist in gastric X/A-like cells [3, 23]. In our study, the kinetics of the circulating levels of nesfatin-1 were opposite to those of ghrelin. Therefore, the

Fig. 6 Data from three dogs which show plasma nesfatin-1 levels before and after feeding. Nesfatin-1 concentrations were unchanged before and after feeding



gastric X/A-like cells may regulate the mechanisms by which nesfatin-1 and ghrelin are secreted.

In this study, nesfatin-1 administration did not affect gastrointestinal motility in the fed state. Moreover, blood nesfatin-1 levels did not change before and after feeding. Post-feeding gastrointestinal motility is overly complex and indefinite for motility regulation.

In conclusion, we showed that nesfatin-1 suppresses gastric contractions in conscious dogs in the fasted state. Nesfatin-1 peaked in late phase I in the fasted state. These results are valuable for elucidating the relationship between nesfatin-1 and gastrointestinal motility. Further research is necessary to investigate the interactions of each digestive hormone, including nesfatin-1, ghrelin, and motilin, with gastrointestinal motility.

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Conflict of interest None.

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ORIGINAL ARTICLE – CLINICAL ONCOLOGY

A phase 3 non-inferiority study of 5-FU/*l*-leucovorin/irinotecan (FOLFIRI) versus irinotecan/S-1 (IRIS) as second-line chemotherapy for metastatic colorectal cancer: updated results of the FIRIS study

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Abstract
Purpose The FIRIS study previously demonstrated non-inferiority of IRIS (irinotecan plus S-1) to FOLFIRI (5-fluorouracil/leucovorin with irinotecan) for progression-free survival as the second-line chemotherapy for metastatic colorectal cancer (mCRC) as the primary endpoint. The overall survival (OS) data were immature at the time of the primary analysis.
Methods Between 30 January 2006 and 29 January 2008, 426 patients with mCRC who failed in first-line chemotherapy were randomly assigned to receive either FOLFIRI or IRIS. After the primary analysis, the follow-up survey was cut off on 29 July 2010, and the final OS data were analysed.
Results With a median follow-up of 39.2 months, the median OS was 17.4 months in the FOLFIRI group and 17.8 months in the IRIS group [hazard ratio (HR) 0.900; 95 % confidence interval (CI) 0.728–1.112]. In the pre-planned subgroup of patients who received prior chemotherapy containing oxaliplatin, the median OS was

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12.7 months in the FOLFIRI group and 15.3 months in the IRIS group (HR 0.755; 95 % CI 0.580–0.983).
Conclusions IRIS is non-inferior to FOLFIRI for OS as second-line chemotherapy for mCRC. IRIS can be an option for second-line chemotherapy of mCRC. (ClinicalTrials.gov Number: NCT00284258).

Keywords Colorectal cancer · FIRIS · Irinotecan · IRIS · S-1

Introduction

At present, the combination of 5-fluorouracil (5-FU)/leucovorin (LV) with either oxaliplatin (FOLFOX) or irinotecan (FOLFIRI) is the mainstream chemotherapy for metastatic colorectal cancer (mCRC) worldwide (O'Neil and Goldberg 2008; National Comprehensive Cancer Network 2014a, b; Tournigand et al. 2004).

In Japan, FOLFOX or FOLFIRI is widely used as the first-line or second-line chemotherapy for mCRC. However, infusional 5-FU-based regimens such as FOLFOX or FOLFIRI are inconvenient because continuous infusion and implantation of an intravenous port system are required. In addition, their use is sometimes complicated by catheter-related infections and thrombosis. Replacement of infusional 5-FU with an oral anticancer drug may be convenient and reduce the burden on patients and healthcare professionals.

In Japan, oral S-1 has been widely used for the treatment of gastrointestinal cancers. In phase 2 studies of IRIS combining S-1 and irinotecan for mCRC, the response rates ranged from 52.5 to 62.5 %, and the median progression-free survival (PFS) was 7.8–8.6 months, suggesting that IRIS may have comparable efficacy to FOLFIRI as a first-line therapy (Goto et al. 2006; Komatsu et al. 2011; Tsunoda et al. 2009; Komatsu et al. 2010: Shiozawa et al. 2010).

The FIRIS study is a phase 3 randomised study to investigate the non-inferiority of IRIS to FOLFIRI, which is a standard second-line chemotherapy for mCRC after failure of fluoropyrimidine chemotherapy with or without oxaliplatin. In the primary analysis, the median PFS was 5.1 months in the FOLFIRI group and 5.8 months in the IRIS group [hazard ratio (HR) 1.077; 95 % confidence interval (CI) 0.879–1.319], demonstrating the non-inferiority of IRIS to FOLFIRI (Muro et al. 2010). Thereafter, in the ESMO Consensus Guidelines for management of patients with colon and rectal cancer, IRIS is listed in the table of the treatment options (Schmoll et al. 2012). However, the survival data of the FIRIS study were immature. In this paper, an updated analysis focusing on overall survival (OS) is reported.

Patients and methods

Study design and treatment

This randomised, open-label, phase 3 study of second-line chemotherapy for patients with mCRC was conducted at 40 institutions in Japan (see "Appendix"). The eligibility criteria and design were described in detail in a previous report (Muro et al. 2010).

The patients were centrally randomised to receive either FOLFIRI or IRIS using the minimisation method with stratification by institution, prior therapy (with oxaliplatin vs. without oxaliplatin), and performance status (PS; 0 vs. 1). In the FOLFIRI group, the patients received *l*-LV (200 mg/m^2) and irinotecan (150 mg/m^2) followed by a bolus injection of 5-FU (400 mg/m^2) on day 1, and then continuous infusion of 5-FU (2,400 mg/m^2) over 46 h, repeated every 2 weeks (4 weeks counted as one course). The dose of irinotecan (150 mg/m^2) given to the FOLFIRI group is the upper limit of the approved dose in Japan (Fuse et al. 2008). The IRIS group received irinotecan (125 mg/m^2) on days 1 and 15 and S-1 [40–60 mg/body, based on the body surface area (BSA): BSA < 1.25 m^2, 40 mg/body; 1.25 m^2 ≤ BSA < 1.5 m^2, 50 mg/body; BSA ≥ 1.5 m^2, 60 mg/body] twice daily for 2 weeks followed by 2 weeks of rest, based on the results of the phase 2 study (Goto et al. 2006). The treatment was continued until one of the following events occurred: disease progression (PD); unacceptable toxicity; or patient's refusal to continue treatment.

The primary objective of the study was to demonstrate the non-inferiority of IRIS to FOLFIRI for PFS.

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The secondary endpoints included OS, response rate, and safety. In addition, pre-planned subgroup analyses were performed.

The protocol of the study was approved by the institutional review board or ethics committee and was conducted in compliance with the Declaration of Helsinki and Japanese ethical guideline for clinical studies. Written informed consent was obtained from all patients participating in the study.

Study assessments

Physical examinations and laboratory tests were performed at baseline and repeated at least every 2 weeks during the treatment. Tumours were assessed at baseline (within 1 month before enrolment), 2, 3, and 4 months after enrolment, and every 2 months thereafter until progression. Progression was defined when any of the following three events occurred: (1) PD based on the response evaluation criteria in solid tumours (RECIST) version 1.0; (2) clinical progression judged by the investigator; or (3) death from any cause without progression. PFS was calculated from the date of randomisation to the date of the events described above.

OS was calculated from the date of randomisation to the date of death from any cause. Surviving patients, including those lost to follow-up, were censored at the date of last confirmation of survival. Toxicity was evaluated based on the Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v3.0).

Statistical analysis

The intent-to-treat (ITT) population consisted of all randomised patients, and the per-protocol set (PPS) population was defined as the ITT population excluding patients who violated protocols to a considerable extent, including major protocol inclusion/exclusion criteria or treatment protocols.

The primary endpoint of PFS was assumed to be 4 months in both groups. By defining a 1-month shorter PFS with IRIS than with FOLFIRI as the acceptance limit for non-inferiority, which was also the minimum difference detected by monthly image examinations, a non-inferiority margin of 1.333 was selected. After the required number of events was calculated with a one-sided α of 0.025 and a power of 80 %, a target sample size of 400 patients was selected.

For the primary endpoint of PFS and the secondary endpoint of OS, the HR for IRIS to FOLFIRI and its 95 % CI were calculated to show the non-inferiority of IRIS to FOLFIRI, respectively. Furthermore, Bayesian analyses were carried out to assess the robustness of these preliminary results. Post hoc analyses for posterior probabilities with log HR within the range of 1.333–1.15 (a stricter threshold) were performed (Spiegelhalter et al. 1994).

For the primary analysis, the collection of the primary endpoint PFS data was cut off on 31 December 2008 and the number of confirmed events was 389 (Muro et al. 2010). The final analysis was performed on 29 July 2010 (2.5 years after the last patient was enrolled, as pre-specified in the protocol).

Subgroup analyses were pre-planned to determine whether therapeutic efficacy interacted with sex, age, histological type, PS, and prior chemotherapy with or without oxaliplatin. PFS and OS were estimated using the Kaplan–Meier method. The 95 % CI for the median PFS and OS was calculated using the method of Brookmeyer and Crowley (Brookmeyer and Crowley 1982). All p values were two-sided. All statistical analyses were performed using SAS version 8.2 (SAS Institute, Cary, NC, USA). This study is registered with ClinicalTrials.gov (Number: NCT00284258).

Results

Patient populations

A total of 426 patients from 40 institutions in Japan were enrolled from January 2006 to January 2008, and randomised to receive either FOLFIRI or IRIS ($n = 213$ in each group; Fig. 1). The PPS population consisted of 203 patients in the FOLFIRI group and 198 in the IRIS group. All patients who received a study treatment [FOLFIRI ($n = 211$) and IRIS ($n = 210$)] were included in the safety evaluation. The baseline characteristics were well balanced between the two groups, as previously reported (Muro et al. 2010).

Treatment

The median number of courses of the protocol treatment was 4.0 (range 1–27) and 4.0 (range 1–23) in the FOLFIRI and IRIS groups, respectively. The median dose intensity relative to the planned dose intensity was irinotecan 78.3 %, bolus 5-FU 76.9 %, and infusional 5-FU 81.5 % in the FOLFIRI group, and irinotecan 78.3 % and S-1 88.9 % in the IRIS group. Treatments were discontinued because of PD in 71.8 % of the FOLFIRI group ($n = 153$) and 67.1 % of the IRIS group ($n = 143$). Treatment discontinuation owing to adverse events was more frequently observed in the IRIS group ($n = 49$, 23.0 %) than in the FOLFIRI group ($n = 28$, 13.1 %). Overall, 179 (84.8 %) patients in the FOLFIRI group and 184 (87.6 %) patients in the IRIS group required at least one dose delay or dose reduction at some point during the treatment course.

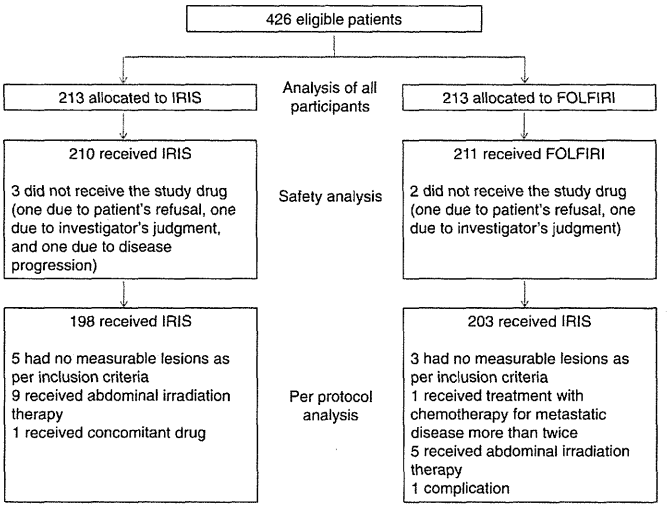

Fig. 1 Consort diagram. *IRIS* irinotecan plus S-1, *FOLFIRI* infusional 5-fluorouracil, folinic acid, and irinotecan

Third-line chemotherapy after failure of the protocol treatment in the second-line therapy was given to 168 (78.9 %) patients in the FOLFIRI group and 153 (71.8 %) patients in the IRIS group. In these patients, molecularly targeted agents were concomitantly used in 58 (27.2 %) patients (bevacizumab, 45; cetuximab, 17) in the FOLFIRI group and 52 (24.4 %) patients (bevacizumab, 38; cetuximab, 16) in the IRIS group, and no marked difference in the use of these agents was evident between the two groups (Table 1).

Table 1 Cancer treatment after discontinuation of the study treatment

Treatment	FOLFIRI *n* (%)	IRIS *n* (%)
No	45 (21.1)	60 (28.2)
Yes	168 (78.9)	153 (71.8)
Bevacizumab		
FOLFOX + bevacizumab	33 (15.5)	29 (13.6)
FOLFIRI + bevacizumab	19 (8.9)	12 (5.6)
5-FU/LV + bevacizumab	8 (3.8)	6 (2.8)
Cetuximab		
FOLFIRI + cetuximab	0 (0)	1 (0.5)
Irinotecan + cetuximab	16 (7.5)	13 (6.1)
FOLFOX	60 (28.2)	61 (28.6)
FOLFIRI	9 (4.2)	25 (11.7)
5-FU/LV	7 (3.3)	10 (4.7)
Irinotecan	8 (3.8)	20 (9.4)
S-1	35 (16.4)	7 (3.3)
Irinotecan + S-1	16 (7.5)	3 (1.4)
Operation	12 (5.6)	11 (5.2)
Radiation therapy	29 (13.6)	18 (8.5)
Other	48 (22.5)	45 (21.1)

FOLFIRI infusional 5-fluorouracil, folinic acid, and irinotecan, *IRIS* irinotecan plus S-1, *FOLFOX* 5-fluorouracil, LV, and oxaliplatin, *5-FU* 5-fluorouracil, *LV* leucovorin

Overall survival

As of 29 July 2010 when the data collection was finally cut off, 352 deaths (FOLFIRI, 178; IRIS, 174) were confirmed with a median follow-up of 39.2 months. A total of 125 censored cases resolved from the last cut-off that we reported. The median OS was 17.4 months in the FOLFIRI group and 17.8 months in the IRIS group (HR 0.900; 95 % CI 0.728–1.112; $p = 0.003$ for a non-inferiority margin of 1.333; Fig. 2a). In the PPS population, the median OS was 17.4 months in the FOLFIRI group and 17.4 months in the IRIS group (HR 0.905; 95 % CI 0.728–1.126). The Bayesian posterior probabilities that the HR of IRIS relative to FOLFIRI would be <1.333 and <1.15 were calculated to be >99.9 % and >98.7 %, respectively.

Progression-free survival

When the data collection was finally cut off, 412 events including an increase of 23 events from the primary analysis were confirmed. The median PFS was 5.1 months in the FOLFIRI group and 5.8 months in the IRIS group. In the ITT population, the HR for IRIS to FOLFIRI was

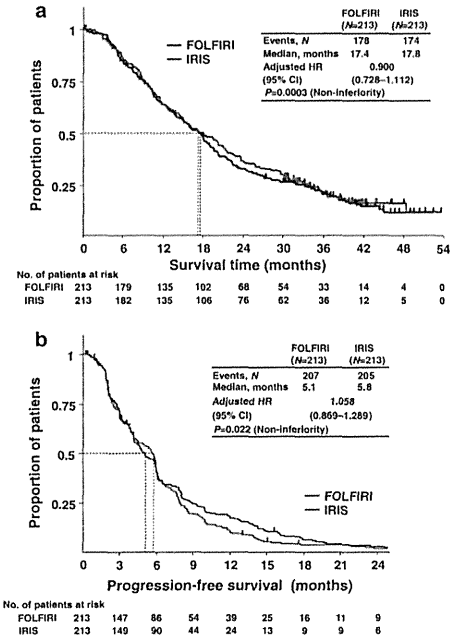

Fig. 2 OS (**a**) and PFS (**b**) in the intention-to-treat population. *IRIS* irinotecan plus S-1, *FOLFIRI* infusional 5-fluorouracil, folinic acid, and irinotecan, *HR* hazard ratio, *CI* confidence interval

1.058 (95 % CI 0.869–1.289; $p = 0.022$) and consistent with the primary analysis (Fig. 2b). In the PPS population, the median PFS was 5.1 months in the FOLFIRI group and 5.7 months in the IRIS group (HR 1.035; 95 % CI 0.843–1.271), being consistent with the primary analysis.

Subgroup analyses

Figure 3 shows the results of the subgroup analyses for OS. Except for the interaction of prior chemotherapy containing oxaliplatin (yes vs. no) and therapeutic effect, no interaction was observed between sex (male vs. female), age (<65 vs. 65–75 years), histological type (adenocarcinoma, well differentiated vs. moderately differentiated vs. poorly differentiated), or PS (0 vs. 1), and the therapeutic effect of IRIS was comparable to that of FOLFIRI.

In the subgroups of patients treated with FOLFIRI ($n = 128$) or IRIS ($n = 129$) who had received prior chemotherapy containing oxaliplatin, the median OS was 15.3 months in the IRIS group and 12.7 months in the FOLFIRI group (adjusted HR 0.755; 95 % CI 0.580–0.983), showing better survival in the IRIS group than in the FOLFIRI group (Fig. 4a). On the other hand, in the subgroups of patients treated with FOLFIRI ($n = 85$) or IRIS ($n = 84$) who had received prior chemotherapy without oxaliplatin, the median OS was more favourable in the FOLFIRI group than in the IRIS group (26.9 vs. 23.6 months; adjusted HR 1.229; 95 % CI 0.866–1.745) (Fig. 4b).

Safety

The results of the updated safety analysis were very similar to those previously reported (Muro et al. 2010). Briefly, specific adverse events were haematological toxicity (grade 3 or 4 neutropenia), which was observed in 52.1 % of the FOLFIRI group and 36.2 % of the IRIS group, and non-haematological toxicity (grade 3 diarrhoea), which was observed in 4.7 % of the FOLFIRI group and 20.5 % of the IRIS group. One treatment-related death from hypotension caused by shock was reported in the FOLFIRI group within 28 days after the end of the protocol treatment, while no treatment-related deaths were reported in the IRIS group.

Discussion

We conducted a phase 3 randomised study to compare FOLFIRI and IRIS as second-line chemotherapies for patients with mCRC. The primary analysis demonstrated the non-inferiority of IRIS to FOLFIRI for PFS as the primary endpoint. The secondary endpoints of OS and response rate were also equivalent between the two groups (Muro et al. 2010), but the data were immature with many cases censored at the primary analysis. In this updated analysis, data obtained 2.5 years after the end of the enrolment period (as pre-specified in the protocol) were included. The non-inferiority of IRIS to FOLFIRI for PFS as the primary endpoint was re-confirmed, and non-inferiority for OS was also demonstrated. In addition, the probabilities of HR < 1.333 and HR < 1.15, which are stricter non-inferiority margins for OS, were estimated to be >99.9 and >98.7 %, respectively, using Bayesian analyses. Our study results are highly robust.

When our study was started, FOLFOX was already one of the standard treatments worldwide, but oxaliplatin had just been launched and was rarely used in an adjuvant setting in Japan. Actually, 85 (39.9 %) patients in the FOLFIRI group and 84 (39.4 %) patients in the IRIS group had received prior chemotherapy without oxaliplatin. Most of these patients received prior chemotherapy in an adjuvant setting including tegafur-uracil with or without LV (27 patients in the FOLFIRI group and 32 in the IRIS group) or 5-FU/LV (11 patients in the FOLFIRI group and 7 in the IRIS group).

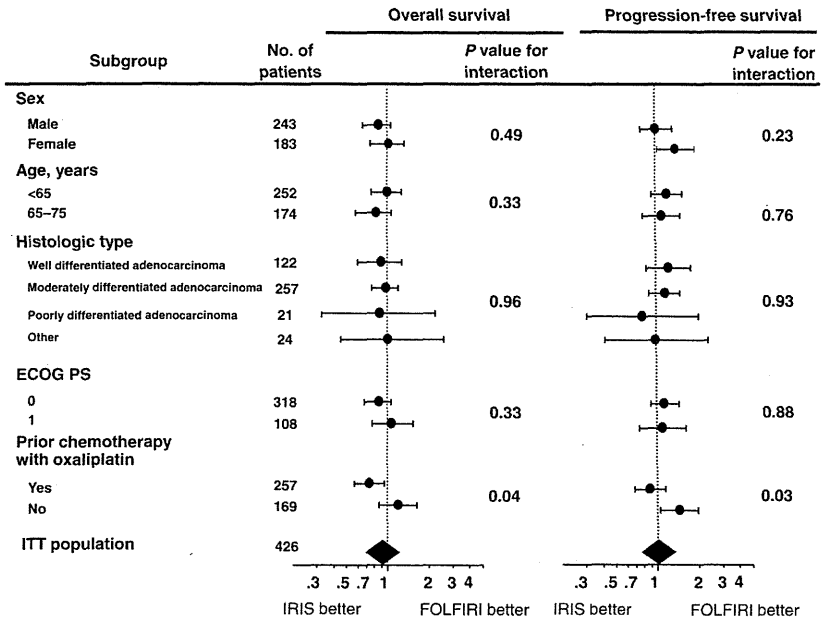

Fig. 3 Subgroup analyses of OS and PFS in the intention-to-treat (ITT) population. *IRIS* irinotecan plus S-1, *FOLFIRI* infusional 5-fluorouracil, folinic acid, and irinotecan

In the subgroup of patients who had received prior oxaliplatin, the adjusted HR for OS of IRIS to FOLFIRI was 0.755 (95 % CI 0.580–0.983), suggesting that IRIS might prolong the survival of patients who failed in first-line chemotherapy with oxaliplatin-containing regimens, compared with FOLFIRI. On the other hand, in the subgroup of patients who had received prior chemotherapy without oxaliplatin, the median OS was longer in the FOLFIRI group than in the IRIS group (adjusted HR 1.229; 95 % CI 0.866–1.745). Interactions between prior chemotherapy and therapeutic effects in the two groups may need to be considered.

There are some possible reasons for the interactions. Resistance to 5-FU/LV shared by patients receiving first-line FOLFOX and second-line FOLFIRI may be overcome to some extent by the dihydropyrimidine dehydrogenase (DPD) inhibitor contained in S-1. On the other hand, it is also speculated that cross-resistance to DPD inhibitory agents may be partly overcome by bolus 5-FU/LV in patients receiving FOLFIRI (Baba et al. 2012), considering the fact that many patients in the subset without prior oxaliplatin received adjuvant chemotherapy with DPD inhibitory agents as a prior therapy. However, further studies, including basic studies, are needed to clarify this finding.

In recent phase 3 trials of molecularly targeted agents used in second-line chemotherapy regimens, the median OS was reported to be 10.7–14.5 months in groups treated with anti-EGFR antibodies. The survival data in the present study seemed to be consistent with the survival data in these recent studies of molecularly targeted agents (Sobrero et al. 2008; Peeters et al. 2010).

In conclusion, this study has demonstrated that IRIS is non-inferior to FOLFIRI not only for PFS, but also for OS as second-line chemotherapy for mCRC. Thus, IRIS should be considered as a treatment option. In particular, IRIS may be a favourable regimen for patients previously treated with chemotherapy containing oxaliplatin. To further improve the outcome, future studies of both first-line and second-line therapies are warranted to evaluate IRIS in combination with molecularly targeted agents such as bevacizumab, cetuximab, and panitumumab.

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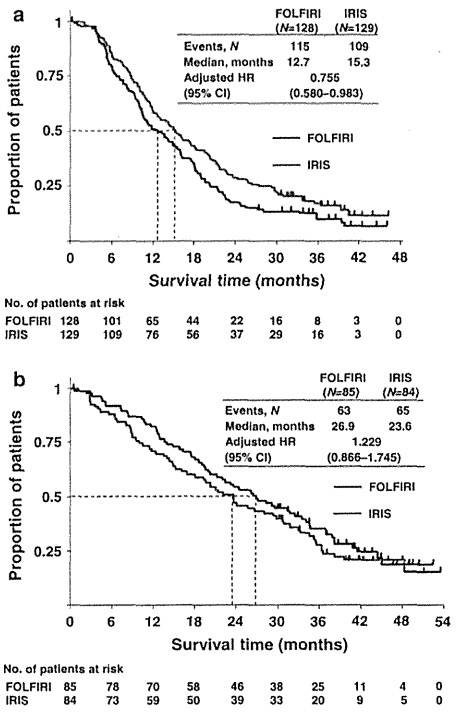

Fig. 4 Survival according to prior chemotherapy with oxaliplatin (**a**) or without oxaliplatin (**b**). *IRIS* irinotecan plus S-1, *FOLFIRI* infusional 5-fluorouracil, folinic acid, and irinotecan, *HR* hazard ratio, *CI* confidence interval

article to the memory of Prof. Hiroya Takiuchi, who contributed to the conception and design of this study. The senior academic authors designed the trial in cooperation with the study sponsors. The sponsors provided funding and organisational support, collected data, and performed analyses, but did not undertake any data interpretation. This report was written by the corresponding author (with additional input from the other authors), who had unrestricted access to the raw study data, gives assurance for the accuracy and completeness of the reported analyses, and had final responsibility for the decision to submit for publication. This work was funded by Taiho Pharmaceutical Co. Ltd., Japan, and Daiichi Sankyo Co. Ltd., Japan.

Conflict of interest The authors declare no conflict of interest.

Appendix (participating institutes): FIRIS Study Group

List of participating institutions in order of patient recruitment:

Shizuoka Cancer Center (Shizuoka, Japan); Aichi Cancer Center Hospital (Nagoya, Japan); National Cancer Center Hospital (Tokyo, Japan); Kochi Health Sciences Center (Kochi, Japan); Gunma Prefectural Cancer Center (Gunma, Japan); Kumamoto University Hospital (Kumamoto, Japan); Kinki University School of Medicine (Osaka, Japan); Chiba Cancer Center (Chiba, Japan); Nagoya Memorial Hospital (Nagoya, Japan); National Hospital Organization Shikoku Cancer Center (Matsuyama, Japan); Saitama Cancer Center (Saitama, Japan); Osaka Medical College Hospital (Takatsuki, Japan); National Kyushu Cancer Center (Fukuoka, Japan); Osaka City General Hospital (Osaka, Japan); Gunma University Graduate School of Medicine (Maebashi, Japan); Hokkaido University Hospital Cancer Center (Sapporo, Japan); National Hospital Organization Kyoto Medical Center (Kyoto, Japan); Keio University Hospital (Tokyo, Japan); Kansai Rosai Hospital (Hyogo, Japan); Tokyo Medical and Dental University (Tokyo, Japan); Osaka Medical Center for Cancer and Cardiovascular Diseases (Osaka, Japan); Aomori Prefectural Central Hospital (Aomori, Japan); Showa University Toyosu Hospital (Tokyo, Japan); Minoh City Hospital (Osaka, Japan); Saiseikai Kumamoto Hospital (Kumamoto, Japan); Toyama University Hospital (Toyama, Japan); National Hospital Organization Kagoshima Medical Center (Kagoshima, Japan); Tonan Hospital (Sapporo, Japan); Kanagawa Cancer Center (Yokohama, Japan); Niigata Cancer Center Hospital (Niigata, Japan); Saku Central Hospital (Nagano, Japan); Hyogo Cancer Center (Hyogo, Japan); Hiroshima University Hospital (Hiroshima, Japan); Tomakomai Nissho Hospital (Hokkaido, Japan); Aichi Cancer Center Aichi Hospital (Aichi, Japan); National Hospital Organization Nagoya Medical Center (Nagoya, Japan); Kobe University Hospital (Kobe, Japan); Yamagata Prefectural Central Hospital (Yamagata, Japan); Yokohama City University Hospital (Yokohama, Japan); and Kitasato University East Hospital (Kanagawa, Japan).

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ORIGINAL ARTICLE – BREAST ONCOLOGY

Prognostic Value of Extracapsular Invasion of Axillary Lymph Nodes Combined with Peritumoral Vascular Invasion in Patients with Breast Cancer

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ABSTRACT

Background. Extracapsular invasion (ECI) of metastatic axillary lymph nodes has been associated with aggressive nodal disease but its prognostic role in breast cancer is unclear. The present study evaluated nodal ECI as a predictor of breast cancer recurrence.

Methods. We evaluated 154 women with histologically proven node-positive breast cancer who were diagnosed with invasive ductal carcinoma, and investigated the relationships between ECI and recurrences and other clinicopathological factors, particularly vascular invasion and the number of lymph node metastases.

Results. The presence of ECI at positive nodes was significantly associated with the number of positive nodes, and with disease recurrence and survival in univariate (but not multivariate) analysis. Interestingly, all ECI^+ patients with distant metastases in our series had peritumoral vascular invasion (PVI), which may have reflected systemic disease; ECI with PVI of the primary tumor strongly predicted recurrent disease and shorter survival.

Conclusion. ECI of axillary metastases combined with PVI indicates high tumor aggressiveness. Patients with ECI and PVI may be considered for stronger adjuvant therapies because of their high risk for distant recurrences.

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The status of axillary lymph nodes is an important prognostic factor in invasive breast cancer,[1] including both the presence and total number of metastatic lymph nodes.[2] Extracapsular invasion (ECI) of tumor cells is sometimes seen histopathologically in axillary contents of patients with node-positive breast cancer, and is associated with greater numbers of metastatic axillary nodes.[3–5] Experimental tumor models and human clinicopathological data have associated solid tumor invasion to near lymphatic vessels with node metastasis.[6–8] Previously, we found ECI in sentinel lymph nodes (SLNs) to strongly predict residual axillary disease or non-SLN metastasis,[5] and therefore to be a critical predictor for locoregional lymphatic progression.

Reportedly, ECI is a prognostic factor for various cancer types, including esophageal, gastric, colorectal, and thyroid cancer.[9–13] In breast cancer, previous reports have also suggested that ECI is a prognostic factor of recurrence-free survival (RFS) and overall survival (OS).[14–17] However, although many reports have associated ECI with higher numbers of positive lymph nodes, ECI is a significant prognostic factor in univariate analysis but not in multivariate analysis.[18–21] Therefore, whether ECI in axillary lymph node metastases correlates with disease recurrence or shorter survival in breast cancer is unclear. Although the higher recurrence rate could be attributed to the association between ECI and high numbers of metastatic lymph nodes, several studies have also associated ECI and distant recurrence,[21–23] which indicates that ECI is a marker for

systemic aggressive disease and suggests that ECI affects both loco regional and distant progression in breast cancer. Because ECI may be associated with both higher likelihood of additional nodal metastasis and systemic aggressive disease, and because vascular invasion of primary tumors reflects systemic disease, which predicts disease recurrence or poor survival, we hypothesized that ECI of positive nodes combined with vascular invasion of primary tumors would affect survival and recurrence risk. In this study, we retrospectively investigated the prognostic significance of ECI in relation to other clinicopathological factors, particularly peri tumoral vascular invasion (PVI) that reflects systemic disease for predicting disease recurrence or shorter survival, in ECI^+ node patients with distant recurrent lesion.

PATIENTS AND METHODS

Patients

From January 2003 to December 2007, a total of 810 patients with invasive breast cancer underwent radical breast surgery at the Gunma Prefectural Cancer Center and Department of General Surgical Science, Gunma University Hospital. Of these 810 patients, 262 had histologically proven node-positive breast cancer, and of these 262 patients, 213 were diagnosed with invasive ductal carcinoma and had not then received neoadjuvant systemic chemotherapy, and their resected margins were all clear. After excluding 59 patients with incomplete clinical information, we finally recruited 154 patients for this study, all of whom gave informed consent. Follow-up was completed in July 2013; seven patients were lost to follow-up.

Details extracted from the database were age, histological types, primary tumor size, nuclear grade, number of involved lymph nodes, lymphatic or vascular invasion, estrogen receptor (ER) and progesterone receptor (PR) status, and human epidermal growth factor receptor 2 (HER2) scores of primary tumors. Recorded treatment details were types of surgery, radiotherapy, chemotherapy, and hormonal therapy given for ER^+ and/or PR^+ tumors. Outcomes were RFS and breast cancer-specific survival (CSS). The first recurrence sites and dates were also recorded. Recurrences were divided into loco regional (including intramammary and regional lymph nodes) and distant metastases. Regional lymph nodes were defined as axillary, subclavicular and supraclavicular lymph nodes, and internal mammary lymph nodes. Chemotherapy treatments were divided into five regimens: six courses of cyclophosphamide, epirubicin and fluorouracil (CEF); four courses of CEF followed by four courses of paclitaxel or docetaxel (taxane); four courses of adriamycin and cyclophosphamide (AC) followed by four courses of taxane; eight courses of taxane; and others.

Definitions

ECI was examined by hematoxylin and eosin (H&E) staining. We determined the presence of ECI by assessing whether tumor cells invaded through the lymph node capsule. If the lymph node capsule was infiltrated but not penetrated, it was defined as ECI absent (electronic supplementary file 1). Pathological staging followed the TNM classification of the International Union for Cancer Control (UICC) [7th edition]. RFS and CSS were calculated from the date of surgery. Lymphatic and venous invasion were categorized according to the Japanese Classification of Carcinoma: no invasion (ly0, v0), minimal invasion (ly1, v1), moderate invasion (ly2, v2), and severe invasion (ly3, v3).[5]

Statistical Analysis

Analyses were performed using the software program JMP 5.0 (SAS Institute, Cary, NC, USA). Student's t test was used to analyze differences between continuous variables, and the Chi-square test was used for categorical variables. Fisher's exact test or the Chi-square test were used for univariate analyses. The Kaplan–Meier method was used to develop survival curves. The log-rank test was used to assess differences between these curves. Any variables that were significant in univariate analyses were included in multivariate analyses. Cox's logistic regression was used for multivariate analyses. A p-value of < 0.05 was considered significant.

RESULTS

Extracapsular Invasion of Positive Nodes was Associated with Lymphatic Expansion in Breast Cancer

The 154 patients with metastatic nodes were divided into two groups based on the presence of ECI of axillary lymph node metastases (Table 1); 59 were in the ECI^+ group (38.3 %), and 95 were in the ECI^- group (61.7 %). The univariate analysis of the relationship between the clinicopathological variables and ECI shows that positive lymph node number ($p < 0.0001$) and lymphatic invasion ($p = 0.0009$) were significantly related to the presence of ECI (Table 1). We found no significant difference for the presence of PVI. Type of surgery, radiotherapy, adjuvant chemotherapy, and hormonal therapy showed no significant differences (data not shown).

TABLE 1 Relations between ECI and clinicopathological characteristics, and recurrences

	ECI positive ($n = 59$) [N (%)]	ECI negative ($n = 95$) [N (%)]	p value
Age (years)	58.4 ± 11.1	55.9 ± 10.6	0.1562
Histopathology			0.8936
Scirrous	38 (64.4)	58 (61.1)	
Papillo-tubular	12 (20.3)	20 (21.1)	
Solid tubular	9 (15.3)	17 (17.9)	
Tumor size (mm)	28.2 ± 13.5	25.6 ± 10.6	0.1857
Nuclear grade			0.1615
1	15 (25.4)	33 (34.7)	
2	18 (30.5)	17 (17.9)	
3	26 (44.1)	45 (47.4)	
Positive LN number	8.30 ± 7.50	1.64 ± 1.70	<0.0001
Lymphovascular invasion			
v0	20 (33.9)	46 (48.4)	0.0917
v1	19 (32.2)	33 (32.2)	
v2	18 (30.5)	14 (14.7)	
v3	2 (3.4)	2 (2.1)	
ly0	1 (1.7)	3 (3.2)	0.0009
ly1	3 (5.1)	29 (30.5)	
ly2	29 (49.1)	40 (42.1)	
ly3	26 (44.1)	23 (24.2)	
ER status positive	50 (84.7)	76 (80.0)	0.4579
PR status positive	43 (72.9)	65 (68.4)	0.5566
HER2 score			
0	7 (11.9)	16 (18.4)	0.1520
1	12 (20.3)	25 (28.7)	
2	26 (44.1)	23 (26.4)	
3	14 (23.7)	23 (26.4)	
Recurrences			
Locoregional			
Total	15 (25.4)	6 (6.3)	0.0008
Intramammary	1 (1.7)	3 (3.2)	0.5790
Axillary	5 (8.5)	1 (1.1)	0.0207
Regional LNs	9 (15.3)	2 (2.1)	0.0021
Distant metastases (without locoregional recurrent)			
Total	16 (27.1)	13 (13.7)	0.0382
Multiple sites	4 (6.8)	4 (4.2)	0.4849
One site	12 (20.3)	9 (9.5)	0.0561
Lung	2 (3.4)	4 (4.2)	0.7980
Liver	1 (1.7)	1 (1.1)	0.7322
Bone	9 (15.3)	2 (2.1)	0.0021
Distant LNs	0 (0.0)	2 (2.1)	0.2619

ECI extracapsular invasion, *LNs* lymph nodes, *ER* estrogen receptor, *PR* progesterone receptor, *HER2* human epidermal growth factor receptor 2

ECI of Positive Nodes was Associated with Disease Recurrence

In the ECI^+ group, 15 patients (25.4 %) had locoregional recurrence (Table 1), of whom 14 had recurrence of lymph node metastasis. In the ECI^- group, six patients (6.3 %) had loco regional recurrence, of whom three had lymph node recurrence. Loco regional recurrence was greater in the ECI^+ group than in the ECI^- group ($p = 0.0008$). On the other hand, 16 patients (27.1 %) had distant metastases in the ECI^+ group, of whom nine had bone metastasis. In the ECI^- group, 13 patients (13.7 %) had distant metastases, of whom two had distant lymph node recurrence. Distant metastases were more frequent in the ECI^+ group per univariate analysis ($p = 0.0382$). When relapse by organ type was considered, recurrence in bone was significantly correlated with ECI ($p = 0.0021$). These results imply that ECI is related to both locoregional recurrence and distant metastasis.

Within a median follow-up period of 84.0 months, both survival curves suggest significantly shorter survival with ECI (Fig. 1), with hazard ratios for ECI^+ patients of 1.79 RFS and 1.98 CSS on univariate analysis. Clinicopathological factors that were significantly associated with RFS and CSS in univariate analysis are shown in Table 2. Multivariate analyses showed higher nuclear grade and absence of PR to be independent negative prognostic factors, but ECI lost its significance in multivariate analysis. In multivariate analyses, adjuvant chemotherapy with cancellation or modified regimens was a negative factor and hormonal therapy was a positive prognostic factor, but characteristics of the tumors, including ECI at positive nodes, were not independent predictors.

Combination of ECI of Positive Nodes and Vascular Invasion of Primary Tumors Affects Prognosis and Recurrence Risk

Although our results did not support independent prognostic values for ECI in RFS and CSS, distant metastases were observed more frequently in the ECI^+ group. Therefore, we further examined clinicopathological features of ECI^+ patients with distant metastases. We found that all of the ECI^+ patients with distant metastatic lesions also had PVI (v^+), which can reflect systemic disease; in contrast, patients with ECI but no PVI (v^-) had no distant metastases. Therefore, we compared clinicopathological factors between v^+ and v^- patients in the ECI^+ group. Univariate analyses of clinicopathological factors between the ECI^+/v^+ and ECI^+/v^- groups showed that tumor size,

TABLE 2 Predictors of relapse free survival and cancer specific survival

	RFS					CSS				
	Univariate analysis			Multivariate analysis		Univariate analysis			Multivariate analysis	
	HR	95 % CI	*p* value	HR	95 % CI	HR	95 % CI	*p* value	HR	95 % CI
ECI positive	1.79	1.35–2.40	<0.0001	1.59	0.99–2.57	1.98	1.39–2.91	0.0001	1.21	0.57–2.55
Age ≤60 years	1.15	0.86–1.56	<0.0001			1.02		0.8822		
Tumor size >20 mm	1.59	1.16–2.24	0.0028	1.25	0.86–1.90	1.82	1.21–2.98	0.0029	0.99	0.57–1.80
Nuclear grade ≥2	3.00	1.81–6.08	<0.0001	4.55	2.05–19.37	3.91	1.81–16.51	<0.0001	2.28	0.96–10.05
Positive LNs ≥4	2.12	1.60–2.83	<0.0001	1.38	0.89–2.21	2.32	1.63–3.37	<0.0001	1.57	0.77–3.36
LVI										
ly ≥2	1.32		0.1186			1.78	1.06–3.65	0.0253	0.95	0.34–4.35
v ≥1	1.42	1.06–1.95	0.0174	1.00	0.69–1.48	1.68	1.13–2.65	0.0084	1.39	0.74–2.93
ER positive	0.59	0.44–0.80	0.0014	1.08	0.43–2.60	0.56	0.39–0.81	0.0033	2.30	0.85–6.16
PR positive	0.58	0.44–0.77	0.0002	0.61	0.41–0.98	0.51	0.36–0.72	0.0002	0.56	0.31–1.08
HER2 score ≥2	1.00		0.9774			1.10		0.5723		
Surgical/radiation therapy										
Mastectomy + RT	1.81		0.0910			1.08		0.8722		
Mastectomy only	1.12		0.4361			1.27		0.2011		
Breast-conserving + RT	0.63		0.0771			0.90		0.7207		
Breast-conserving only	0.95		0.7827			0.74		0.1991		
Adjuvant chemotherapy										
Exposured	1.08		0.6165			1.03		0.8588		
CEF × 6	1.01		0.6416			0.73		0.1935		
CEF × 4/T × 4	0.80		0.1756			0.93		0.7371		
AC × 4/T × 4	0.93		0.8993			0.99		0.9865		
T × 8	0.79		0.4920			0.97		0.9360		
Others	1.56	0.99–2.40	0.0133			1.66	1.03–2.53	0.0359	2.05	1.16–3.52
Hormonal therapy exposure	0.52	0.35–0.85	0.0122	0.58	0.25–1.54	0.42	0.27–0.71	0.0022	0.31	0.12–0.85

HR hazard ratio, *CI* confidence interval, *RFS* recurrence-free survival, *CSS* cancer-specific survival, *ECI* extracapsular invasion, *LNs* lymph nodes, *LVI* lymphovascular invasion, *ER* estrogen receptor, *PR* progesterone receptor, *HER2* human epidermal growth factor receptor, *RT* radiotherapy

nuclear grade, and lymphatic invasion tended to be associated with tumor progression in the ECI^+/v^+ group (Table 3). Although types of surgery and radiation therapy did not differ between the groups (data not shown), and more patients underwent adjuvant chemotherapy in the ECI^+/v^+ group than in the ECI^+/v^- group, distant metastases were observed only in the ECI^+/v^+ group ($p = 0.0008$). The RFS and CSS curves for the presence of ECI and vascular invasion of primary tumor are shown in Fig. 2. Although the RFS and CSS curves in the v^+ group were significantly lower than in the v^- group in all patients with metastatic nodes (electronic supplementary file 2), curves for the ECI^- group were similar to those in the ECI^-/v^+ group, irrespective of the presence of peritumoral invasion (Fig. 2). The ECI^+/v^+ group had the poorest RFS curve among the groups. In contrast, the curve for the ECI^+/v^- group was similar to that of the ECI^- group for both RFS and CSS. These results suggested that ECI combined with vascular invasion of the primary tumor strongly affects prognosis and distant recurrence risk. The RFS rate of ECI^+ patients without PVI was comparable to those in both ECI^- groups.

DISCUSSION

ECI is reportedly found in 24.0–65.4 % of histological examinations of lymph node metastasis in breast cancers,[15,21,22] and was found in 38.3 % of patients in this study. Many studies have associated ECI with higher numbers of positive nodes.[5–8] Recruitment by metastatic nodes of degradation factors that permit cancer cells to break through the lymph node capsule is characteristic of very aggressive breast cancers. However, ECI may primarily represent a higher likelihood of additional nodal metastasis. Although several studies have shown ECI to be an independent prognostic factor in patients with breast

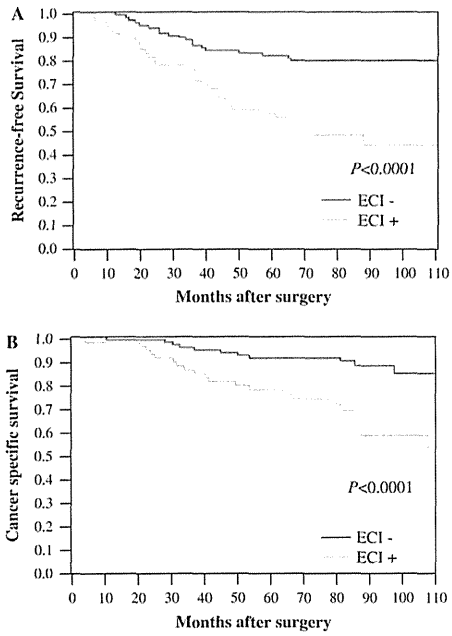

FIG. 1 Time to tumor recurrence determined from Kaplan–Meier curves varies among patients depending on the presence of ECI in positive nodes. **a** recurrence-free survival curve, and **b** breast cancer-specific survival curve. *ECI* extracapsular invasion

cancer,[14–17] other studies have shown ECI to be a significant prognostic factor in univariate, but not multivariate, analysis.[18–21] Thus, whether ECI in axillary lymph node metastases is a prognostic factor for breast cancer is controversial. This study therefore evaluated ECI^+ axillary lymph nodes as predictors of disease recurrence in breast cancer.

The investigation produced the following key results: (a) ECI in positive nodes was significantly associated with greater numbers of positive nodes; (b) ECI was also associated with disease recurrence or decreased survival but lost its prognostic significance in multivariate analysis; and (c) all cases of distant metastases in our series also had vascular invasion. The last result (c) may reflect systemic disease; ECI combined with vascular invasion of the primary tumor strongly affects prognosis and recurrence risk. These results also imply that ECI in axillary metastases with vascular invasion indicates high systemic aggressiveness.

Lymph node metastasis is an important prognostic factor in breast cancer, and the number of metastatic nodes affects

TABLE 3 Clinicopathological characteristics and recurrences in ECI positive group, with or without peritumoral vascular invasion

	ECI positive/V^+ (n = 39) [N (%)]	ECI positive/V^- (n = 20) [N (%)]	Univariate p value	Multivariate p value
Age (years)	57.9 ± 11.7	59.5 ± 10.3	0.5996	
Histopathology			0.3164	
Scirrous	27 (69.2)	11 (55.0)		
Solid tubular	4 (10.3)	5 (25.0)		
Papillo-tubular	8 (20.5)	4 (20.0)		
Tumor size (mm)	30.4 ± 14.7	22.0 ± 8.0	0.0023	0.0772
Nuclear grade			0.0247	0.3862
1	7 (18.0)	8 (40.0)		
2	10 (25.6)	8 (40.0)		
3	22 (56.4)	4 (20.0)		
Positive LN number	9.15 ± 1.20	6.65 ± 1.67	0.2280	
Lymphatic invasion			0.0058	0.8894
ly0	0 (0.0)	1 (5.0)		
ly1	0 (0.0)	3 (15.0)		
ly2	17 (43.6)	12 (60.0)		
ly3	22 (56.4)	4 (20.0)		
ER status positive	31 (79.5)	19 (95.0)	0.1167	
PR status positive	28 (71.8)	15 (75.0)	0.7932	
HER2 score			0.5972	
0	5 (12.8)	2 (10.0)		
1	8 (20.5)	4 (20.0)		
2	15 (38.5)	11 (55.0)		
3	11 (28.2)	3 (15.0)		
Locoregional recurrence				
Total	10 (25.6)	5 (25.0)	0.9573	
Intramammary	1 (2.6)	0 (0.0)	0.4701	
Axillary	3 (7.7)	2 (10.0)	0.7632	
Regional LNs	6 (15.4)	3 (15.0)	0.9690	
Distant metastases (without locoregional recurrent)				
Total	16 (41.0)	0 (0.0)	0.0008	
Multiple sites	4 (10.3)	0 (0.0)	0.1380	
One site	12 (30.8)	0 (0.0)	0.0054	
Lung	2 (5.1)	0 (0.0)	0.3028	
Liver	1 (2.6)	0 (0.0)	0.4701	
Bone	9 (23.1)	0 (0.0)	0.0196	
Distant LNs	0 (0.0)	0 (0.0)		

ECI extracapsular invasion, *LNs* lymph nodes, *ER* estrogen receptor, *PR* progesterone receptor, *HER2* human epidermal growth factor receptor 2

OS.[1,2] Our results concord with many studies that have shown a relationship between ECI and higher numbers of positive nodes.[14–17] ECI is thought to be a mechanism for node-to-node expansion in loco regional lymphatic progression. Earlier, we found the presence of ECI at SLNs to strongly predict residual axillary disease or non-SLN

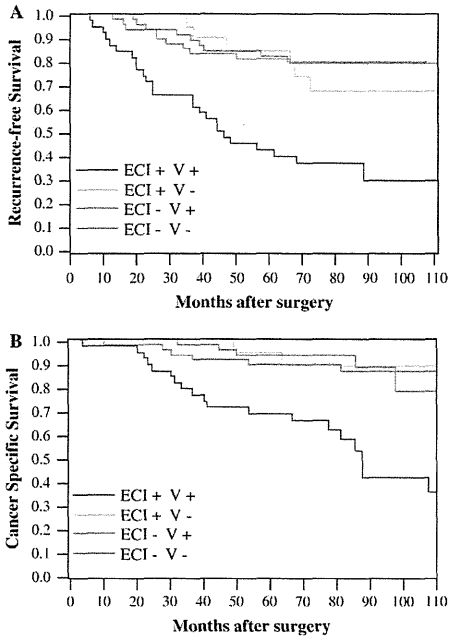

FIG. 2 a Recurrence-free survival curves, and b cancer-specific survival curves for the combined presence of ECI and vascular invasion of the primary tumor. *ECI* extracapsular invasion

metastasis.[5] In the present study, lymph nodes dissected during surgery showed loco regional recurrences in regional lymph nodes were more frequent in the ECI^+ group. In contrast, in 29 patients, distant metastases without loco regional recurrences were significantly associated with ECI in univariate analysis, but not in multivariate analysis, as the number of involved lymph nodes is a strong prognostic factor. Several studies have also correlated ECI with disease recurrence only in patients with fewer metastatic nodes,[21–23] which implies ECI is a marker for aggressive nodal disease; ECI was also seen in some patients with systemic aggressive disease.

Lymphovascular invasion (LVI) is reported to be a prognostic factor in patients with breast cancer.[22,24–26] We found ECI of positive nodes was related to lymphatic invasion but not to vascular invasion that may reflect systemic disease (Table 1). Therefore, ECI may not be directly associated with PVI. Although all ECI^+ patients in our study with distant metastases had PVI, patients with no vascular invasion had no distant metastases, despite the presence of ECI. Furthermore, ECI combined with vascular invasion was a highly significant predictor of outcome. Neri et al. stated that peritumoral LVI and ECI were predictors of shorter DFS and OS in multivariate analyses, and were strongly related with pN category and increased risk of distant recurrences.[22] Although several reports have suggested a relationship between ECI and LVI,[22,24] they did not separate vascular invasion and lymphatic invasion. Because ECI and lymphatic invasion are closely associated, vascular invasion may be more representative of systemic disease than lymphatic invasion. We therefore investigated the utility of vascular invasion as an additional useful prognostic indicator. We found that ECI and vascular invasion were independent variables; the combination of both factors (ECI^+/v^+) indicated significantly worse prognosis. ECI of positive nodes may be associated with higher likelihood of additional nodal metastasis and potentially systemic aggressive disease. In patients with ECI, those with vascular invasion appear to be at higher risk for systemic disease.

Notably, when recurrence was observed in bone tissue, ECI was significantly correlated with relapse. Organ specificity may be important in establishing metastasis. The potential of ECI as a bone metastasis marker in breast cancer should be the focus of a future study.

This study has several potential limitations. It used retrospective data collection methods and had relatively few patients as study subjects.

CONCLUSIONS

Additional research is needed to explore the significance of ECI in OS or metastatic disease and the role of ECI in locoregional lymphatic progression.

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