continued. Tumour lesions were assessed radiologically at baseline, week 4, week 7, every 6 weeks from week 7 to 18 months, and every 12 weeks thereafter until disease progression according to RECIST 1.1.

| | Erlotinib plus bevacizumab group (n=75) | Erlotinib alone group (n=77) |
|---|---|---|
| **Age (years)** | | |
| Median | 67·0 (59–73) | 67·0 (60–73) |
| <75 | 63 (84%) | 62 (81%) |
| ≥75 | 12 (16%) | 15 (19%) |
| **Sex** | | |
| Male | 30 (40%) | 26 (34%) |
| Female | 45 (60%) | 51 (66%) |
| **Smoking status** | | |
| Never smoker | 42 (56%) | 45 (58%) |
| Former light smoker | 9 (12%) | 6 (8%) |
| Other | 24 (32%) | 26 (34%) |
| **ECOG performance status** | | |
| 0 | 43 (57%) | 41 (53%) |
| 1 | 32 (43%) | 36 (47%) |
| **Histopathological classification** | | |
| Adenocarcinoma | 74 (99%) | 76 (99%) |
| Large-cell carcinoma | 0 | 1 (1%) |
| Adenosquamous carcinoma | 1 (1%) | 0 |
| **Clinical stage at screening** | | |
| IIIB | 1 (1%) | 0 |
| IV | 60 (80%) | 62 (81%) |
| Postoperative recurrence | 14 (19%) | 15 (19%) |
| **EGFR mutation type** | | |
| Exon 19 deletion | 40 (53%) | 40 (52%) |
| Exon 21 Leu858Arg mutation | 35 (47%) | 37 (48%) |

Data are n (%) or median (IQR). ECOG=Eastern Cooperative Oncology Group.

*Table 1:* **Baseline demographics and clinical characteristics**

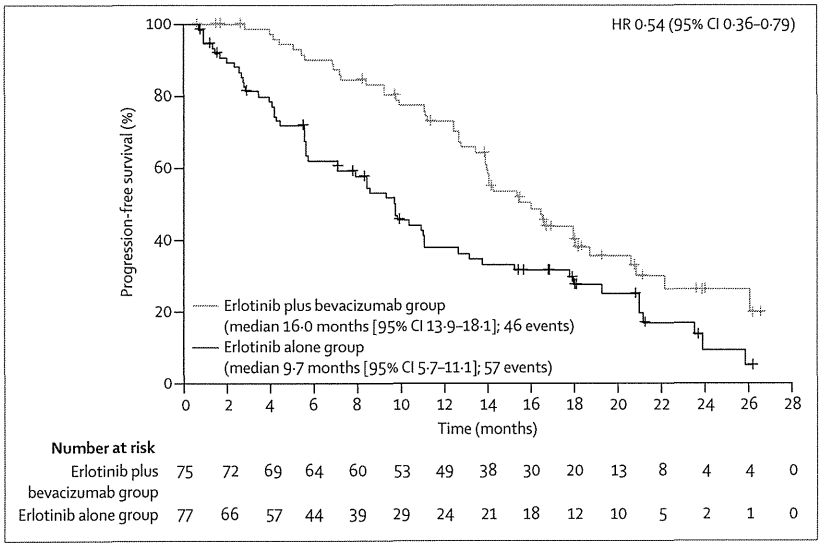


*Figure 2:* **Progression-free survival, as determined by independent review committee, in the modified intention-to-treat population**
HR=hazard ratio.

Patient-reported outcomes were assessed with the Functional Assessment of Cancer Therapy for patients with Lung cancer (FACT-L) scale until disease progression. An independent review committee of clinicians and radiologists masked to treatment assignment reviewed all tumour images and determined tumour response and progression status. Laboratory studies including blood and urine tests were done at days 1, 8, and 15 in cycles 1 and 2, and day 1 in cycle 3 and thereafter. Adverse events were monitored throughout the study period and were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTC-AE) version 4.03.

## Outcomes

The primary endpoint was progression-free survival, as determined by an independent review committee. Secondary endpoints were overall survival, tumour response (the proportion of patients with an objective response and disease control, and duration of response) according to RECIST 1.1, quality of life, symptom improvement measured by the FACT-L scale, and safety profile.

## Statistical analysis

A median progression-free survival of 13 months was estimated for the erlotinib alone group, and 89 events were deemed necessary to detect a hazard ratio (HR) of 0·7 in favour of erlotinib plus bevacizumab, with a one-sided significance level of 0·2 and a power of 0·8. The target sample size was set at 150 patients (75 patients in both groups), allowing for dropouts. Median progression-free survival was estimated by the Kaplan-Meier method and compared between groups with an unstratified log-rank test. Greenwood's formula was used to calculate 95% CIs. HRs were calculated by unstratified Cox proportional hazard methodology.

In the safety analysis, adverse events were converted to Medical Dictionary for Regulatory Activities (version 14.0) preferred terms, and tabulated by grade. Changes in laboratory test data with time were summarised in tables and graphs.

All patients who received at least one dose of the study treatment were included in the safety analysis population. The modified intention-to-treat population for the efficacy analysis included all patients who received at least one dose of study treatment and had tumour assessment at least once after randomisation. Statistical analyses were done with SAS version 9.2.

The study is registered with the Japan Pharmaceutical Information Center, number JapicCTI-111390.

## Role of the funding source

The study was designed and funded by Chugai Pharmaceutical Co Ltd and monitored by a clinical research organisation (Niphix Corp, Tokyo, Japan) who obtained all data and did all initial data analyses; further analysis and interpretation was done by the funder, with

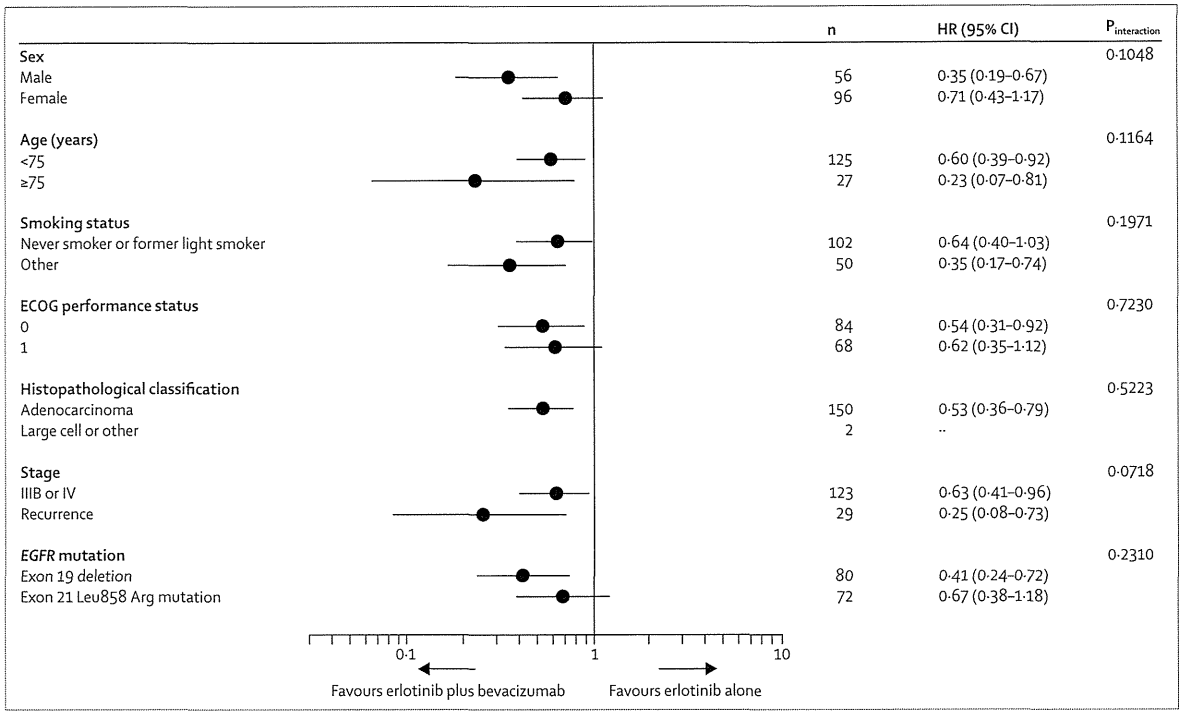


*Figure 3:* **Forest plot of hazard ratios for progression-free survival by baseline characteristics**
HR=hazard ratio.

| | Erlotinib plus bevacizumab group (n=75) | Erlotinib alone group (n=77) |
|---|---|---|
| Complete response | 3 (4%) | 1 (1%) |
| Partial response | 49 (65%) | 48 (62%) |
| Stable disease | 22 (29%) | 19 (25%) |
| Progressive disease | 0 | 6 (8%) |
| Non-evaluable | 1 (1%) | 3 (4%) |

RECIST=Response Evaluation Criteria in Solid Tumors.

*Table 2:* **Best RECIST response, as determined by independent review committee**

input from the authors and investigators. The initial draft of the report was reviewed and commented on by all authors and by employees of Chugai Pharmaceutical Co Ltd. NobuY had full access to all data, and had final responsibility for the decision to submit the results for publication.

## Results

Between Feb 21, 2011, and March 5, 2012, 154 patients were enrolled, of whom 77 were randomly assigned to receive erlotinib plus bevacizumab and 77 to erlotinib alone. Two patients withdrew before treatment started and were excluded (one had multiple thrombosis and the other had increased pleural effusion). Thus, data from 152 patients (75 patients in the erlotinib plus bevacizumab group and 77 in the erlotinib alone group) were included in the analysis population (figure 1). The cutoff date for the primary analysis was June 30, 2013, when 103 progression events had occurred; median follow-up was 20·4 months (IQR 17·4–24·1).

The baseline characteristics of patients were well balanced between the groups (table 1). Median age was 67 years (IQR 60–73), and 27 (18%) patients were aged 75 years or older. *EGFR* mutation subtypes were balanced between the two groups.

Progression-free survival was significantly prolonged with erlotinib plus bevacizumab compared with erlotinib alone (log-rank test p=0·0015; figure 2). When subgroup analyses were done by baseline clinical characteristics, most patient subgroups seemed to have greater benefit from erlotinib plus bevacizumab compared with erlotinib alone. No significant difference was noted between any of the subgroups ($p_{interaction}$>0·05 for all subgroups; figure 3).

Analysis of progression-free survival by mutation subtype showed that in patients whose tumours had an exon 19 deletion (40 [53%] of 75 patients in the erlotinib plus bevacizumab group and 40 [52%] of 77 patients in the erlotinib alone group), median progression-free survival was significantly longer with erlotinib plus bevacizumab than with erlotinib alone (18·0 months [95% CI 14·1–20·6] *vs* 10·3 months [95% CI 8·0–13·1]; HR 0·41 [95% CI 0·24–0·72]; p=0·0011; appendix p 1). In patients whose tumours harboured the Leu858Arg mutation (35 [47%] patients in the erlotinib plus bevacizumab group; 37 [48%] patients in the erlotinib alone group), median progression-free survival was numerically longer with erlotinib plus bevacizumab than with erlotinib alone, but

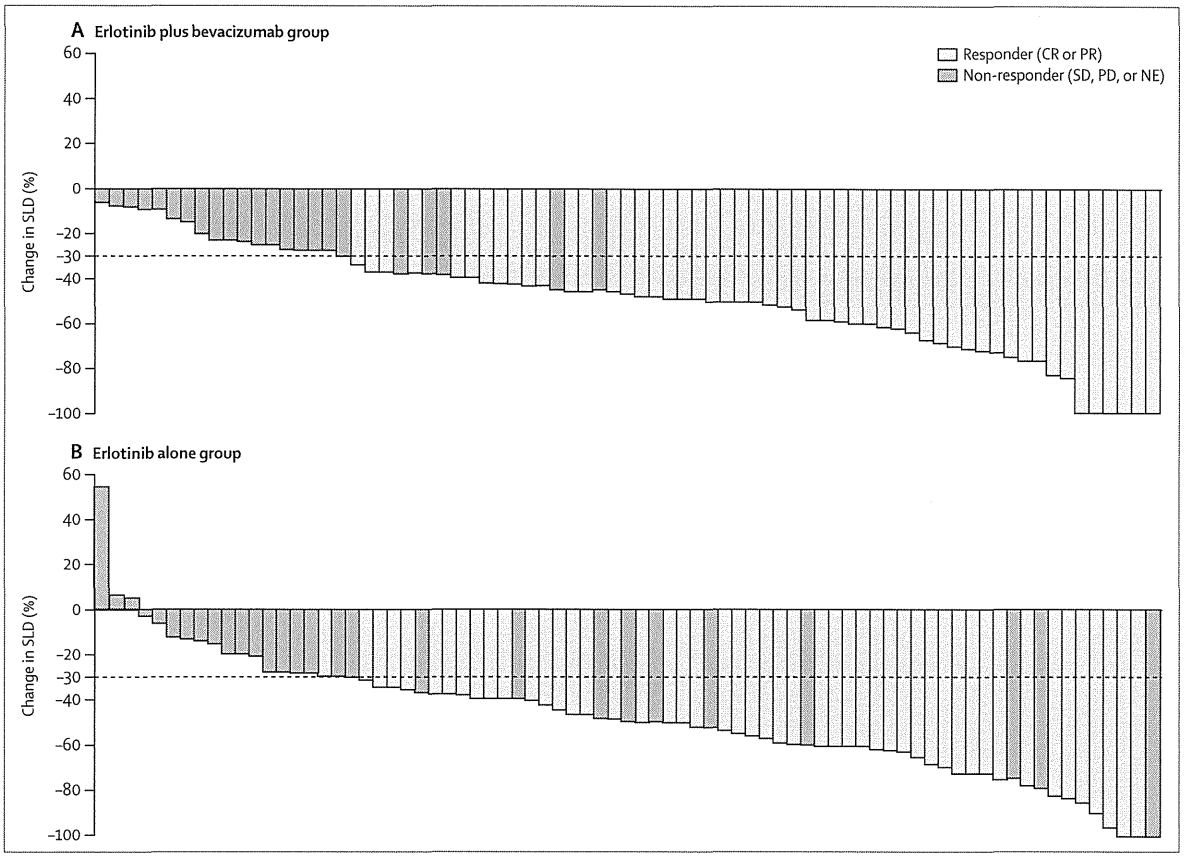


*Figure 4:* **Waterfall plot of best percentage change from baseline in the sum of longest tumour diameters**
Responders were confirmed by Response Evaluation Criteria in Solid Tumors. CR=complete response. PR=partial response. SD=stable disease. PD=progressive disease. NE=non-evaluable. SLD=sum of longest diameters.

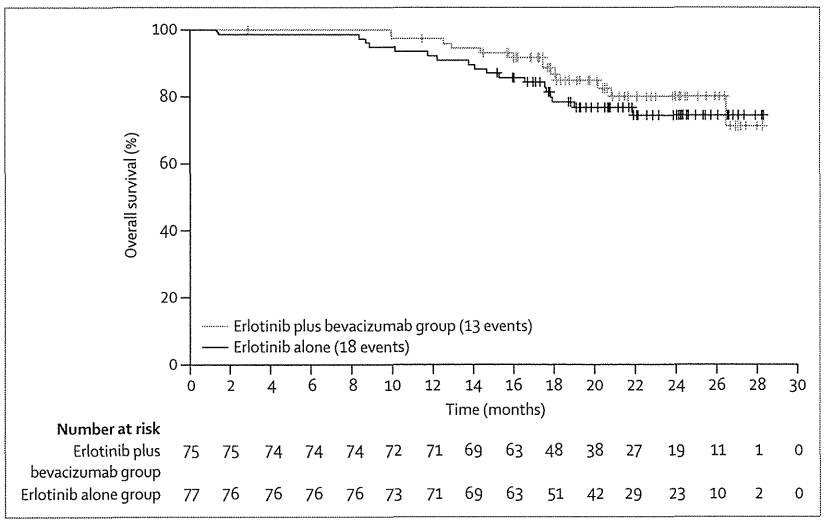


*Figure 5:* **Overall survival, as determined by independent review committee, in the modified intention-to-treat population**

the difference was not significant (13·9 months [95% CI 11·2–20·9] *vs* 7·1 months [95% CI 4·3–15·2], respectively; HR 0·67 [95% CI 0·38–1·18]; p=0·1653; appendix p 2).

52 (69% [95% CI 58–80]) patients in the erlotinib plus bevacizumab group had an objective response, as did 49 (64% [52–74]) patients in the erlotinib alone group (p=0·4951), although median duration of response was not significantly longer with erlotinib plus bevacizumab than with erlotinib alone (13·3 months [95% CI 11·6–16·5] *vs* 9·3 months [6·9–13·8]; p=0·1118). A greater proportion of patients achieved disease control with erlotinib plus bevacizumab (74 [99%] *vs* 68 [88%]; p=0·0177). Best responses to treatment are shown in table 2.

Figure 4 shows change in tumour size from baseline in the two groups. All patients in the erlotinib plus bevacizumab achieved tumour reduction, but three patients in the erlotinib alone group did not. Of patients who had a 30% or greater reduction in tumour size during treatment, six (8%) patients in the erlotinib plus bevacizumab group and 12 (16%) patients in the erlotinib alone group did not meet the criteria for complete or partial response according to RECIST.

Overall survival data are immature at present and so we cannot present any statistical analyses. At data cutoff, only 13 events (17%) had occurred in the erlotinib plus bevacizumab group and 18 events (23%) in the erlotinib alone group (figure 5).

| | Erlotinib plus bevacizumab group (n=75) | | | | | Erlotinib alone group (n=77) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | All | Grade 1–2 | Grade 3 | Grade 4 | Grade 5 | All | Grade 1–2 | Grade 3 | Grade 4 | Grade 5 |
| Rash | 74 (99%) | 55 (73%) | 19 (25%) | 0 | 0 | 76 (99%) | 61 (79%) | 15 (19%) | 0 | 0 |
| Diarrhoea | 61 (81%) | 60 (80%) | 1 (1%) | 0 | 0 | 60 (78%) | 59 (77%) | 1 (1%) | 0 | 0 |
| Paronychia | 57 (76%) | 55 (73%) | 2 (3%) | 0 | 0 | 50 (65%) | 47 (61%) | 3 (4%) | 0 | 0 |
| Dry skin | 56 (75%) | 54 (72%) | 2 (3%) | 0 | 0 | 45 (58%) | 45 (58%) | 0 | 0 | 0 |
| Stomatitis | 47 (63%) | 46 (61%) | 1 (1%) | 0 | 0 | 46 (60%) | 44 (57%) | 2 (3%) | 0 | 0 |
| Haemorrhagic event | 54 (72%) | 52 (69%) | 2 (3%) | 0 | 0 | 22 (29%) | 22 (29%) | 0 | 0 | 0 |
| Liver function disorder or abnormal hepatic function | 33 (44%) | 27 (36%) | 5 (7%) | 1 (1%) | 0 | 39 (51%) | 25 (32%) | 7 (9%) | 7 (9%) | 0 |
| Hypertension | 57 (76%) | 12 (16%) | 45 (60%) | 0 | 0 | 10 (13%) | 2 (3%) | 8 (10%) | 0 | 0 |
| *Pruritus* | 34 (45%) | 33 (44%) | 1 (1%) | 0 | 0 | 32 (42%) | 32 (42%) | 0 | 0 | 0 |
| Weight decreased | 33 (44%) | 33 (44%) | 0 | 0 | 0 | 19 (25%) | 19 (25%) | 0 | 0 | 0 |
| Decreased appetite | 26 (35%) | 25 (33%) | 1 (1%) | 0 | 0 | 26 (34%) | 25 (32%) | 1 (1%) | 0 | 0 |
| Proteinuria | 39 (52%) | 33 (44%) | 6 (8%) | 0 | 0 | 3 (4%) | 3 (4%) | 0 | 0 | 0 |
| Dysgeusia | 20 (27%) | 20 (27%) | 0 | 0 | 0 | 17 (22%) | 17 (22%) | 0 | 0 | 0 |
| Nasopharyngitis | 20 (27%) | 20 (27%) | 0 | 0 | 0 | 15 (19%) | 15 (19%) | 0 | 0 | 0 |
| Constipation | 17 (23%) | 17 (23%) | 0 | 0 | 0 | 15 (19%) | 14 (18%) | 1 (1%) | 0 | 0 |
| Alopecia | 13 (17%) | 13 (17%) | 0 | 0 | 0 | 14 (18%) | 14 (18%) | 0 | 0 | 0 |
| Nausea | 12 (16%) | 12 (16%) | 0 | 0 | 0 | 15 (19%) | 15 (19%) | 0 | 0 | 0 |
| Vomiting | 14 (19%) | 14 (19%) | 0 | 0 | 0 | 7 (9%) | 7 (9%) | 0 | 0 | 0 |
| Malaise | 10 (13%) | 10 (13%) | 0 | 0 | 0 | 10 (13%) | 10 (13%) | 0 | 0 | 0 |
| Insomnia | 8 (11%) | 8 (11%) | 0 | 0 | 0 | 8 (10%) | 8 (10%) | 0 | 0 | 0 |
| Pyrexia | 7 (9%) | 7 (9%) | 0 | 0 | 0 | 9 (12%) | 9 (12%) | 0 | 0 | 0 |
| Upper respiratory tract infection | 9 (12%) | 9 (12%) | 0 | 0 | 0 | 7 (9%) | 7 (9%) | 0 | 0 | 0 |
| *Conjunctivitis* | 8 (11%) | 8 (11%) | 0 | 0 | 0 | 7 (9%) | 7 (9%) | 0 | 0 | 0 |
| Peripheral oedema | 8 (11%) | 8 (11%) | 0 | 0 | 0 | 6 (8%) | 6 (8%) | 0 | 0 | 0 |
| Fatigue | 10 (13%) | 9 (12%) | 1 (1%) | 0 | 0 | 3 (4%) | 3 (4%) | 0 | 0 | 0 |
| Nail disorder | 9 (12%) | 9 (12%) | 0 | 0 | 0 | 4 (5%) | 4 (5%) | 0 | 0 | 0 |
| Dry eye | 8 (11%) | 8 (11%) | 0 | 0 | 0 | 3 (4%) | 3 (4%) | 0 | 0 | 0 |
| Dysphonia | 8 (11%) | 8 (11%) | 0 | 0 | 0 | 1 (1%) | 1 (1%) | 0 | 0 | 0 |

Data are n (%).

*Table 3:* Adverse events reported by 10% or more patients for grades 1 and 2 and all adverse events for grades 3–5 (safety population)

68 (91%) patients in the erlotinib plus bevacizumab group and 41 (53%) patients in the erlotinib group had grade 3 or 4 adverse events. The most common adverse events of any grade in the erlotinib plus bevacizumab group were rash, diarrhoea, hypertension, and paronychia, and in the erlotininb alone group were rash, diarrhoea, and paronychia (table 3). The most common grade 3 or worse adverse events in the erlotinib plus bevacizumab group were hypertension, rash, proteinuria, and liver function disorder or abnormal hepatic function, and in the erlotinib group were rash, liver function disorder or abnormal hepatic function, and hypertension (table 3). Substantially higher (>40%) incidences of hypertension, haemorrhagic events, and proteinuria were noted in the erlotinib plus bevacizumab group compared with the erlotinib alone group (table 3). Serious adverse events were reported by 18 (24%) patients in the erlotinib plus bevacizumab group and 19 (25%) patients in the erlotinib group.

12 (16%) patients in the erlotinib plus bevacizumab group and 14 (18%) patients in the erlotinib group discontinued erlotinib because of adverse events. 31 (41%) patients discontinued bevacizumab because of adverse events (figure 1). Ten patients discontinued both erlotinib and bevacizumab because of adverse events in the erlotinib plus bevacizumab group. Of these patients, seven discontinued erlotinib and bevacizumab simultaneously because of adverse events (liver function disorder or abnormal hepatic function in two patients, and infection, pancreatic cancer, rash, interstitial lung disease, and cerebral infarction in one patient each). In the remaining three patients, bevacizumab was initially discontinued, and patients continued on erlotinib monotherapy, although this was also subsequently discontinued. The dose of erlotinib was reduced to 100 mg for 34 (45%) of 75 patients in the erlotinib plus bevacizumab group and 33 (43%) of 77 patients in the erlotinib alone group; and to 50 mg for 17 (23%) of patients in the erlotinib plus bevacizumab group and eight (10%) patients in the erlotinib alone group.

The major adverse events leading to discontinuation of erlotinib in both groups were liver function disorder or abnormal hepatic function (two [3%] patients in the erlotinib plus bevacizumab group, eight [10%] in the

*Panel:* Research in context

**Systematic review**

We searched PubMed for articles published in English until Feb 1, 2014 (with no restrictions for the starting date), using the search terms "bevacizumab", "erlotinib", "NSCLC", and "EGFR". We identified two studies that had assessed the efficacy of erlotinib plus bevacizumab in the first-line setting.[19,20] However, no previous study had assessed the efficacy of the combination of erlotinib and bevacizumab as first-line therapy for patients with activating *EGFR* mutation-positive NSCLC.

**Interpretation**

To our knowledge, this study is the first to show that the combination of erlotinib and bevacizumab can significantly prolong progression-free survival compared with erlotinib alone in patients with non-squamous *EGFR* mutation-positive NSCLC. Some degree of increased toxicity, particularly hypertension, proteinuria, and haemorrhagic events, was noted with the addition of bevacizumab. Our findings suggest that the combination of erlotinib and bevacizumab could be a new first-line regimen in *EGFR* mutation-positive NSCLC. Two clinical trials, BELIEF (NCT01562028) and ACCRU RC1126 (NCT01532089) are ongoing and the results are awaited to confirm the efficacy and safety shown in our study.

erlotinib alone group), interstitial lung disease (two [3%], three [4%]), and rash (two [3%], none). Major adverse events leading to discontinuation of bevacizumab were proteinuria (11 [15%] patients), haemorrhagic events (nine [12%]), and hypertension (two [3%]). Most haemorrhagic events were low-grade epistaxis or haemorrhoidal bleeding. All of the 11 patients who discontinued bevacizumab because of proteinuria had grade 3 or lower events, and five of these patients recovered during the study period. All of the nine patients who discontinued because of haemorrhagic events had grade 3 or lower events; eight patients improved or recovered during the study period.

The median duration of erlotinib treatment was 431 days (range 21–837) in the erlotinib plus bevacizumab group and 254 days (18–829) in the erlotinib group, whereas median duration of bevacizumab was 325 days (1–815). The median duration of bevacizumab in patients who discontinued treatment because of proteinuria was 329 days (113–639) and because of haemorrhagic events was 128 days (23–357).

The relative dose intensity of erlotinib (calculated as [totally administered dose / total treatment duration] / 150×100) was similar in both groups (95·3% [range 34·7–100·0] in the erlotinib plus bevacizumab group and 98·7% [33·3–100·0] in the erlotinib alone group), whereas that of bevacizumab (calculated as totally administered dose/planned dose×100) was 93·9% (72·4–99·7).

Haemoptysis was reported in six (8%) patients in the erlotinib plus bevacizumab group (five [7%] patients had grade 1 events and one [1%] had a grade 2 event); one patient (1%) had a grade 1 event in the erlotinib alone group. Interstitial lung disease was reported for five (3%) of all patients. One patient in the erlotinib alone group had grade 3 interstitial lung disease, but all other cases were grade 1 or 2, and all patients recovered. During the study period, one patient in the erlotinib group died by drowning, and a potential association with the study drug was confirmed.

No significant difference was noted between the two groups in terms of quality of life, including total FACT-L score, trial outcome index score, and all other subscores, since the standard deviations at each time point overlapped (appendix pp 3–9).

## Discussion

In this study, the addition of bevacizumab to erlotinib significantly prolonged progression-free survival in patients with NSCLC with activating *EGFR* mutation-positive disease compared with erlotinib alone. To our knowledge, this is the first randomised study to show a clinically significant treatment effect of combining an EGFR tyrosine-kinase inhibitor with another biological drug in patients with activating *EGFR* mutation-positive NSCLC (panel). We noted clear separation of the Kaplan-Meier survival curves from the start of treatment, despite the use of erlotinib in both groups.

Multivariate analysis according to baseline patient characteristics showed a consistent treatment benefit, with longer progression-free survival noted with erlotinib plus bevacizumab across most subgroups of patients. Previous studies have reported that erlotinib tends to be more effective in tumours with *EGFR* exon 19 deletions versus those with Leu858Arg mutations,[7,8,21] which is consistent with our results.

No new safety signals were identified and the incidence of adverse events (any grade) and serious adverse events was similar between the two groups. There were more grade 3 or worse adverse events in the erlotinib plus bevacizumab group. Discontinuation of bevacizumab because of adverse events was more common than that reported in previous studies.[13,14] One possible reason for this discrepancy could be the longer duration of treatment than in previous studies: the median treatment duration of bevacizumab was 325 days (16 cycles), which is substantially longer than that in previous studies. Furthermore, proteinuria was one of the major adverse events that led to discontinuation of bevacizumab, and the time to onset of bevacizumab discontinuation because of proteinuria tended to be in the later treatment phase (median 329 days [range 113–639]). Nevertheless, despite the high incidence of bevacizumab discontinuation because of adverse events, most of these events (mainly proteinuria and haemorrhagic events) were deemed non-serious and reversible.

The incidence of grade 3 or greater hypertension and proteinuria were higher than those in previous studies, again possibly related to the prolonged duration of treatment. Another potential factor that could explain the difference in the incidence of hypertension is in the definition of grading used; we used CTC-AE version 4.03, whereas previous studies[14,16] used CTC-AE version 3. Akhtar and colleagues[22] showed that the change in CTC-AE version from 3 to 4 could lead to a significant

shift in the severity of adverse events in clinical trials. Furthermore, despite the somewhat higher incidence of hypertension observed in this study, only two (3%) of 75 patients discontinued bevacizumab administration because of hypertension.

Although we noted no significant difference in the proportion of patients achieving an objective response between the erlotinib plus bevacizumab group and erlotinib alone groups, all patients in the erlotinib plus bevacizumab group had a reduction in tumour size. Of those patients who had a greater than 30% reduction in the sum of longest diameter of their target lesions from baseline, more patients in the erlotinib alone group failed to meet the criteria for complete or partial response. These findings suggest that the addition of bevacizumab to erlotinib might help to maintain the tumour-suppressing effect after reduction in tumour size, which might explain the difference in progression-free survival between the two groups.

One possible mechanism to explain this effect could be improved drug delivery. Bevacizumab changes tumour vessel physiology, resulting in increased intratumoral uptake of drugs.[23,24] The results of a preclinical study suggested that patients on lower doses of EGFR tyrosine-kinase inhibitors tend to develop treatment resistance earlier than those who receive higher doses.[25,26] Therefore, achieving a higher intratumoral concentration of erlotinib could delay the appearance of resistant cells. Another possible mechanism that could explain these findings is the effective blocking of angiogenesis signalling via the VEGF receptor and EGFR signalling pathways, which is thought to promote tumour growth.[27,28] In addition to synergistic inhibition of tumour growth signalling, VEGF signal inhibition is still effective for tumours harbouring EGFR tyrosine-kinase inhibitor resistance mutations. In preclinical studies, blocking the VEGF receptor signalling pathway overcame resistance for EGFR signalling blockage by Thr790Met EGFR mutation in vivo.[29,30]

Another treatment strategy that has been recently investigated is the combination of an EGFR tyrosine-kinase inhibitor with chemotherapy. Wu and colleagues[31] reported that platinum doublet chemotherapy with intercalated erlotinib increased progression-free survival compared with platinum doublet chemotherapy alone. In a subset analysis of the *EGFR* mutation-positive population in this study, progression-free survival was 16·8 months. In our study, median progression-free survival with erlotinib and bevacizumab was 16·0 months. The first-line use of erlotinib and bevacizumab could allow chemotherapy to be reserved for subsequent lines of treatment, which might further improve survival outcomes in these patients.

Our study has several limitations. First, the analysis of *EGFR* mutations was not done at a central laboratory and various methods were used, including the peptide nucleic acid, locked nucleic acid PCR clamp method, the PCR invader method, and the cycleave method. However, on the basis of previous evidence, these methods are generally judged to provide consistent results.[32] Second, because some patients are still receiving the first-line treatment and overall survival data are still immature, assessment of subsequent treatment effects after progression is not possible. Data relating to post-study treatment will be reported in due course with updated overall survival results. Third, we did not use the EQ-5D questionnaire developed by the EuroQol group for quality-of-life assessment. Therefore, we could not formally estimate quality-adjusted life-years for a cost-effectiveness analysis. The health economics related to the combined use of erlotinib and bevacizumab remains unclear and should be discussed in future studies. Additionally, follow-up for overall survival is still ongoing and these results are needed before the clinical value of this combination can be determined.

In summary, our study provides, to the best of our knowledge, the first evidence that the addition of bevacizumab to erlotinib confers a significant improvement in progression-free survival when used as first-line treatment for patients with non-squamous NSCLC with activating *EGFR* mutation-positive disease. Some degree of increased toxicity, particularly hypertension, proteinuria, and haemorrhagic events, seems to be associated with the addition of bevacizumab. Our findings suggest that the combination of erlotinib and bevacizumab could be a new first-line regimen in *EGFR* mutation-positive NSCLC, and that further investigation of the regimen is warranted. Two clinical trials, BELIEF (NCT01562028) and ACCRU RC1126 (NCT01532089), are ongoing and the results are awaited to confirm the efficacy and safety shown in our study.

#### Contributors

NobuY was the principal investigator. TS, TK, MN, KG, NoboY, IO, TY, KT, RH, MF, and NobuY contributed to the study design and data analysis and data interpretation. TS, TK, MN, KG, SA, YH, NoboY, TH, MM, KN, SN, IO, and NobuY contributed to patient recruitment and data collection. NobuY, TS, KT, and RH prepared the initial draft of the report input from other authors. All authors approved the final version of the report.

#### Declaration of interests

TS received research grants and honoraria from Chugai Pharmaceutical. TK received research grants and honoraria from Chugai Pharmaceutical; honoraria from Eli Lilly, Ono Pharmaceutical, Novartis Pharma, Taiho Pharmaceutical, and AstraZeneca; and research grants from Nippon Boehringer Ingelheim, Kyowa Hakko Kirin, Pfizer, and Shionogi. MN received research grants and honoraria from Chugai Pharmaceutical, Pfizer, Novartis Pharma, Taiho Pharmaceutical, Nippon Boehringer Ingelheim, and AstraZeneca; research grants from MSD and Bristol-Myers Squibb. KG received research grants and honoraria from Chugai Pharmaceutical, Taiho Pharmaceutical and Nippon Boehringer Ingelheim; honoraria from AstraZeneca, Sanofi, Novartis Pharma, Pfizer, Yakult Honsha, Ono Pharmaceutical and Eli Lilly. SA received honoraria from Chugai Pharmaceutical, Eli Lilly, Taiho Pharmaceutical, Sawai Pharmaceutical, and Novartis Pharma. YH received research grants and honoraria from Chugai Pharmaceutical, Ono Pharmaceutical, and Taiho Pharmaceutical; honoraria from AstraZeneca, Eli Lilly, Novartis Pharma, and Takeda Pharmaceutical; research grants form Yakult Honsha, MSD, Kyowa Hakko Kirin, and Daiichi Sankyo. NoboY received research grants form Chugai Pharmaceutical, Pfizer, Takeda Bio, Astellas Pharma, Taiho Pharmaceutical, and Bristol-Myers Squibb. TH received research grants form Chugai Pharmaceutical, AstraZeneca, Nippon Boehringer Ingelheim, Pfizer, Eli Lilly, Takeda Bio, Novartis Pharma, Ono Pharmaceutical, Daiichi Sankyo, Merck Serono, Kyowa Hakko Kirin, Dainippon Sumitomo Pharma, Bristol-Myers Squibb, and Esai.

MM received honoraria from Chugai Pharmaceutical and AstraZeneca; research grants and honoraria from Nippon Boehringer Ingelheim. KN received honoraria from Chugai Pharmaceutical, AstraZeneca, Nippon Boehringer Ingelheim, and Eli Lilly. SN declares no competing interests. IO received honoraria from Chugai Pharmaceutical, Eli Lilly, Pfizer, and Taiho Pharmaceutical. TY received honoraria from Chugai Pharmaceutical, Taiho Pharmaceutical, Takeda Pharmaceutical, and Bristol-Myers Squibb. KT and RH are employees of Chugai Pharmaceutical. MF received honoraria from Chugai Pharmaceutical. NobuY received honoraria from Chugai Pharmaceutical, Nippon Boehringer Ingelheim, and AstraZeneca.

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# Treatment of Patients with Adult T Cell Leukemia/Lymphoma with Cord Blood Transplantation: A Japanese Nationwide Retrospective Survey

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A B S T R A C T

Allogeneic bone marrow and peripheral blood stem cell transplantations are curative treatment modalities for adult T cell leukemia/lymphoma (ATLL) because of the intrinsic graft-versus-ATLL effect. However, limited information is available regarding whether cord blood transplantation (CBT) induces a curative graft-versus-ATLL effect against aggressive ATLL. To evaluate the effect of CBT against ATLL, we retrospectively analyzed data from 175 patients with ATLL who initially underwent single-unit CBT. The 2-year overall survival (OS) rate was 20.6% (95% confidence interval [CI], 13.8% to 27.4%). A multivariate analysis revealed that the development of graft-versus-host disease (GVHD) was a favorable prognostic factor for OS (hazard ratio, .10; 95% CI, .01 to .94; $P = .044$). Furthermore, the 2-year OS (42.7%; 95% CI, 28.1% to 56.6%) of patients with grade 1 to 2 acute GVHD was higher than that of patients without acute GVHD (24.2%; 95% CI, 11.2% to 39.8%; $P = .048$). However, the cumulative incidence of treatment-related mortality (TRM) was high (46.1%; 95% CI, 38.2% to 53.7%), and early death was particularly problematic. In conclusion, CBT cures patients with ATLL partly through a graft-versus-ATLL effect. However, novel interventions will be required, particularly in the early phase, to reduce TRM and optimize GVHD.



## INTRODUCTION

Adult T cell leukemia/lymphoma (ATLL), an aggressive peripheral T cell neoplasm caused by the human T cell lymphotropic/leukemia virus type-1, has an extremely poor prognosis [1]. Intensive chemotherapy and autologous stem cell transplantation have not been shown to improve this prognosis [2,3]. As a curative treatment, allogeneic hematopoietic stem cell transplantation (allo-HSCT) can confer long-term remission via a graft-versus-ATLL effect in a proportion of patients with ATLL [4-7]. Recent reports have demonstrated that allo-HSCT using bone marrow (BM) or peripheral blood stem cells (PBSC) from a related or unrelated donor can effectively treat ATLL, yielding a 3-year overall survival rate

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(OS) of approximately 30% [8-16]. However, patients with ATLL typically lack a suitable HLA-identical sibling donor because both the recipients and donors are typically elderly and because the aggressive ATLL tumor burden reduces the available time to find a suitable unrelated donor within the Japan Marrow Donor Program. Umbilical cord blood, which can serve as an alternative to BM or PBSC as a source of stem cells, has been used primarily to treat children; however, the number of unrelated-donor cord blood transplantation (CBT) procedures used to treat adult patients with ATLL is increasing in Japan. The rapid availability of CBT may provide a great advantage for patients who require urgent allo-HSCT to treat aggressive ATLL [17].

Currently, the outcome of CBT in patients with acute leukemia is comparable to that of other graft sources [18,19]; however, there are few reports on the outcomes of CBT in patients with ATL [20,21]. Moreover, it is difficult to draw firm conclusions regarding the efficacy of this procedure because of the small number of cases. Therefore, to evaluate the role of CBT for ATLL in a larger and more recent cohort, we performed a nationwide retrospective study of patients with ATLL who underwent CBT as the initial allo-HSCT.

## PATIENTS AND METHODS

### *Data Collection*

We analyzed nationwide survey data from the Japan Society for Hematopoietic Cell Transplantation regarding patients with ATLL who had undergone an initial CBT between March 2001 and December 2009 (n = 175). This analysis included the patients' clinical characteristics, such as the age at transplantation, gender, disease status at transplantation, date of transplantation, time from diagnosis to transplantation, conditioning regimens, and number of infused cells. The number of mismatches was counted with respect to HLA-A, HLA-B (low-resolution typing), and DRB1 (high-resolution typing). The present study was approved by the data management committees of the Japan Society for Hematopoietic Cell Transplantation as well as the institutional ethics committee of the Kyushu University Graduate School of Medical Sciences.

### *Definitions*

*OS* was defined as the time from transplantation until death, and patients who remained alive at the time of the last follow-up were censored. The causes of death were reviewed and categorized as either ATLL-related or transplantation-related mortality (TRM). *ATLL-related mortality* was defined as death caused by a relapse or progression of ATLL, whereas *TRM* was defined as any death related to transplantation other than ATLL-related mortality, according to the judgment of each institution. The patients were divided into 2 groups according to the conditioning regimen: full-intensity conditioning (FIC) and reduced-intensity conditioning (RIC). FIC and RIC were defined according to the proposals of Giralt et al. [22] and Bacigalupo et al. [23], respectively, with slight modifications. In the present study, conditioning regimens that included ≥5 Gy of total body irradiation (TBI) in a single fraction or ≥8 Gy of TBI in multiple fractions, oral busulfan (BU) at >8 mg/kg, intravenous BU at >6.4 mg/kg, or melphalan (Mel) at >140 mg/m$^2$ were considered FIC; all others were classified as RIC.

### *Statistical Analysis*

Descriptive statistics were used to summarize the variables related to patient demographics and transplantation characteristics. The probability of the OS time was estimated according to the Kaplan-Meier method. To evaluate the influences of confounding factors on acute graft-versus-host disease (GVHD) and survival, the log-rank test and proportional hazards modeling were used for the univariate and multivariate analyses, respectively. The Cox proportional hazard model was used for the multivariate analyses of OS in which all independent variables were incorporated in the model, followed by the use of a stepwise selection method [24]. Fine and Gray proportional hazard modeling was used to estimate the effects of the same variables used in the multivariate analysis for OS on the cumulative incidence rates of TRM and ATLL-related mortality [25,26]. In these regression models, the occurrence of GVHD was treated as a time-dependent covariate [27]. In the analysis of acute GVHD, patients were assigned to the "no acute GVHD group" at the time of transplantation and transferred to the "acute GVHD group" at the onset of the maximum grade of acute GVHD. The landmark method was used to evaluate the effects of GVHD on OS and the cumulative incidence of disease-associated and treatment-related deaths among patients who remained alive at 60 days for acute GVHD and at 100 days for chronic GVHD after transplantation. Factors associated with at least borderline significance ($P \leq .10$) in the univariate analysis were subjected to a multivariate analysis using a backward stepwise covariate selection. All $P$ values were 2-tailed, and $P$ values ≤.05 were considered statistically significant. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University), a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0) [28].

## RESULTS

### *Patient Characteristics*

The characteristics of 175 ATLL patients who received a single CBT are shown in Table 1. The median age at CBT was 55 years (range, 27 to 79 years). The cohort comprised 70 women and 105 men with the following ATLL statuses at CBT: complete remission (CR; n = 50), not in CR (n = 116), and unknown (n = 9). The conditioning regimen intensity was classified as FIC in 63 (36%) patients and RIC in 128 (62%) patients. FIC was further subdivided into 2 groups as follows: TBI (n = 47) or non-TBI (n = 15). RIC was also subdivided into 3 groups as follows: fludarabine (Flu) + Mel (n = 75), Flu + BU (n = 15), and other types (n = 15). Cyclosporine and tacrolimus were administered for prophylaxis to 90 (51%) and 77 patients (44%), respectively. Cyclosporine-based prophylaxis was subdivided into 3 groups as follows: (1) cyclosporine

**Table 1**
Patient Characteristics at Cord Blood Transplantation

| Variables | No. of Patients (n = 175) |
|---|---|
| Age at transplantation, median (range), yr | 55 (27-79) |
| Gender | |
| Male | 105 |
| Female | 70 |
| Disease status at transplantation | |
| CR | 50 |
| Not in CR | 116 |
| Unknown | 9 |
| Conditioning regimen | |
| FIC | 63 |
| RIC | 108 |
| Unknown | 4 |
| GVHD prophylaxis | |
| Cyclosporine-based | 90 |
| Tacrolimus-based | 77 |
| Unknown | 8 |
| Time from diagnosis to transplantation, d | |
| <200 | 94 |
| ≥200 | 75 |
| Unknown | 6 |
| Year of transplantation | |
| <2005 | 71 |
| ≥2005 | 104 |
| HLA matching* | |
| 0 mismatched loci | 5 |
| 1 mismatched locus | 36 |
| 2 mismatched loci | 73 |
| ≥3 mismatched loci | 42 |
| Unknown | 19 |
| ABO matching | |
| Matched | 56 |
| Minor mismatched | 49 |
| Major mismatched | 69 |
| Unknown | 1 |
| Nucleated cells infused per $10^7$/kg, median (range) | 2.58 (.36-5.34) |
| CD34-positive cells infused per $10^5$/kg, median (range) | .85 (.07-5.39) |

* Number of mismatches was counted among HLA-A, -B (low-resolution typing), and DRB1 (high-resolution typing).

alone (n = 33), (2) cyclosporine + short-term methotrexate (MTX) (n = 45), and (3) cyclosporine + mycophenolate mofetil (MMF; n = 12). Tacrolimus-based prophylaxis was subdivided into 4 groups as follows: (1) tacrolimus alone (n = 37), (2) tacrolimus + short-term MTX (n = 32), (3) tacrolimus + MMF (n = 5), (4) and tacrolimus + prednisolone (n = 3). Ninety-four patients (54%) received CBT < 200 days after diagnosis. One hundred twenty-four (71%) patients underwent CBT with 2 HLA-mismatched loci. The numbers of infused nucleated and CD34-positive cells were $2.58 \times 10^7$/kg (range, .36 to $5.34 \times 10^7$/kg) and $.85 \times 10^5$/kg (range, .07 to $5.39 \times 10^5$/kg), respectively. Engraftment evaluation was possible in 125 patients (71%) within a median interval of 19 days after CBT (range, 7 to 46 days). Among the survivors, the median follow-up duration was 22.5 months (range, 0 to 74.5 months).

### *Prognostic Factors for Survival*

The OS rates of 175 patients with ATLL who received CBT were 30.2% (95% confidence interval [CI], 23.0% to 37.4%) at 1 year and 20.6% (95% CI, 13.8% to 27.4%) at 2 years (Figure 1A). The cumulative incidence rates of ATLL-related mortality and TRM at 2 years were 31.9% (95% CI, 24.8% to 39.3%) and 46.4% (95% CI, 38.5% to 54.0%), respectively (Figure 1B). The following confounding factors affected survival: age, gender, disease status at transplantation, days from diagnosis to transplantation, date of transplantation, age at transplantation, conditioning regimen, number of infused nucleated and CD34-positive cells, ABO compatibility, HLA compatibility, GVHD prophylaxis, and the development of acute GVHD. A univariate analysis revealed that higher OS ($P < .05$) correlated with CR at transplantation, minor ABO incompatibility, the addition of other agents to calcineurin inhibitors (MTX or MMF), and the development of acute GVHD (Table 2). A multivariate analysis was performed to further examine the effects of an age <55 years, the development of acute GVHD as a time-dependent covariate coincident with CR at transplantation, minor ABO incompatibility, and the addition of other agents to calcineurin inhibitors (Table 3). Compared with the absence of GVHD, the development of acute GVHD was associated independently with higher OS (hazard ratio [HR], .10; 95% CI, .01 to 0.94; $P = .044$).

### *Effects of Acute GVHD on Survival*

To further validate the effect of acute GVHD on OS, we examined survival according to the acute GVHD grade in a landmark analysis. The median time to onset of acute GVHD of any grade after transplantation was 21 days (range, 5 to 100 days). Acute GVHD occurred in 80 patients (46%) as

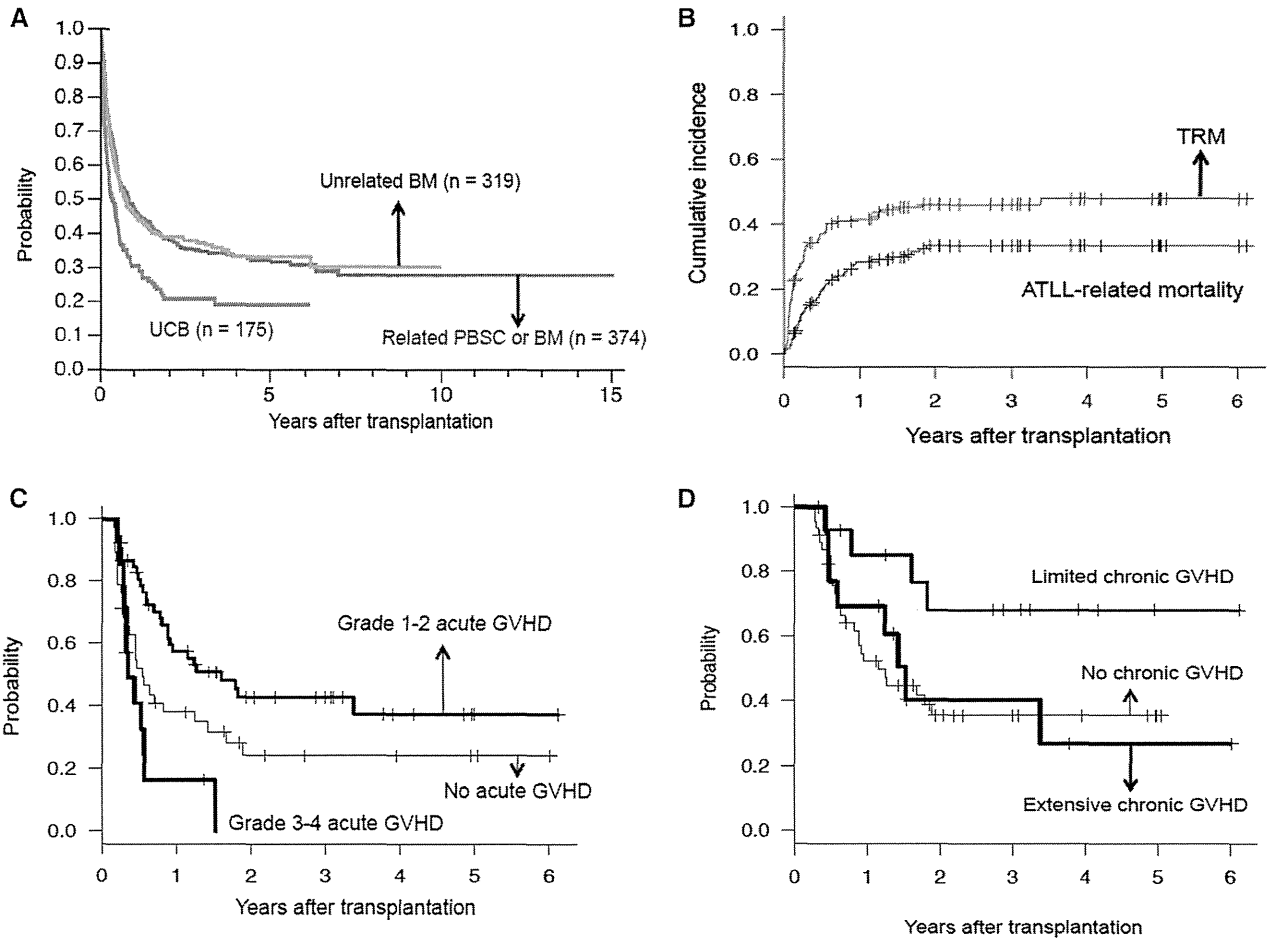


**Figure 1.** Survival, adult T cell leukemia/lymphoma (ATLL)-related mortality rates, and transplantation-related mortality (TRM) rates of patients receiving cord blood transplantation (CBT). (A) Kaplan-Meier curves of the estimated overall survival rates (OS) of ATLL patients treated with CBT. UCB, umbilical cord blood; PBSC, peripheral blood stem cells; BM, bone marrow, GVHD, graft-versus-host disease. (B) Cumulative incidence curves of ATLL-related mortality and TRM in patients treated with CBT. (C) Landmark plots of OS to determine the effects of acute GVHD. (D) Landmark plots of OS to determine the effects of chronic GVHD.

**Table 2**
Univariate Analysis of Risk Factors for Overall Survival

| Variables | | No. | OS | | |
|---|---|---|---|---|---|
| | | | Two-Year OS (%) | 95% CI | *P* Value |
| Age 1 | <60 yr | 134 | 23.0 | 15.0-31.0 | .080 |
| | ≥60 yr | 41 | 12.0 | 6.0-22.4 | |
| Age 2 | <55 yr | 85 | 25.4 | 15.0-35.8 | .100 |
| | ≥55 yr | 90 | 15.6 | 7.0-24.2 | |
| Sex | Female | 70 | 22.3 | 11.5-33.1 | .453 |
| | Male | 105 | 19.4 | 10.8-28.0 | |
| Disease status at transplantation | CR | 50 | 40.3 | 25.5-55.1 | .003 |
| | Not in CR | 116 | 14.3 | 7.1-21.7 | |
| Time from diagnosis to transplantation | <200 d | 94 | 22.4 | 12.8-32.0 | .752 |
| | ≥200 d | 75 | 19.9 | 9.7-30.1 | |
| Yr of transplantation | <2005 | 71 | 17.6 | 8.2-27.0 | .160 |
| | ≥2005 | 104 | 23.1 | 13.5-31.5 | |
| Conditioning regimen | FIC | 63 | 20.2 | 9.8-30.6 | .740 |
| | RIC | 108 | 20.2 | 11.8-28.6 | |
| Infused nucleated cell dose ($\times 10^7$/kg) | <2 | 19 | 10.8 | 0-29.3 | .290 |
| | ≥2 | 145 | 22.6 | 14.9-30.3 | |
| Infused CD34 cell dose ($\times 10^5$/kg) | <1 | 97 | 23.3 | 13.9-32.7 | .396 |
| | ≥1 | 66 | 19.1 | 8.0-30.2 | |
| ABO matching | Matched | 56 | 12.8 | 3.4-22.2 | .024 |
| | Minor mismatched | 49 | 30.5 | 15.5-45.5 | |
| | Major mismatched | 69 | 20.5 | 9.9-31.1 | |
| HLA matching | 0 mismatched | 5 | 30.0 | 0-77.4 | .525 |
| | 1 mismatched | 36 | 21.6 | 5.6-37.6 | |
| | 2 mismatched | 73 | 24.6 | 14.3-35.9 | |
| | ≥3 mismatched | 42 | 18.1 | 3.9-32.3 | |
| GVHD prophylaxis 1 | Cyclosporine-based | 90 | 21.9 | 12.5-31.4 | .710 |
| | Tacrolimus-based | 77 | 20.3 | 10.0-30.4 | |
| GVHD prophylaxis 2 (cyclosporine/tacrolimus + other drug) | No | 70 | 12.4 | 4.8-20.0 | .003 |
| | Yes | 97 | 32.7 | 21.1-44.3 | |
| Acute GVHD | No | 59 | 16.8 | 5.7-27.9 | <.0001 |
| | Yes | 80 | 29.4 | 18.2-40.6 | |

follows: grade 1, n = 23 patients; grade 2, n = 37 patients; grade 3, n = 14 patients; and grade 4, n = 6 patients. There was no significant difference in OS between patients with grades 1 and 2 GVHD ($P = 1.00$), in contrast to the difference between patients with grades 1 and 3 GVHD ($P = .013$). Moreover, based on the previous national survey analysis of the effect of acute GVHD on survival in patients with ATLL [5,15], the effect of acute GVHD on OS in the present study was evaluated using landmark plots (landmark day 60) according to the following 3 categories: (1) no acute GVHD (n = 38), (2) grade 1 to 2 acute GVHD (n = 53), and (3) grade 3 to 4 acute GVHD (n = 14). The 2-year OS rates for patients according to the acute GVHD grade were as follows: 24.2% (95% CI, 11.2% to 39.8%) without acute GVHD; 42.7% (95% CI, 28.1% to 56.6%) with grade 1 to 2 GVHD; and 0% with grade 3 to 4 GVHD (Figure 1C). These analyses demonstrated that the development of grade 1 to 2 acute GVHD was associated with higher OS compared with the absence of acute GVHD ($P = .048$), whereas the development of grade 3 to 4 acute GVHD was associated with lower OS compared with that in patients with grade 1 to 2 acute GVHD ($P = .0003$). The cumulative 2-year ATLL-related mortality rates according to the GVHD grades were as follows: 32.6% (95% CI, 19.7% to 46.1%) for grade 1 to 2 acute GVHD; 29.8% (95% CI, 8.2% to 55.6%) for grade 3 to 4 acute GVHD; and 45.9% (95% CI, 29.0% to 61.3%) for no acute GVHD. There was a trend toward a lower risk of relapse or progression in those who developed grade 1 to 2 acute GVHD relative to those without GVHD. Among patients with non-CR at transplantation, there was also a trend toward higher 2-year OS (36.7%; 95% CI, 18.7% to 54.9%) in those who developed grade 1 to 2 acute GVHD than in those without GVHD (15.6%; 95% CI, 3.4% to 35.9%). These data suggested a graft-versus-ATLL effect induced by CBT.

**Table 3**
Multivariate Analysis of Risk Factors for OS

| Variables | OS | | |
|---|---|---|---|
| | HR | 95% CI | *P* Value |
| Age, yr | | | |
| <55 | 1 | | |
| ≥55 | 1.15 | .63-2.09 | .652 |
| Disease status at transplantation | | | |
| CR | 1 | | |
| Not in CR | 1.38 | .73-2.63 | .190 |
| ABO matching | | | |
| Matched | 1 | | |
| Minor mismatched | .56 | .25-1.24 | .152 |
| Major mismatched | .77 | .39-1.48 | .337 |
| GVHD prophylaxis (cyclosporine/ tacrolimus + other drug) | | | |
| No | 1 | | |
| Yes | .76 | .42-1.38 | .365 |
| Acute GVHD (time-dependent covariate) | | | |
| No | 1 | | |
| Yes | .10 | .01-.94 | .044 |

### *Effects of Chronic GVHD on Survival*

Chronic GVHD was evaluated in 74 patients who survived for at least 100 days after transplantation. Chronic GVHD occurred in 28 patients (37%) with a median time to onset of 115 days (range, 73 to 1287 days) after CBT. The effect of chronic GVHD on OS was evaluated using landmark plots (landmark day 100), and the 2-year OS results were as follows: no chronic GVHD (n = 46), 35.6% (95% CI, 21.0% to 50.0%); limited chronic GVHD (n = 15), 68.1% (95% CI, 35.4%

to 86.8%); and extensive chronic GVHD (n = 13), 40.4% (95% CI, 13.4% to 66.4%) (Figure 1D). There was a trend toward a higher OS among patients with limited chronic GVHD, but there were no significant differences relative to patients without chronic GVHD ($P = .10$) and those with extensive chronic GVHD ($P = .12$).

### *Cause of Death*

At the last follow-up, 46 patients remained alive and 129 were deceased. The median follow-up time among the survivors was 22.5 months (range, 0 to 74.5 months). Disease progression (n = 52) was the leading cause of death. Infection was the cause of death in 40 patients (31%; bacterial, n = 27 patients; fungal, n = 3; viral, n = 8; and others, n = 2). Viral infection-related deaths were caused by the following pathogens: cytomegalovirus, n = 3; adenovirus, n = 2; human herpesvirus-6, n = 2; and varicella-zoster virus, n = 1. Among the 27 patients who succumbed to bacterial infection, 16 died before engraftment at a median of 17 days after CBT (range, 7 to 38 days). Among the 20 patients who developed severe acute grade 3 to 4 GVHD, 2 remain alive without disease progression. However, 9 of the 20 patients died of GVHD, 5 of disease progression, and 4 of infection.

The Fine and Gray proportional hazards model was applied to identify the variables affecting ATLL-related mortality and TRM. The pretransplantation variables included age, gender, disease status at CBT, days from diagnosis to transplantation, age at transplantation, conditioning regimen, number of infused nucleated cells, ABO compatibility, HLA compatibility, and GVHD prophylaxis. The following pretransplantation factors associated with a higher risk of ATLL-related mortality were identified in a multivariate analysis: not in CR at CBT (HR, 3.37; 95% CI, 1.12 to 10.2; $P = .032$) and an age > 55 years at CBT (HR, 2.32; 95% CI, .98 to 5.48; $P = .054$). The following pretransplantation factors were associated with a marginally higher risk of TRM: lower number of infused nucleated cells ($\geq 2 \times 10^7$/kg versus $< 2 \times 10^7$/kg; HR, .56; 95% CI, .30 to 1.02; $P = .059$) and GVHD prophylaxis with a calcineurin inhibitor alone (additional agents plus calcineurin inhibitors versus calcineurin inhibitors alone; HR, .60; 95% CI, .34 to 1.07; $P = .064$).

## DISCUSSION

We present here the results of the largest retrospective study of ATLL patients receiving CBT; these results have extended our knowledge relative to that gained from other studies, which were limited by the numbers of cases [15,20,21]. Because graft source selection is strongly influenced by the donor availability, it is difficult to directly compare the outcomes of CBT with those of other allo-HSCT modalities. Nevertheless, the outcome of CBT for ATLL in the previous nationwide survey, with a 3-year OS rate of 17%, was clearly unsatisfactory because the study period corresponded with the developmental phase of CBT in adult patients [15]. Recent improvements in the outcome of CBT have been expected after optimization of the number of cells used for CBT and the improved HLA-compatibility of cord blood units [29-31]. Consequently, a recent nationwide survey data of adults with acute non-ATLL leukemia revealed no differences in the outcome of CBT in comparison with those of other allo-HSCT modalities [18,19]. However, the updated data (through December 2009) indicated that CBT for ATLL remained associated with a poorer 3-year OS of 20.6%, compared with OS of 34.4% among the 374 patients who received related BM or PBSC and 37.1% among the 319 patients who received unrelated BM ($P < .0001$) (Figure 1A). Therefore, the aim of the present study focused on the feasibility of CBT in the context of a larger cohort of patients with ATLL.

In the present study, 2 important findings were identified regarding CBT for ATLL. First, CBT cured patients with ATLL partly through a graft-versus-ATLL effect. Second, the high rate of TRM (approximately 50%) remains a significant problem. The OS curve for ATLL patients who received CBT reached a plateau by 3 years, suggesting long-term survival of selected patients, although the outcome of CBT for ATLL (3-year OS, 20%) did not compare favorably with those of other allo-HSCT modalities. Regarding the prognostic factors affecting survival, our present univariate analysis identified the 5 following significant variables associated with higher OS: (1) age, (2) disease status at transplantation, (3) ABO compatibility, (4) addition of agents such as MTX or MMF to calcineurin inhibitors for GVHD prophylaxis, and (5) development of acute GVHD. Further, the multivariate analysis revealed that the development of acute GVHD was independently associated with better OS relative to the absence of acute GVHD. A landmark analysis showed that the development of grade 1 to 2, or so called mild-to-moderate acute GVHD, was associated with better OS when compared with the absence of acute GVHD. There was also a trend toward a lower risk of relapse or progression with the development of acute GVHD when compared with the absence of GVHD and better OS in patients with limited chronic GVHD. Taken together, these data suggest the presence of a curative graft-versus-ATLL effect conferred by CBT.

However, it is typically difficult for physicians to optimize the effects of acute GVHD to prevent disease progression via graft-versus-ATLL. Therefore, a more realistic attempt would be the control of pretransplantation factors that might affect the CBT outcome and, thus, enhance the benefit of allo-HSCT. The multivariate analysis performed herein with respect to ATLL-related deaths identified disease status at CBT as the most important factor. ATLL usually resists conventional chemotherapy and must be treated soon after diagnosis because of the rapid proliferation of tumor cells, which generates a high tumor burden [2,3]. In the future, novel agents, such as mogamulizumab, a humanized anti-CCR4 monoclonal antibody, might improve CBT-associated survival by decreasing the tumor burden before transplantation [32-35]. Another possibility for improving survival might be reducing the time from diagnosis to transplantation while patients with ATLL remain chemosensitive. Moreover, CBT provides a considerable advantage for patients who require urgent allo-HSCT to combat aggressive ATLL.

In the present study, we have shown that CBT is feasible and curative. However, the high rate of TRM remained a significant problem. Bacterial infection caused the highest incidence of death (21%) during the neutropenic period. The infusion of lower numbers of nucleated cells ($< 2 \times 10^7$/kg), which is usually associated with delayed engraftment, was marginally associated with TRM. Neutrophil recovery is slower in patients treated via CBT, and immunosuppressed patients with ATLL might be at an increased risk of developing more frequent opportunistic infections [36]. Improved supportive care to prevent bacterial infection is required after CBT for patients experiencing a prolonged neutropenic period. The ongoing development of better graft engineering [37] or double-CBT [38] might facilitate rapid neutrophil recovery and, thus, help to reduce the TRM rate in CB recipients.

The present study has several limitations. First, our results concerning the effect of chronic GVHD on survival should be interpreted with caution because the relatively small number of patients who developed chronic GVHD did not allow us to evaluate the effect of this condition on survival in a multivariate analysis. Instead, we were limited to performing a landmark analysis of OS according to the severity of chronic GVHD. Certainly, we detected a trend toward higher OS in patients with limited chronic GVHD when compared with patients without chronic GVHD, suggesting the possible presence of a graft-versus-ATLL effect. However, these results might be biased because of insufficient statistical power. Our future studies will assess the effect of chronic GVHD on the outcome of CBT for the treatment of ATLL after a long-term follow-up. Although the present study employed, to our knowledge, the largest cohort of CBT-treated patients to date and our results demonstrated that CBT is a feasible and effective treatment, this was a retrospective analysis. Therefore, this finding requires confirmation in prospective studies. To establish reliable criteria for CBT administration, a prospective multicenter clinical trial is underway in Japan to evaluate the safety and efficacy of CBT combined with Flu, Mel, and low-dose TBI (4 Gy) along with GVHD prophylaxis (tacrolimus and MMF [39]).

In conclusion, CBT is feasible and effective for patients with ATLL and acts via a graft-versus-ATLL effect. However, the outcome of CBT is unsatisfactory when compared with those of other allo-HSCT modalities. The high rate of TRM must be reduced, and the development of novel strategies is required to further improve the outcome of CBT.

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ORIGINAL ARTICLE

# Evaluating the 21-gene assay Recurrence Score® as a predictor of clinical response to 24 weeks of neoadjuvant exemestane in estrogen receptor-positive breast cancer

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**Abstract**
*Background* The aim of this study was to investigate the association between the results of the Recurrence Score (RS) assay and the clinical response to neoadjuvant endocrine therapy in postmenopausal women with breast cancer.
*Methods* Core biopsy samples at baseline and post-treatment surgical samples were obtained from 80 and 77 of 116 patients, respectively, enrolled in the multicenter prospective study of neoadjuvant exemestane therapy (JFMC34-0601). The 21-gene assay was performed after appropriate manual microdissection. The estrogen receptor (ER), progesterone receptor, HER2 and Ki-67 were assayed by immunohistochemistry at a central laboratory. Clinical response was assessed based on the RECIST (Response Evaluation Criteria In Solid Tumors) guideline.
*Results* Sixty-four core biopsy samples and 52 resection samples met the RS quality requirements. The clinical response rate in those patients with a low RS result (low RS group; 19/32, 59.4 %) was significantly higher than that in those patients with a high RS result (high RS group; 3/15, 20.0 %) ($P = 0.015$) and similar to that in patients with an intermediate RS result (intermediate RS group; 10/17, 58.8 %). The rates of breast-conserving surgery (BCS) were 90.6 % (29/32) in the low RS group, 76.5 % (13/17) in the intermediate RS group and 46.7 % (7/15) in the high RS group. The odds ratio for BCS adjusted for continuous

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baseline Ki-67 was 0.114 [95 % confidence interval (CI) 0.014–0.721; $P = 0.028$] between the high and low RS groups. RS values in pre-treatment samples were highly correlated with those in post-treatment samples (Spearman correlation coefficient 0.745, 95 % CI 0.592–0.846).
*Conclusion* Our results demonstrate the predictive value of the RS for clinical response to neoadjuvant exemestane therapy in postmenopausal women with ER-positive breast cancer.

**Keywords** Recurrence Score · Neoadjuvant endocrine therapy · Ki-67 · Clinical response · Breast-conserving surgery rate

## Introduction

There are several potential advantages to neoadjuvant therapy of breast cancer in terms of improving outcomes in women with operable and inoperable early-stage disease [1, 2]. Both neoadjuvant chemotherapy and endocrine therapy have been shown to enable less extensive resection and improve rates of breast-conserving surgery (BCS) [3–6]. The ACOSOG Z1031 trial, which compared three aromatase inhibitors (AIs) in neoadjuvant settings, showed that 51 % (81/159) of the patients who were designated candidates for mastectomy experienced downstaging to BCS [7]. Neoadjuvant endocrine therapy is now an acceptable option for postmenopausal patients with endocrine-responsive disease [8].

Despite the use of standard biomarkers, the considerable heterogeneity of response to therapy still represents a challenge to clinicians in terms of choosing the most suitable neoadjuvant therapy. As such, tools to improve the identification of those patients who will respond to therapy would represent a major clinical advance. Although the Ki-67 labeling index (LI) shows some consistency in predicting response to chemotherapy, its ability to predict response to neoadjuvant endocrine therapy is controversial [9, 10].

We previously reported results from a neoadjuvant exemestane study in postmenopausal women [11]. In that study, the target response rate was 51 % (59/116), and 40 (77 %) of 59 patients who would have required mastectomy were converted to BCS. Neither baseline Ki-67 LI nor changes in Ki-67 LI were associated with clinical response in the study.

The Onco*type* DX® assay (Genomic Health, Redwood City, CA) has been shown to be able assess recurrence risk in women with hormone receptor-positive (HR+), lymph node-negative or -positive, early stage breast cancer who are treated with adjuvant endocrine therapy [12–15]. It has also been shown to predict the likelihood of benefit from adjuvant chemotherapy [12, 16]. Accordingly, the assay is included in clinical guidelines for use in patients with HR+ lymph node-negative disease; however, its applicability to HR+ postmenopausal women with lymph node positive disease is considered controversial, pending results of the RxPONDER trial [8, 17–19]. Additionally, studies in the neoadjuvant setting have shown that the test can be used to predict the response to chemotherapy [20, 21]. More recently, a study suggested that the Recurrence Score (RS) value may predict responses to neoadjuvant endocrine therapy with either tamoxifen or anastrozole [22]. The Onco*type* DX assay may improve the clinician's ability to discriminate between clinically similar tumors based on the tumor's underlying biology. Consequently, the aim of this study was to investigate the clinical usefulness of the RS assay results in the prediction of response to neoadjuvant endocrine therapy.

## Methods

### Study design

This was a prospectively designed study using archived tumor tissues from the previously conducted JFMC34-0601 study. The primary objective was to assess the association between the results of the RS assay at baseline and clinical response, by comparing the response rates between patients with a low RS result (<18; low RS group) and those with a high RS result (≥31; high RS group). Secondary objectives included assessment of the associations of continuous baseline RS, quantitative estrogen receptor (ER) by reverse transcriptase (RT)-PCR and Ki-67 with clinical response and with BCS, as well as associations of changes from baseline to post-treatment values of these markers with clinical response. The study protocol was approved by the Ethics Committee of each participating institution. Informed consent was obtained from all patients. The study was performed in accordance with the Helsinki Declaration.

### Patient cohort and tumor samples

Eligibility criteria for the parent JFMC34-0601 study included age 55–75 years, ER+ and stage II or IIIa invasive breast cancer (T2-3, N0-2, M0). Patients were confirmed positive for ER or progesterone receptor (PgR) by immunohistochemistry (≥10 % nuclear staining). The study treatment was 25 mg/day exemestane for 16 weeks, with a possible 8-week extension based on the assessment of clinical response. Patients with progressive disease (PD) were withdrawn from the study. At week 24, patients underwent surgery, except those with PD, who had the option of selecting another treatment approach.

### Clinical outcomes measures

Clinical response was assessed by comparing the longest diameter of the target lesions with the baseline measurement, based on the Response Evaluation Criteria in Solid Tumors (RECIST) guideline version 1.0, by caliper measurement of palpable lesions and ultrasound as previously described [11]. Briefly, complete response (CR) was defined by the disappearance of all target lesions; partial response (PR) by at least a 30 % decrease in the sum of diameters of the target lesions; PD by at least a 20 % increase in the sum of diameters of the target lesions; stable disease (SD) by neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD.

### Biomarker assessments

The Onco*type* DX® 21-gene assay was performed on core biopsy and resection samples by Genomic Health [14].

Immunohistochemistry assays of Ki-67, ER and PgR were performed at one central location and the results assessed by three independent pathologists as described previously [11]. In brief, immunohistochemistry staining was performed using a Histofine kit (Nichirei, Tokyo, Japan). Ki-67 was stained using the following antibody dilution: 1:100 (Dako, Glostrup, Denmark), and the Ki-67 LI was obtained by counting 500–1,000 tumor cells at the sites of hot spots. Ki-67 groups were defined post hoc as <10, 10–30 and >30 %, respectively. ER and PgR immunoreactivity were scored according to Allred's procedure.

Expression of HER2 was determined by the HercepTest (Dako, Glostrup, Denmark). Positive HER2 status was defined as either 3+ or 2+ with confirmed c-erbB2 gene amplification by the fluorescence in situ hybridization (FISH) test.

### Statistical analyses

Analyses of baseline markers included all patients with an evaluable RT-PCR result from core biopsies. Analyses of changes from baseline to post-treatment markers included the subset of patients with results from both core biopsies and surgical resections. Changes in continuous markers were defined as "post-treatment value–pre-treatment value". In the primary analysis, the rates of clinical response were compared between the high and low baseline RS groups using Fisher's exact test. Logistic regression models were fit to both clinical response and surgery type. Odds ratio (OR) estimates are presented with Wald $p$ values and 95 % confidence intervals (CIs). All $P$ values are two-sided. In exploratory analyses, the Spearman rank correlation coefficient (and associated 95 % CI) was calculated for the baseline continuous RS and either the post-treatment RS or baseline continuous Ki-67 as determined by immunohistochemistry. A paired $t$ test was applied to compare the baseline and post-treatment RS values. A two-sample $t$ test was used to compare the percentage reduction in tumor size between the high and low RS groups. Fisher's exact test was used to compare the conversion rate from mastectomy to BCS among risk groups.

## Results

A total of 116 patients were enrolled in JFMC34-0601 between March 2006 and December 2007, of whom 102 completed 24 weeks of neoadjuvant exemestane treatment [11]. Core biopsy and resection samples were obtained for 80 (69 %) and 77 (66 %) patients, respectively. Of the 157 samples sent for Onco*type* DX testing, two were deemed ineligible based on the blinded Genomic Health pathology review, insufficient RNA (<375 ng) was extracted from 18 samples (15 core biopsy and 3 resection samples), and standard quality metrics were not met for eight samples (all resections). This left 64 core biopsy samples, of which 52 had matching resection samples with evaluable RT-PCR results.

Baseline characteristics and clinical outcomes for the 64 patients are shown in Table 1. Forty-nine (76.6 %) patients had BCS, and 32 patients (50 %) had been candidates for BCS before the treatment. Four patients refused surgery after exemestane therapy and are treated as not BCS patients.

In the primary analysis, the clinical response rate in the low RS group (19/32, 59.4 %) was significantly higher than that in the high RS group (3/15, 20.0 %) ($P = 0.015$) (Table 2). The clinical response rate in the intermediate risk group (10/17, 58.8 %) was similar to that in the low risk group. Logistic regression revealed that the OR for clinical response between the intermediate and low RS groups was 0.977 (95 % CI 0.296–3.233, $P = 0.970$) and that the OR between the high and low RS groups was 0.171 (95 % CI 0.040–0.728, $P = 0.017$). In an exploratory analysis, the percentage reduction in tumor size determined by ultrasound was compared between the low and high RS groups. Patients in the low RS group showed an average reduction in tumor size of 31.8 % while those in the high RS group showed an average reduction of 12.5 %; this difference was significant between the groups ($P = 0.045$). The average reduction (27.6 %) in patients in the intermediate risk group was similar to that in the low risk group.

When treated as a continuous variable, the baseline RS Score was significantly associated with clinical response in a logistic regression analysis ($P = 0.042$; Table 3). There

**Table 1** Baseline patient characteristics and clinical outcomes ($n = 64$)

| Feature | *n* (%) |
|---|---|
| Age (years) | |
| 55–64 | 34 (53.1) |
| 65–74 | 25 (39.1) |
| 75–77 | 5 (7.8) |
| Tumor stage at baseline | |
| T2 | 62 (96.9) |
| T3 | 2 (3.1) |
| Stage | |
| IIA | 47 (73.4) |
| IIB | 15 (23.4) |
| IIIA | 2 (3.1) |
| ER by IHC (Allred score) | |
| 4 | 1 (1.6) |
| 5 | 3 (4.7) |
| 6 | 5 (7.8) |
| 7 | 14 (21.9) |
| 8 | 41 (64.1) |
| ER status by RT-PCR | |
| ER− ($\leq 6.5 C_T$) | 1 (1.5) |
| ER+ ($>6.5 C_T$) | 63 (98.4) |
| PgR by IHC (Allred score) | |
| 0 | 4 (6.25) |
| 4 | 7 (10.94) |
| 5 | 4 (6.25) |
| 6 | 8 (12.5) |
| 8 | 12 (18.75) |
| NE | 10 (15.63) |
| PgR status by RT-PCR | |
| PgR− ($\leq 5.5$ $C_T$) | 14 (21.9) |
| PgR+ ($>5.5$ $C_T$) | 50 (78.1) |
| HER2 by IHC/FISH | |
| Negative | 50 (78.1) |
| Positive | 2 (3.1) |
| Unknown | 12 (18.8) |
| RS risk group | |
| Low (<18) | 32 (50.0) |
| Intermediate (18–30) | 17 (26.6) |
| High (≥31) | 15 (23.4) |
| Ki-67 by IHC (%) | |
| <10 | 28 (43.8) |
| 10–30 | 23 (35.9) |
| >30 | 13 (20.3) |
| Clinical response | |
| Complete response (CR) | 0 |
| Partial response (PR) | 32 (50.0) |
| Stable disease (SD) | 24 (37.5) |
| Progressive disease (PD) | 5 (7.8) |
| NE | 3 (4.7) |
| Surgery type | |
| Breast-conserving | 49 (76.6) |
| Mastectomy | 11 (17.2) |
| No surgery | 4 (6.3) |

*ER* estrogen receptor, *IHC* immunohistochemistry, *RT* reverse transcriptase, *PgR* progesterone receptor, *NE* not evaluable, *FISH* fluorescence in situ hybridization, $C_T$ cycling threshold score, *RS* recurrence Score

was a trend between continuous baseline ER as determined by RT-PCR and clinical response ($P = 0.076$). Continuous baseline Ki-67 by IHC was not associated with clinical response ($P = 0.273$).

The associations between changes from baseline to post-treatment values of continuous markers and clinical response were examined in logistic regression analyses. Changes in the RS, ER as determined by RT-PCR, and Ki-67 as determined by IHC were not associated with clinical response ($P = 0.240$, 0.343 and 0.629, respectively).

Analysis of the RS categories and BCS is shown in Table 2. The OR for BCS between the intermediate and low RS groups was 0.336 (95 % CI 0.066–1.722, $P = 0.19$) and that between the high and low RS groups was 0.091 (95 % CI 0.019–0.432, $P = 0.003$). The logistic regression analyses of continuous baseline RS, ER by RT-PCR and Ki-67 by IHC with BCS are shown in Table 3. The continuous baseline RS was significantly associated with BCS in both the unadjusted ($p = 0.001$) and covariate-adjusted (for tumor size and PgR) ($P = 0.004$) analyses. The continuous baseline ER by RT-PCR was also significantly associated with BCS in both the unadjusted ($P = 0.001$) and covariate-adjusted ($P = 0.023$) analyses. Continuous baseline Ki-67 by IHC was significantly associated with BCS in the unadjusted analysis ($P = 0.024$) but lost its significance when adjusted for tumor size and PgR ($P = 0.060$). When both the continuous RS values and continuous Ki-67 were included in the logistic regression model for BCS, the RS retained its statistical significance ($P = 0.012$) whereas Ki-67 did not ($P = 0.868$). The conversion rate from mastectomy planned at baseline to BCS performed after the treatment was 88 % (15/17) in the low RS group, 70 % (7/10) in the intermediate RS group and 20 % (1/5) in the high RS group. The rate was significantly different among groups ($P = 0.010$).

The associations between RS and Ki-67, and their respective and joint associations with BCS were examined in exploratory analyses. Figure 1a shows a scatterplot of baseline Ki-67 as determined by IHC versus the baseline RS results. The Spearman correlation coefficient was 0.672 (95 % CI 0.506–0.785). All patients with PD had a high RS

**Table 2** Clinical response and breast-conserving surgery according to categorical baseline Recurrence Score

| RS risk group | Clinical response | | |
|---|---|---|---|
| | Proportion (response rate)[a] (%) | Odds ratio (95 % CI) | *P* value |
| Low (RS <18) | 19/32 (59.4) | 1 | n/a |
| Intermediate (RS 18–30) | 10/17 (58.8) | 0.977 (0.296, 3.233) | 0.970 |
| High (RS ≥31) | 3/15 (20.0) | 0.171 (0.040, 0.728) | 0.017 |
| RS risk group | Breast-conserving surgery | | |
| | Proportion (BCS rate) (%) | Odds ratio (95 % CI) | *P* value |
| Low (RS <18) | 29/32 (90.6) | 1 | n/a |
| Intermediate (RS 18–30) | 13/17 (76.5) | 0.336 (0.066, 1.722) | 0.19 |
| High (RS ≥31) | 7/15 (46.7) | 0.091 (0.019, 0.432) | 0.003 |

Data are presented as the number of patients with the percentage in parenthesis

*CI* confidence interval, *BCS* breast-conserving surgery, *n/a* not available

[a] Primary analysis: $P = 0.015$ by Fisher's exact test for comparison of clinical response rates between the low and high RS groups

**Table 3** Continuous baseline Recurrence Score and estrogen receptor by reverse transcriptase-PCR and Ki-67 by immunohistochemistry and clinical response and breast-conserving surgery

| Endpoint/analysis | Continuous marker | | | | | |
|---|---|---|---|---|---|---|
| | RS (50 units) | | ER by RT-PCR (log2 increase) | | Ki-67 by IHC (%) | |
| | Odds ratio (95 % CI) | *P* value | Odds ratio (95 % CI) | *P* value | Odds ratio (95 % CI) | *P* value |
| Clinical response/unadjusted | 0.205 (0.044, 0.946) | 0.042 | 1.436 (0.963, 2.141) | 0.076 | 0.981 (0.948, 1.015) | 0.273 |
| BCS/unadjusted | 0.055 (0.009, 0.323) | 0.001 | 1.786 (1.150, 2.774) | 0.001 | 0.957 (0.921, 0.994) | 0.024 |
| BCS/covariate-adjusted[a] | 0.016 (<0.001, 0.259) | 0.004 | 1.881 (1.090, 3.245) | 0.023 | 0.953 (0.907, 1.002) | 0.060 |

*RT* reverse transcriptase

[a] Adjusted for tumor size and PgR Allred score, which were significantly associated with BCS in the univariable analyses

(range 32–73) while three of five PD patients had an intermediate Ki-67 LI (Fig. 1a).

No statistically significant difference was observed between baseline and post-treatment RS values ($P = 0.484$). A scatterplot is shown in Fig. 1b. The Spearman correlation analysis showed a high correlation (correlation coefficient 0.745, 95 % CI 0.592–0.846).

## Discussion

In this study, we demonstrated the predictive value of the RS results for response to neoadjuvant endocrine therapy. Among our patient cohort, those with low scores showed a better response to neoadjuvant endocrine therapy than those with high scores. Since patients with high RS results have been shown to benefit from chemotherapy, the 21-gene assay may provide additional information that could facilitate the selection of neoadjuvant treatment with endocrine therapy for cancer patients with a low RS and chemotherapy for those with a high RS.

ER Allred scores have been reported to correlate with response rates to neoadjuvant letrozole or tamoxifen. The P024 trial of neoadjuvant letrozole or tamoxifen showed that tumors with low ER Allred scores still responded to letrozole [23]. Conversely, some tumors with higher ER levels did not respond to endocrine therapy [23, 24]. Gene expression-based profiles categorize HR+, HER2− breast cancers into two subtypes: luminal-A and -B [25]. However, the classification, which is based on PAM50, has been reported not to relate to clinical response or the likelihood of BCS after neoadjuvant AI treatment [7].

In our study, the RS was the only predictive factor for clinical responses to neoadjuvant endocrine therapy and the most potent predictive factor for BCS in the covariate-adjusted analysis. These results are consistent with those from other studies which suggest that a low RS can predict benefit from endocrine therapy [22, 24]. The study by Kim et al. [24] compared the outcomes of the tamoxifen and

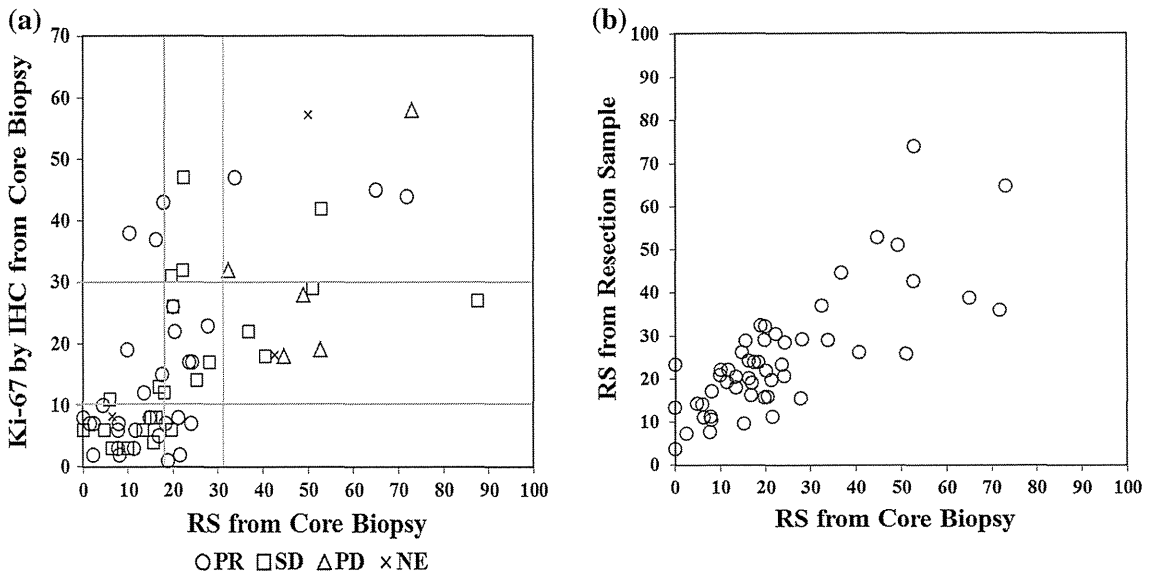


**Fig. 1** **a** Scatterplot of the baseline Recurrence score (*RS*) and baseline Ki-67, with the Spearman correlation coefficient. The Spearman correlation coefficient between the baseline RS and baseline Ki-67 was 0.672 [95 % confidence interval (CI) 0.506–0.785]. None of five patients with tumor progression was in the low or intermediate RS groups. **b** Scatterplot of the baseline RS and post-treatment RS, with the Spearman correlation coefficient. The baseline RS was highly correlated with RS in the post-treatment samples (Spearman correlation coefficient 0.745, 95 % CI 0.592–0.846). *PR* Partial response, *SD* stable disease, *PD* progressive disease, *NE* Not evaluable

placebo arms of the NSABP B14 trial and demonstrated that higher levels of quantitative ER expression, as determined by RT-PCR, correlated with a greater benefit from adjuvant tamoxifen, as measured by distant recurrence.

Our results indicate that the values of the RS before and after endocrine therapy were highly correlated. Since a number of studies have suggested that post-treatment biomarkers such as Ki-67 LI and ER have better prognostic values than pre-treatment biomarkers, post-treatment biomarkers are receiving increasing interest in clinical trials as a tool for patient stratification [26–28]. Dowsett et al. [26] reported the results of an unplanned, exploratory investigation of the relationship between post-treatment Ki-67 (2 weeks) and recurrence-free survival (RFS) using archived tumors from the IMPACT study. Their results indicate that post-treatment Ki-67, larger baseline tumor size and post-treatment ER level are significantly correlated with DFS. Ellis et al. [27] analyzed the ability of post-treatment Ki-67 and other factors (tumor size, grade, nodal status, and post-treatment ER expression) to predict RFS and breast cancer-specific survival using archived tumors from the P024 study. Another interesting study (ACOSOG Z1031, Cohort B) has been conducted to determine whether patients with a high Ki-67 value after 2 weeks of neoadjuvant AI treatment show a higher than expected pathogenic CR rate to neoadjuvant chemotherapy than would be typically observed for those patients with unselected ER-rich tumors. The results will tell us whether an assessment of Ki-67 2 weeks after neoadjuvant AI treatment will be useful for the identification of a chemotherapy-sensitive subgroup of ER+ tumors. However, even if this is the case, intervention of a 2-week AI treatment and re-biopsy are necessary. Although further investigations are needed, the comparative stability of the RS would improve the overall decision-making process regarding the complete treatment before the initiation of treatment.

The main limitation of this was its small sample size. The availability of tumor samples from the parent study was limited and recovery of mRNA was not uniformly adequate. Further investigation in larger prospective studies would better define candidates for neoadjuvant endocrine therapy. Another limitation was the absence of any assessment of lymph node response. Although nodal response is clinically relevant, one of the major purposes of neoadjuvant endocrine therapy is improvement in surgical outcome. That said, however, the clinical response at the primary site and the BCS rate are also of clinical importance for the assessment of the effect of neoadjuvant endocrine therapy.

In conclusion, this study showed that RS results have predictive value for the clinical response to neoadjuvant exemestane therapy. The 21-gene assay would appear to be a promising tool for providing useful information to guide the clinician in choosing neoadjuvant treatment for systemic therapy, with neoadjuvant endocrine treatment for patients with low RS disease and neoadjuvant chemotherapy treatment for patients with high RS disease.