2-year OS. PFS and OS were assessed using the Kaplan–Meier method, and the groups were compared using the log-rank test. A multivariate Cox regression analysis was performed to assess the effects of treatment and the various baseline prognostic factors on PFS and OS. The heterogeneity of treatment effect on the survival outcomes was also examined across the different risk groups based on the R-IPI. The patients with B cell lymphoma were analysed according to pathological diagnosis; therefore, the variables for patients with DLBCL and those with FL were also assessed separately. The analysis is based on follow-up until January 2007. The prognostic variables were compared between the groups using the Mann–Whitney *U*-test for continuous variables and the chi-squared test for categorical variables. All *P* values are two-tailed. Statistical analysis was performed using STATA 8.1 (StataCorp. LP, College Station, TX, USA) and Review Manager (REVMAN; version 5.0. Copenhagen Denmark: The Nordic Cochrane Centre, The Cochrane Collaboration, 2008). *P* values < 0·05 were considered significant.

Results

All B cell lymphoma patients

A total of 1229 patients with newly diagnosed mature B cell lymphoma were enrolled in the study. Of these, 1126 patients (91·6%) received systemic chemotherapies. Patients given rituximab alone for induction were also included. Patients who received systemic therapies were the subject of this analysis, so that patients given radiation alone or eradication of *Helicobacter pylori* alone for induction were excluded. The pathological classifications are listed in Table I. The breakdown of the pathological classification was significantly different between the groups with and without rituximab for induction therapy (Table I). The ratio of patients with FL was higher in the rituximab group. This was caused by the different approval dates of rituximab for indolent B cell lymphoma and aggressive B cell lymphoma. Therefore, direct comparison of the clinical outcomes between these two groups was not considered appropriate, and the analyses were performed separately for each pathological group. Overall, 762 (67·7%) of these patients were diagnosed as having DLBCL, and 215 (19·1%) were diagnosed with FL. Thus, 86·8% (977/1126) of the patients were classified as having DLBCL or FL, so that these two diseases represented the majority of mature B cell lymphoma.

Table I. Pathological subtype of patients (n = 1126).

Histology at diagnosis	Rituximab group (n = 348)	Non-rituximab group (n = 778)	Total (n = 1126) %
DLBCL	184	578	762 (67·7)
Burkitt lymphoma	1	17	18 (1·6)
Follicular lymphoma	111	104	215 (19·1)
Small lymphocytic lymphoma	1	9	10 (0·9)
Lymphoplasmacytic lymphoma	5	8	13 (1·2)
Splenic marginal zone lymhoma	0	3	3 (0·3)
MALT-lymphoma	14	20	34 (3·0)
Nodal marginal zone B cell lymphoma	9	0	9 (0·8)
Mantle cell lymphoma	18	26	44 (3·9)
Others	5	13	18 (0·7)

DLBCL, diffuse large B-cell lymphoma; MALT-lymphoma, extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue.

DLBCL

A total of 762 DLBCL patients were enrolled. Of these, 184 patients received rituximab as part of the first-line treatment in combination with chemotherapy (rituximab group), and 578 patients were treated by chemotherapy alone (non-rituximab group). This difference in patient number was caused by the date of rituximab approval (September 2003 for aggressive B cell lymphoma) and the time period of the study (from January 2000 to December 2004). After approval, almost all DLBCL patients were treated with rituximab, but rituximab was available for only 1 year and 4 months of the 5-year study period. The patients' characteristics are listed in Table II. The ratio of cases receiving anthracyclin containing regimens in each group was not significantly different (rituximab group, 183/184; non-rituximab group, 560/578; P = 0·057). The prognostic variables (IPI and IPI subgroup) were not different between the rituximab group and the non-rituximab group (Table II). The median follow-up time for living patients was 22 months for the non-rituximab group (range, 1–50 months) and 22 months for the rituximab group (range, 1–84 months). PFS was markedly improved in the rituximab group compared with the non-rituximab group [hazard ratio (HR), 0·58; 95% confidence interval (CI), 0·44–0·77; P < 0·001, Fig 1]. The 2-year estimated PFS was 64·4% (95% CI, 56·41–71·3%) in the rituximab group and 48·7% (95% CI, 44·4–52·9%) in the non-rituximab group. OS was also improved in the rituximab group compared with the non-rituximab group (HR, 0·52; 95% CI, 0·37–0·73; P < 0·001, Fig 1). The 2-year estimated OS was 78·0% (95% CI, 70·5–83·7%) in the rituximab group and 61·7% (95% CI, 57·42–65·7%) in the non-rituximab group. Looking only at the patients who received an anthracyclin-containing regimen (CHOP or a CHOP-like regimen), the PFS and OS were compared between the rituximab group and the non-rituximab group in each R-IPI risk group. R-IPI is the revised prognostic model for DLBCL in patients receiving R-CHOP; it identifies three distinct prognostic groups (very good, good and poor). Among DLBCL patients receiving an anthracyclin-containing regimen, the ratio of these risk groups in the rituximab group and the non-rituximab group was not significantly different (Table II). For the R-IPI very good risk

Table II. Characteristics of DLBCL patients (n = 762).

Characteristic	Rituximab group (n = 184)	Non-rituximab group (n = 578)	P
Age (years), median (range)	67 (20–96)	68 (16–95)	0·947*
Gender male/female	100/84	300/278	0·563†
PS at diagnosis			
0	58	182	0·309*
1	74	195	
2	26	100	
3	22	75	
4	4	26	
LDH > normal	101	346	0·233†
Extranodal site > 1	42	130	0·925†
Clinical stage			
I	30	92	0·797*
II	60	176	
III	32	118	
IV	62	192	
IPI			
L	66	174	0·141*
LI	41	138	
HI	37	115	
H	40	151	
Receiving anthracyclin-containing regimen	183	560	0·057†
R-IPI			
Very good	26	60	0·251*
Good	80	244	
Poor	77	256	

PS, ECOG performance status; LDH, lactate dehydrogenase; IPI, International Prognostic Index (L, low; LI, low-intermediate; HI, high-intermediate; H, high); R-IPI, Revised International Prognostic Index.
*Mann–Whitney U-test.
†Chi-squared test.

group, the PFS and OS of the rituximab group were not statistically different from those of the non-rituximab group (HR, 1·38; 95% CI, 0·40–4·72; P = 0·61, HR, 1·89; 95% CI, 0·42–8·49; P = 0·40 respectively) (Fig 2). However, for the R-IPI higher risk groups (good and poor), PFS was significantly improved by the addition of rituximab (HR, 0·58; 95% CI, 0·35–0·96; P = 0·035, HR, 0·54; 95% CI, 0·38–0·76; P < 0·001 respectively) (Figs 3 and 4). OS was also improved in the R-IPI poor risk group (HR, 0·48; 95% CI, 0·32–0·72; P < 0·001), and an improvement in the R-IPI good risk group was also noted, but it was not statistically significant (HR, 0·52; 95% CI, 0·26–1·05; P = 0·069). We also performed a forest plot to explore the heterogeneity between these subgroups. There was no evidence of substantial heterogeneity in the relative treatment effect on PFS and OS between different risk groups based on the R-IPI (The P value for heterogeneity was 0·35 and 0·23 respectively) (Fig 5). These results suggest that rituximab improved the clinical outcome of all DLBCL patients.

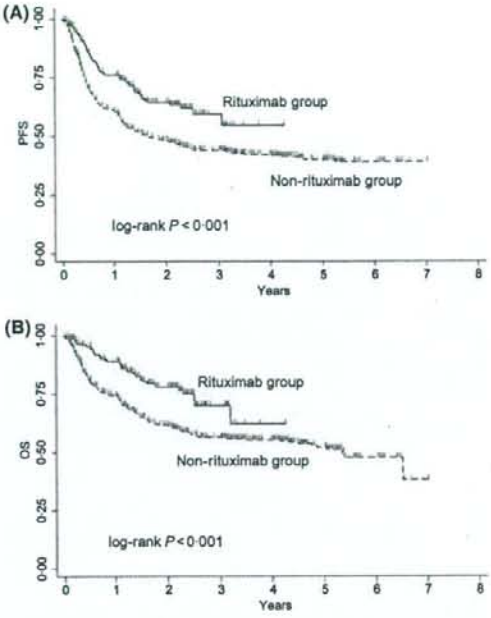

Fig 1. Progression-free survival (A) and overall survival (B) of 762 DLBCL patients. The rituximab group received rituximab in addition to systemic chemotherapy as first remission induction. The non-rituximab group received systemic chemotherapy alone as first remission induction.

Follicular lymphoma

A total of 215 FL patients were enrolled. Of these, 111 patients were in the rituximab group, and the other 104 were in the non-rituximab group. The patient number in each group was almost equal because of the date of rituximab approval (September 2002 for indolent B cell lymphoma) and the time period of the study (from January 2000 to December 2004). After approval, almost all FL cases were treated with rituximab, so that rituximab was available for 2 years and 4 months of the 5-year study period. The patients' characteristics are listed in Table III. The ratio of cases receiving an anthracyclin-containing regimen in each group was not significantly different (rituximab group, 104/111; non-rituximab group, 91/104; P = 0·159). Only three (age, LDH level, Ann-Arbor clinical stage) of the five prognostic variables that make up the FLIPI could be evaluated. These variables were not different between the rituximab group and the non-rituximab group (Table III). The median follow-up time for living patients was 37 months for the non-rituximab group (range, 1–72 month) and 41 months for the rituximab group (range, 1–80 months). PFS was markedly improved in the rituximab group compared with the non-rituximab group (HR, 0·45; 95% CI, 0·30–0·69; P < 0·001, Fig 6). The 2-year estimated PFS was 77·6% (95% CI, 68·1–84·5%) in the rituximab group and 56·3% (95% CI,

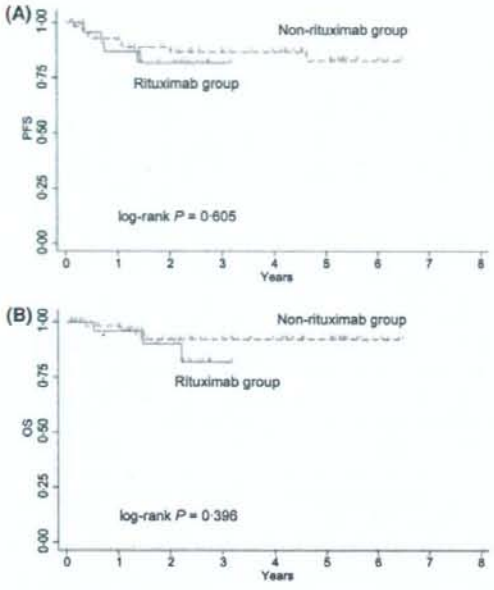

Fig 2. Progression-free survival (A) and overall survival (B) of 86 DLBCL patients (R-IPI very good risk). The rituximab group received rituximab in addition to systemic chemotherapy as first remission induction. The non-rituximab group received systemic chemotherapy alone as first remission induction.

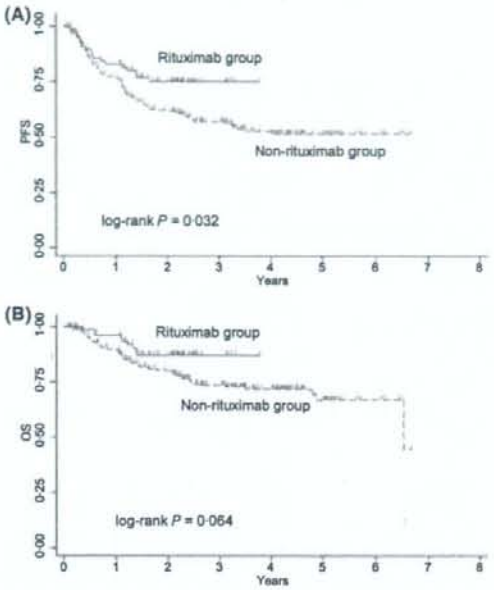

Fig 3. Progression-free survival (A) and overall survival (B) of 324 DLBCL patients (R-IPI good risk). The rituximab group received rituximab in addition to systemic chemotherapy as first remission induction. The non-rituximab group received systemic chemotherapy alone as first remission induction.

45·9–65·5%) in the non-rituximab group. OS was also improved in the rituximab group compared with the non-rituximab group (HR, 0·35; 95% CI, 0·17–0·72; $P = 0·003$, Fig 5). The 2-year estimated OS was 94·3% (95% CI, 87·8–97·4%) in the rituximab group and 81·7% (95% CI, 72·5–88·0%) in the non-rituximab group.

A multivariate analysis was performed to assess the effect of rituximab on clinical outcome after controlling for prognostic variables. After controlling for the prognostic variables included in R-IPI and IPI itself, rituximab remained an independent prognostic predictor of both PFS (risk ratio, 0·56; 95% CI, 0·43–0·74; $P < 0·001$) and OS (risk ratio, 0·50; 95% CI, 0·36–0·70; $P < 0·001$) in DLBCL. In FL, rituximab was also an independent prognostic predictor of both PFS (risk ratio, 0·49; 95% CI, 0·32–0·74; $P = 0·001$) and OS (risk ratio, 0·44; 95% CI, 0·21–0·92; $P = 0·028$) after adjustment for prognostic variables (age, LDH level and clinical stage).

Discussion

This retrospective survey showed that the addition of rituximab significantly improved PFS and OS in patients with FL and DLBCL when used as part of first remission induction therapy. This survey was carried out among 20 hospitals belonging to CHG–NHO. The clinical data of all patients diagnosed with NHL during this study were accumulated, and the PFS and OS of B cell lymphoma patients receiving systemic chemotherapies with and without rituximab were analysed. Rituximab was approved in September 2002 for indolent B cell lymphoma and in September 2003 for aggressive B cell lymphoma in Japan. The period of this survey was from January 2000 to December 2004 (5 years); therefore, differences in clinical outcomes could be compared between the rituximab group and the non-rituximab group. NHL patients were enrolled without regard to the chemotherapeutic regimen. During the study period, 1229 mature B cell lymphoma patients were newly diagnosed, and 1126 (92%) received systemic chemotherapy. Of the 1126 patients, 977 were diagnosed with DLBCL or FL, so that these cases accounted for 86·8% of the 1126 cases of mature B cell lymphoma receiving systemic chemotherapy. Thus, the clinical outcomes of these subjects reflect those of almost the entire mature B cell lymphoma population in clinical practice.

So far, many clinical studies have shown the benefits of rituximab in the treatment of B cell lymphoma. In 1999, a single arm phase II study of a combination of rituximab and CHOP for untreated indolent B cell lymphoma was reported (Czuczman *et al*, 1999). The response rate was 95% (38 of 40), and long-term remissions were observed (Czuczman *et al*, 2004). Several randomized phase III studies have demonstrated

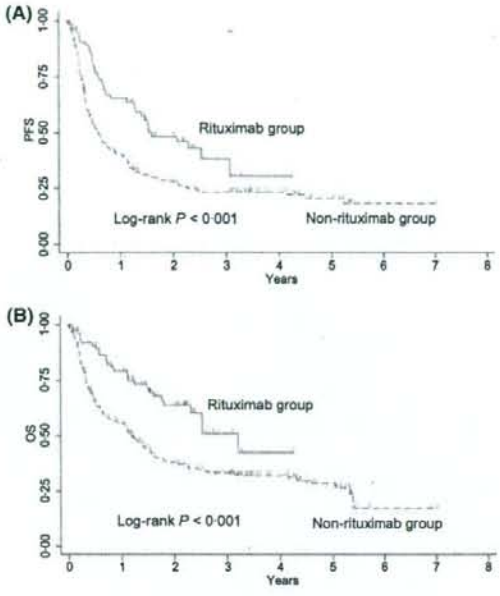

Fig 4. Progression-free survival (A) and overall survival (B) of 333 DLBCL patients (R-IPI poor risk). The rituximab group received rituximab in addition to systemic chemotherapy as first remission induction. The non-rituximab group received systemic chemotherapy alone as first remission induction.

the advantages of the addition of rituximab to chemotherapy, both in previously untreated patients, as well as in relapsed/refractory indolent B cell lymphoma patients (Forstpointner *et al*, 2004; Hiddemann *et al*, 2005; Lenz *et al*, 2005; Marcus *et al*, 2005; Rivas-Vera *et al*, 2005; van Oers *et al*, 2006; Herold *et al*, 2007; Schulz *et al*, 2007). The German Low-Grade Lymphoma Study Group (GLSG) conducted a phase III study comparing CHOP combined with rituximab to CHOP alone, and they showed significant improvements in remission rates, PFS and OS in the combination group (Hiddemann *et al*, 2005). Other studies also showed that chemotherapy with rituximab provided a better PFS than chemotherapy alone. Recently, the Cochrane Hematological Malignancies Group performed a comprehensive systematic review and meta-analysis to compare the efficacy of chemotherapy with rituximab to the identical chemotherapy alone in patients with indolent B cell lymphoma or mantle cell lymphoma (Schulz *et al*, 2007). This analysis included seven well-controlled, randomized studies comparing rituximab–chemotherapy combination therapy with chemotherapy alone, and indicated that the rituximab–chemotherapy combination provided superior OS to chemotherapy alone.

For DLBCL, many phase III studies have proven the benefits of the addition of rituximab to chemotherapy. The Groupe d'Etude des Lymphomes de l'Adulte study showed superiority of CHOP and rituximab to CHOP alone in elderly, advanced, previously untreated, DLBCL patients with respect to PFS and OS (Coiffier *et al*, 2002). The advantage of rituximab in

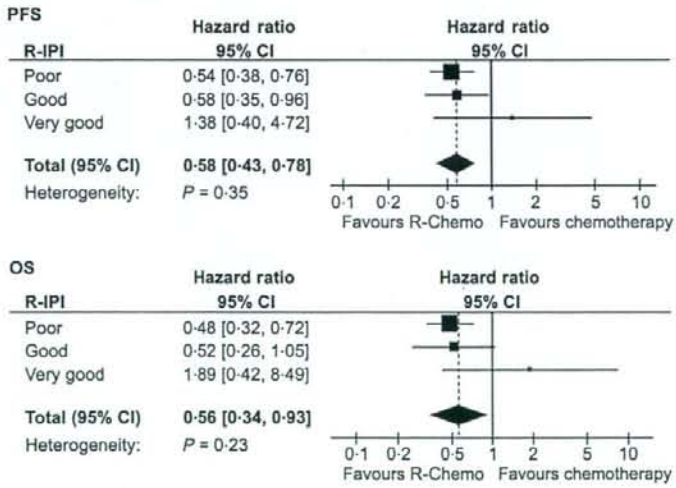

Fig 5. Disease control for DLBCL in each R-IPI risk group receiving rituximab with chemotherapy (R-chemo) or chemotherapy alone. Disease control is shown as the hazard ratio (HR) for a disease event (progression or death). Solid squares represent risk estimates for the each R-IPI risk group. The size of squares represents the weight assigned to each R-IPI risk group and is proportional to inverse variance of the estimate. Horizontal lines indicate 95% confidence intervals (CIs). The diamond indicates the 95% CIs for the overall HR. Values less than 1·0 indicate HRs that favour R-chemo.

Table III. Characteristics of follicular lymphoma patients (n = 215).

Characteristics	Rituximab group (n = 111)	Non-rituximab group (n = 104)	P
Age (years), median (range)	56 (26–83)	57 (23–91)	0·497*
Gender male/female	49/62	48/56	0·767†
PS at diagnosis			
0	60	53	0·395*
1	38	31	
2	8	13	
3	4	6	
4	1	1	
LDH > normal	42	47	0·274†
Clinical stage			
I	4	7	0·065*
II	28	15	
III	41	32	
IV	38	50	
Receiving anthracyclin-containing regimen	104	91	0·159†

PS, ECOG performance status; LDH, lactate dehydrogenase.
*Mann–Whitney *U*-test.
†Chi-squared test.

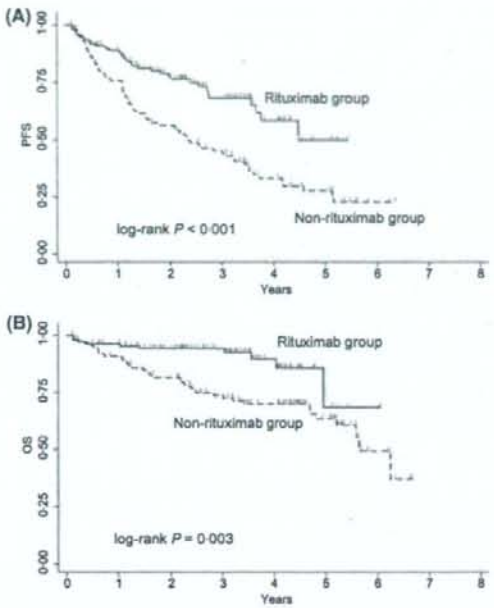

Fig 6. Progression-free survival (A) and overall survival (B) of 215 follicular lymphoma patients. The rituximab group received rituximab in addition to systemic chemotherapy as first remission induction. The non-rituximab group received systemic chemotherapy alone as first remission induction.

combination with a CHOP-like regimen for the younger DLBCL population was indicated by the intergroup cooperative study (MInT study) (Pfreundschuh *et al*, 2006). Therefore, the clinical merits of the use of rituximab in the induction treatment of mature B cell lymphoma have now been established by these well controlled, phase III studies, but the actual benefits of rituximab benefits in clinical practice have not been addressed. Prospective clinical trials for treatment have critical inclusion and exclusion criteria, and patients with poor PS or organ dysfunction are usually excluded. One population-based retrospective analysis, by the British Columbia Cancer Registry, assessed the effect of rituximab in combination with CHOP for DLBCL and demonstrated improvement in treatment outcome in clinical practice (Sehn *et al*, 2005). However, this study was limited to patients who were treated with curative intent. The present study serially enrolled all patients with mature B cell lymphoma who were newly diagnosed, and all patients receiving systemic chemotherapy, whether or not the intent was curative, were included in the analysis to evaluate the effect of rituximab. This approach reflects the actual state of management of mature B cell lymphoma patients in clinical practice.

In DLBCL, PFS and OS were better in the rituximab group than in the non-rituximab group. When DLBCL was classified by R-IPI, the benefit of rituximab was statistically identified in the good and poor risk group but not in the very good risk group. The favourable effect of rituximab seemed to be restricted in higher risk patients, but the significant heterogeneity between these subgroups was not identified by the forest plot (Fig 5). This finding might be a result of small patient numbers in the very good risk group. To clarify whether rituximab contributes to the clinical outcomes of the very good risk group or not, more cases need to be analysed.

In conclusion, this retrospective analysis showed that the use of rituximab for remission induction therapy significantly improved OS and PFS in patients with FL or DLBCL, who constitute the majority of mature B cell lymphoma patients. This study was planned to elucidate the state of NHL management in clinical practice and found that rituximab appeared to dramatically improve clinical outcomes in patients with mature B cell lymphoma.

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Phase III study to evaluate the use of high-dose chemotherapy as consolidation of treatment for high-risk postoperative breast cancer: Japan Clinical Oncology Group study, JCOG 9208

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A randomized controlled trial was conducted to evaluate the efficacy of high-dose chemotherapy (HDC) as consolidation of the treatment of high-risk postoperative breast cancer. Patients under 56 years of age with stage I to IIIB breast cancer involving 10 or more axillary lymph nodes were eligible. The primary endpoint was relapse-free survival (RFS). Between May 1993 and March 1999, 97 patients were enrolled, and two patients became ineligible. The median age of the 97 patients was 46 years (range 27–55 years), and 72 (74%) were premenopausal. The median number of involved axillary nodes was 16 (range 10–49). All patients had undergone a radical mastectomy. Major characteristics were well balanced between the treatment arms. Forty-eight patients in the standard-dose (STD) arm received six courses of cyclophosphamide, doxorubicin, and 5-fluorouracil followed by tamoxifen. Forty-nine patients were assigned to undergo HDC with cyclophosphamide and thiotepa after six courses of cyclophosphamide, doxorubicin, and 5-fluorouracil followed by tamoxifen; however, 15 of these patients (31%) did not undergo HDC. HDC was well tolerated without any treatment-related mortality. At a median follow-up of 63 months, the 5-year RFS of 47 eligible patients in the STD arm and 48 eligible patients in the HDC arm was 37% and 52% on an intent-to-treat basis, respectively ($P = 0.17$). Five-year overall survival of all randomized patients was 62% for the STD arm and 63% for the HDC arm ($P = 0.78$). Although the prespecified values of the two arms were not so accurate as to allow detection of the observed difference, no advantage of HDC was observed in terms of RFS or overall survival. (*Cancer Sci* 2008; 99: 145–151)

Preclinical studies have suggested that doses of cytotoxic chemotherapy correlate with the cure of cancer patients.[1] Among several kinds of dose-intensification strategies, high-dose chemotherapy (HDC) with autologous hematopoietic stem cell support has been extensively investigated in clinical oncology. In addition, HDC was shown to produce survival advantages in certain types of malignant neoplasms, including relapsed aggressive non-Hodgkin's lymphoma responding to salvage chemotherapy,[2] and untreated multiple myeloma[3,4] in randomized controlled studies.

Adjuvant chemotherapy has been shown to improve relapse-free survival (RFS) and overall survival (OS) in patients with primary breast cancer[5] and dose-intensification was found to be associated with superior outcomes in some populations.[6] However, the prognosis of patients with extensive axillary lymph node involvement is still poor despite conventional-dose adjuvant chemotherapy. Thus, such patients have been considered to be appropriate candidates for clinical trials of HDC.

Several uncontrolled studies have suggested a survival advantage for HDC in the adjuvant treatment of high-risk primary breast cancer with extensive axillary lymph node involvement.[7–11] At the time of writing, 12 adequately conducted randomized controlled trials comparing HDC with standard-dose (STD) or conventional-dose chemotherapy in high-risk postoperative breast cancer patients have been reported.[12–23] In 10 of them, the advantage of HDC was not shown. However, two of them have shown improved RFS from HDC[18,22] and one study has shown an OS benefit.[22] Thus, its role in the treatment of high-risk primary breast cancer is still inconclusive and deserves further attention.

Based on the promising results of uncontrolled phase II trials of HDC for high-risk primary breast cancer, especially those of the Duke series including patients enrolled into the Cancer and Leukemia Group B (CALGB) study 8782, reported by Peters *et al.*[8] phase I/II studies of cyclophosphamide and thiotepa with autologous bone marrow reinfusion[24,25] and our own earlier feasibility study of HDC of cyclophosphamide and thiotepa with autologous stem cell reinfusion against metastatic breast cancer[26] the Japan Clinical Oncology Group (JCOG)[27] conducted a randomized controlled study to evaluate the efficacy of HDC of cyclophosphamide and thiotepa as consolidation of the treatment for high-risk postoperative breast cancer.

Patients and Methods

Patients. The study was designed for women between 15 and 55 years of age with breast cancer, stage I to IIIB, involving 10 or more axillary nodes, histologically confirmed by level II or further dissection. Eligible patients had to have a performance status rating of 0 or 1 according to the Eastern Cooperative Oncology Group (ECOG) criteria.[28] Exclusion criteria were prior chemotherapy, radiotherapy, and endocrine therapy. Patients

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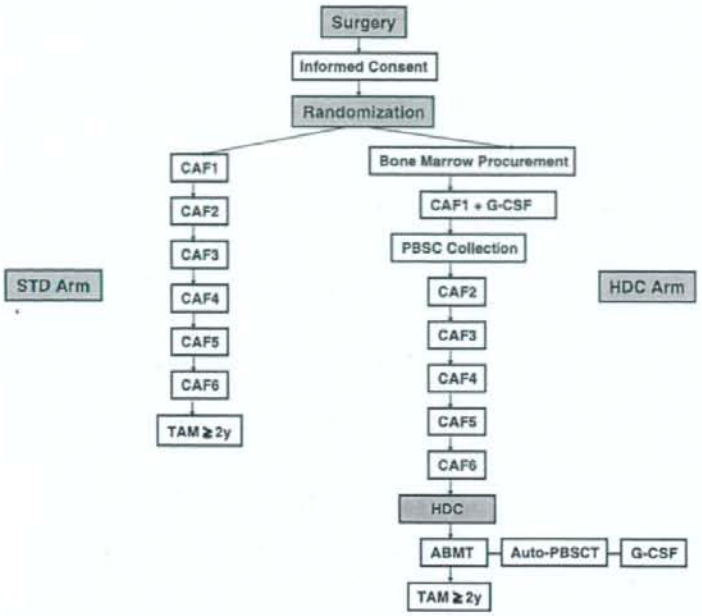

Fig. 1. Trial design of Japan Clinical Oncology Group study, JCOG 9208. ABMT, autologous bone marrow transplantation; CAF, cyclophosphamide, doxorubicin, 5-fluorouracil; G-CSF, granulocyte colony-stimulating factor; HDC, high-dose chemotherapy; PBSC, peripheral blood stem cell; STD, standard-dose; TAM, tamoxifen.

were required to have adequate bone marrow, hepatic, renal, cardiac, and respiratory functions (leukocyte count $\geq 3.5 \times 10^9$/L; hemoglobin ≥10 g/dL; platelet count $\geq 100 \times 10^9$/L; aspartate aminotransferase and alanine aminotransferase ≤4 times the upper normal limit; total bilirubin ≤1.5 times the upper normal limit; blood urea nitrogen and serum creatinine within normal limits; creatinine clearance ≥60 mL/min; no severe cardiac disorder on electrocardiogram; ejection fraction ≥50%; and PaO_2 ≥70 mmHg). Physical examination, chest X-ray, abdominal ultrasound examination, brain computed tomography and a radionuclide bone scan had to be negative for distant metastases. Negative result for bone marrow aspiration or biopsy from the posterior iliac bone was also required.

Patients meeting any one of the following criteria were excluded from the trial: contralateral breast cancer; active concurrent cancer; active peptic ulcer; seropositive for hepatitis B virus surface antigen, hepatitis C virus antibody, or HIV antibody; liver cirrhosis; pulmonary fibrosis or chronic obstructive lung disease; severe psychiatric disorder; diabetes mellitus requiring insulin treatment; uncontrollable hypertension (diastolic pressure ≥110 mmHg); hypercalcemia (serum Ca ≥11 mg/dL); pregnancy or lactation; history of cardiac failure or renal failure; or evidence of concurrent bacterial and fungal infection.

This clinical trial was planned to be conducted at 11 centers belonging to the Autologous Bone Marrow Transplantation Study Group and the Breast Cancer Study Group of JCOG. The JCOG 9208 study protocol and the informed consent document complying with JCOG guidelines and policies were approved by the Clinical Trial Review Committee of JCOG and by the institutional review committee of each participating institution before the start of the study.[27] All patients provided their written or oral consent before the start of the study. Registration involved a telephone call or facsimile from the participating physicians to the JCOG Statistical/Data Center, National Cancer Center, Tokyo, Japan (1991–1997, Statistical Center; 1998–, Data Center).[27] The attending physicians were responsible for submitting periodic data reports on toxicity, relapse, and survival.

Treatment. As shown in Fig. 1, eligible patients were randomly assigned to the STD or HDC arm at the time of enrolment by minimization method to balance the numbers of positive axillary nodes (10–19 or 20–), menopausal status (pre or post) and institution between the arms.

Patients assigned to the STD arm were planned to receive six courses of cyclophosphamide, doxorubicin and 5-fluorouracil (CAF) at 21-day intervals. Each course consisted of intravenous injection with cyclophosphamide 500 mg/m^2, doxorubicin 40 mg/m^2, and 5-fluorouracil 500 mg/m^2. The first course of CAF chemotherapy had to be initiated within 10 weeks after primary surgery.

Patients assigned to the HDC arm underwent bone marrow procurement under general anesthesia before CAF chemotherapy within 9 weeks after primary surgery. Typically, 1 week after primary surgery, they received the first course of CAF together with lenograstim (granulocyte colony-stimulating factor) to collect peripheral blood stem cells (PBSC) as previously described.[29] Lenograstim was given subcutaneously daily from day 8 after CAF chemotherapy until the day of the last leukapheresis. Leukapheresis was carried out once or twice when the leukocyte count increased to greater than 10×10^9/L as described previously.[26] At least 3 weeks after the sixth course of CAF chemotherapy, the patients underwent HDC consisting of cyclophosphamide 2000 mg/m^2/day and thiotepa 200 mg/m^2/day for three consecutive days (days –5 to –3). The doses of cyclophosphamide and thiotepa were determined based on the results of combination phase I/II studies[24,25] and our own feasibility study.[26] Autologous bone marrow and PBSC were thawed and infused on day 0 and 1, respectively. All patients received oral antibiotics, antifungal agents, sulfamethoxazole/trimethoprim and oral acyclovir (200 mg × 5, daily) prophylactically. Irradiated platelet transfusions were given to maintain the platelet count above 20×10^9/L, and irradiated red blood cells were given if necessary. Then 5 μg/kg lenograstim was started on day 2.

Following the above-described therapy, all patients received tamoxifen 20 mg/day for at least 2 years, irrespective of receptor status. Radiation therapy was not planned. All toxicities were

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graded according to the toxicity grading criteria of JCOG,[30] a modified and expanded version of the National Cancer Institute – Common Toxicity Criteria version 1.0.

Baseline evaluation included staging examination (mammography, bone scintigram, brain computed tomography, chest X-ray, abdominal ultrasonography, and bone marrow aspiration/biopsy), complete medical history, physical examination, complete blood cell count, serum chemistry, urinary analysis, tumor marker, and estrogen receptor/progesterone receptor. Restaging evaluation, including chest X-ray, bone scintigram, abdominal ultrasonography, and tumor marker, was conducted every 3–4 months for the first 3 years, and every 6 months for the subsequent 2 years. Central monitoring was carried out every 6 months throughout the study.

Study design and statistical analysis. The primary endpoint was RFS and secondary endpoints were OS and toxicity. RFS was defined as the time from randomization to the first observation of relapse or death due to any cause. OS was defined as the time from randomization to the time of death due to any cause. Survival curves were estimated by the Kaplan–Meier method and compared using the log–rank test.

All eligible patients were analyzed as a data set. To detect a 40% increase in RFS at 5 years of the HDC arm compared with 30% of the STD arm at a significance level of 5% by two-sided log–rank test and a power of 80%, 25 patients are required in each arm. Three years of accrual time and 4 years of follow-up time from the last patient enrolment were assumed initially. As up to 25% of patients in the HDC arm might fail to receive HDC, we estimated a requirement of 100 patients in total (50 patients in each arm) in order to have sufficient statistical power at the beginning of the study.

Patient enrolment into this trial was closed in March 1999, and the actual accrual period was 5.8 years. The follow-up time from the last patient enrolment was amended to 2 years, as approved by the JCOG Data and Safety Monitoring Committee in September 2000.[27] Statistical re-calculation revealed 90% power to detect a 30% increase in RFS at 5 years or 60% power to detect a 20% increase with a significance level of 5% by one-sided log–rank test. No interim analysis was carried out.

Results

Patients. Between May 1993 and March 1999, a total of 97 patients were enrolled from eight institutions. Two patients were ruled ineligible, as one had stage IV disease and the other was enrolled after the start of chemotherapy. Median age was 46 years (range 27–55 years), and 72 patients (74%) were premenopausal. The median number of involved axillary nodes was 16 (range 10–49), and 41 patients (42%) had 20 or more positive axillary nodes. All patients had undergone a radical mastectomy. Forty-eight patients were assigned to receive six courses of CAF (STD arm), and 49 patients were assigned to receive additional HDC with autologous stem cell support (HDC arm). The treatment groups were well balanced in terms of characteristics such as age, menopausal status, performance status, number of positive axillary nodes, stage, and hormone receptor status (Table 1).

Fifteen patients (31%) in the HDC arm did not receive HDC, including seven recurrences during or immediately after CAF therapy, seven refusals and one ineligible patient (Fig. 2). One patient in the HDC arm did not receive high-dose cyclophosphamide on day –3 due to the development of grade 4 arrhythmia (complete atrioventricular block). In addition to the one ineligible, five patients in the STD arm did not complete the planned six courses of CAF therapy, consisting of three recurrences and two refusals. Therefore, of the 97 patients enrolled, 76 (80%) of 95 eligible patients completed the planned treatments.

Major deviations from the protocol were: CAF chemotherapy given despite the presence of grade 2 leukopenia (four patients in the STD arm and nine in the HDC arm); CAF given despite hepatic transaminase elevation >4 times the upper normal limit (one patient in each arm); interval shortening and/or prolongation between the cycles of CAF (four patients in the STD arm and three in the HDC arm); initiation of CAF more than 10 weeks after primary surgery (one HDC patient); and a larger dose (140% of the planned doses) of cyclophosphamide and 5-fluorouracil in the first cycle of CAF (one HDC patient).

Table 1. Characteristics of all randomized patients in the Japan Clinical Oncology Group study, JCOG 9208

Treatment arm		Standard-dose	High-dose
No. of enrolled patients		48	49
Median age in years (range)		47 (27–55)	46 (29–55)
Menopause	Pre/post	34/14	38/11
PS	0/1	41/7	46/3
No. of positive axillary nodes	Median (range)	18 (10–46)	16 (10–49)
	10–19	28	28
	20–	20	21
Stage	I	2	2
	IIA	8	12
	IIB	18	16
	IIIA	10	9
	IIIB	10	9
	IV	0	1†
ER	+/–/unknown	29/19/0	25/22/2
PgR	+/–/unknown	25/22/1	22/24/3

†Ineligible. ER, estrogen receptor; No., number; PgR, progesterone receptor; PS, performance status (0 or 1 according to the Eastern Cooperative Oncology Group criteria[28]).

RFS and OS. Seven years after patient recruitment was completed, 52 (54%) of the 97 enrolled patients were alive. Sixty-one (64%) of the 95 eligible patients relapsed or died, 33 (70%) of 47 patients in the STD arm and 28 (58%) of 48 in the HDC arm. Primary analysis was carried out for all 95 eligible patients. At 5 years, RFS of 47 eligible patients in the STD arm and 48 eligible patients in the HDC arm was 37% (95% confidence interval [CI], 23–51%) and 52% (95% CI, 37–66%), respectively (two-sided log–rank, $P = 0.17$) (Fig. 3). Estimated median RFS time was 32 months (95% CI, 23–79 months) for the STD arm and 70 months (95% CI, 36 months–) for the HDC arm. Five-year survival of all randomized patients was 62% (95% CI, 48–76%) for the STD arm and 63% (95% CI, 50–77%) for the HDC arm ($P = 0.78$) (Fig. 4). Estimated median survival time was 87 months for the STD arm (95% CI, 55 months–) and was 110 months for the HDC arm (95% CI, 57 months–).

Toxicity. The HDC treatment was well tolerated, without any treatment-related mortality. All 34 patients receiving HDC actually developed grade 4 leukopenia and grade 4 neutropenia; 27 (79%) developed grade 4 and the other seven grade 3 thrombocytopenia. Hematological status was restored in all patients. Non-hematological toxicities of HDC in 34 patients are shown in Table 2. Three patients developed grade 4 non-hematological toxicities. One developed grade 4 diarrhea on day 4 (9 days after the start of HDC) and recovered 2 days later. Another showed transient grade 4 elevation of hepatic transaminase on day 13 (18 days after the start of HDC). The third patient developed grade 4 arrhythmia (complete atrioventricular block) on day –3 (the third day of HDC), and completely recovered by day 11 (14 days later).

Of 93 patients who actually underwent CAF therapy, seven patients (8%) developed grade 4 neutropenia, but none developed

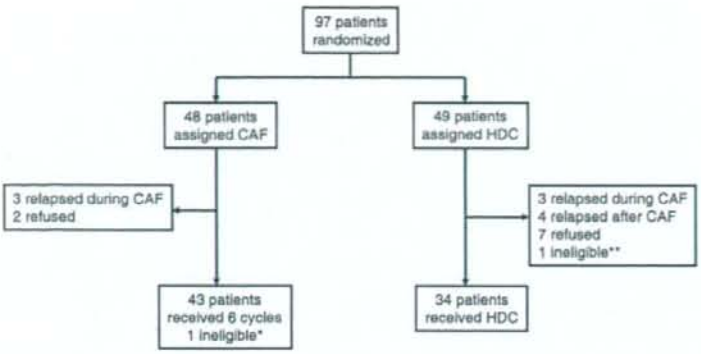

Fig. 2. Trial profile of Japan Clinical Oncology Group study, JCOG 9208. *Registered after the start of cyclophosphamide, doxorubicin, 5-fluorouracil (CAF; violation). **Bone marrow involvement was revealed before the start of CAF.

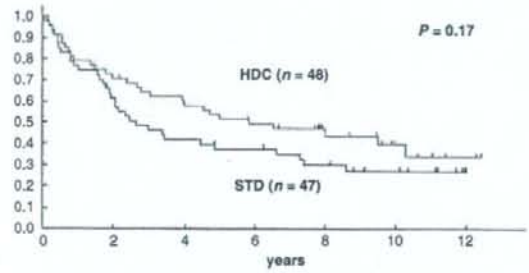

Fig. 3. Relapse-free survival (RFS) of all eligible patients in the Japan Clinical Oncology Group study, JCOG 9208. At 5 years, the intent-to-treat RFS of 47 eligible patients in the standard-dose (STD) arm and 48 eligible patients in the high-dose chemotherapy (HDC) arm was 37% and 52%, respectively (one-sided log-rank, $P = 0.17$). Estimated median RFS time was 36 months for the STD arm and 60 months for the HDC arm.

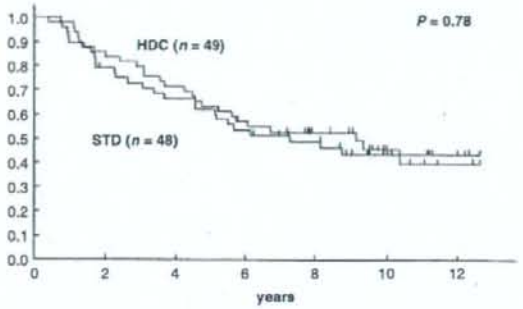

Fig. 4. Overall survival (OS) of all randomized patients in the Japan Clinical Oncology Group study, JCOG 9208. Five-year OS of all randomized patients was 62% for the standard-dose arm and 63% for the high-dose chemotherapy arm (one-sided log-rank, $P = 0.78$).

grade 4 non-hematological toxicities. All the toxicities of CAF therapy were transient.

Discussion

In the present phase III study, we evaluated the efficacy of HDC in high-risk postoperative patients involving 10 or more axillary nodes, using a common CAF regimen as an induction therapy, and HDC as a consolidation after CAF therapy. So far, 13 randomized controlled studies to evaluate the use of HDC in high-risk primary breast cancer have been reported[12–23] including the first report of our study.[31] In the present report, we have updated the analysis of the study, now with a median follow-up of 63 months. However, our study was unable to show any advantage of HDC in terms of RFS or OS.

In our first report, the 4-year RFS of the STD arm was 43% and that of the HDC arm was 61%, showing a trend favoring the latter, although there was no statistical significance between the two arms ($P = 0.12$).[31] In this analysis, the 5-year RFS of the STD and HDC arms was 37% and 52%, respectively, again

Table 2. Non-hematological toxicities of high-dose chemotherapy in 34 patients in the Japan Clinical Oncology Group study, JCOG 9208

Toxicity	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	Grade 4 (%)	Any grade (%)
Nausea/vomiting	3 (9)	9 (26)	22 (65)	0	34 (100)
Diarrhea	10 (29)	11 (32)	9 (26)	1 (3)	31 (91)
Mucositis	16 (47)	3 (9)	5 (15)	0 (0)	24 (71)
Arrhythmia	3 (9)	1 (3)	1 (3)	0 (0)	6 (18)
Infection	9 (26)	9 (26)	2 (6)	1 (3)	20 (59)
Bilirubin	0	4 (12)	1 (3)	0 (0)	5 (15)
AST	15 (44)	12 (35)	5 (15)	0 (0)	32 (94)
ALT	10 (29)	13 (38)	7 (21)	1 (3)	31 (91)

No therapy-related death was observed during high-dose chemotherapy. All toxicities were graded according to the toxicity grading criteria of JCOG,[30] a modified and expanded version of the National Cancer Institute – Common Toxicity Criteria version 1.0. AST, aspartate aminotransferase; ALT, alanine aminotransferase.

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without statistical significance. When we designed this randomized study in 1992, we anticipated a 5-year RFS of 30% for the STD arm, based on the results of consecutive clinical trials conducted by CALGB (CALGB 7581 and CALGB 8082)[8,32,33] and the historical series in the National Cancer Center Hospital in Japan; the 5-year RFS and 10-year RFS of patients involving 10 or more axillary nodes were 30% and 19%, respectively (unpublished data). However, we expected a 5-year RFS of 70% for the HDC arm, based on the results of the phase II study by the Duke group.[8] When we took a closer look at these results, and in particular the selection biases in phase II studies, it seemed likely that the expected difference in RFS between the two arms was too large. As the present study was small and did not have sufficient statistical power to detect small differences (90% power to detect 30% increase in RFS at 5 years or 60% power to detect 20% increase with a significance level of 5% by one-sided test), there remains a possibility that a smaller advantage for the HDC was missed. However, the absence of a trend favoring the HDC arm in OS ($P = 0.75$) suggests that the survival advantage for the HDC would be minimal even if it exists.

The 4-year RFS of 61% for the HDC arm was similar to that of the collected data of the Autologous Blood and Marrow Transplant Registry (ABMTR).[34] However, the RFS data for the HDC arm in the present study was inferior to that of the Duke series (63% of 5-year RFS in the present study *versus* 71% in 5-year event-free survival [EFS] in the Duke series).[16] In the Duke series, only 10% of patients had 20 or more axillary node metastases (median, 14), whereas it was 41% of patients in the present study (median, 16). The higher RFS in the Duke series could be explained partly because they contained more patients with lower risk than the present study. Another possible explanation is that cyclophosphamide and thiotepa of the HDC regimen used in the present study might be less active than cyclophosphamide, carmustine, and cisplatin used in the Duke series.[7,8] The cyclophosphamide and thiotepa regimen was most common in HDC for stage II or III or inflammatory breast cancer, followed by the cyclophosphamide, thiotepa, and carboplatin regimen, according to analysis of ABMTR.[34] Although these two regimens have never been directly compared in a randomized fashion, the analysis of 3451 metastatic breast cancer patients in ABMTR suggested that the HDC regimen did not affect prognosis.[34] Furthermore, two other studies recruiting patients with 10 or more positive axillary nodes[19,20] showed a 6-year RFS of 48% and a 4-year RFS of 52% for the HDC arms, respectively, similar to our results.

In contrast to the RFS results in the HDC arm, the 5-year RFS of 37% for the STD arm was higher than initially anticipated. According to the abstract for the annual meeting of ASCO in 1992 by Peters *et al.*[8] 3-year EFS of the historical control series from CALGB using adjustment for duration of follow-up and selected for age less than 56 years, involvement of 10 or more axillary nodes, and freedom from failure of at least 5 months was 30% in CALGB 8082 and 38% in CALGB 7581.[8,32,33] In an intergroup phase III study, 6-year RFS of 257 patients with 10 or more positive nodes in the conventional-dose arm was 46%[19] and in a German study, 4-year RFS in the conventional-dose arm was 42%.[20] Thus, it is unlikely that RFS in the conventional-dose arm was too high in the present study. As Peto commented on the trend towards a sizeable reduction in breast cancer mortality during the last decade, small improvements might add up to a large beneficial effect[35] in addition to patient selection[36,37] and stage migration.[38]

In the present study, all patients received tamoxifen 20 mg/day for at least 2 years, irrespective of receptor status. In the German study[20] tamoxifen was not planned in the initial protocol, although it was amended.to prescribe tamoxifen for patients with positive hormone-receptor status simultaneously in the HDC and STD arms. According to the Dutch study protocol[15] all patients originally received tamoxifen (40 mg/day) for 2 years. Because of the increasing evidence for treatment with tamoxifen in hormone receptor-positive patients, the protocol was amended and only patients with hormone receptor-positive cancer continued to receive tamoxifen for an additional 3 years. On the contrary, in the ECOG study[19] tamoxifen (20 mg/day) was to be given for 5 years to hormone receptor-positive patients in line with current recommendations. Furthermore, in the present study, adjuvant radiotherapy was originally prohibited, as regional radiotherapy had not been established when the protocol was designed. In the German study[20] as well as the Dutch study[15] it was not initially specified. In contrast, 50 Gy of regional radiotherapy was to be given in the ECOG study.[19] Thus, even in terms of tamoxifen treatment and regional radiotherapy after chemotherapy, protocols in the trials were varied. The results from the single trials and the meta-analysis were inconclusive. HDC should be further investigated in the context of contemporary therapies such as taxanes, dose-dense therapy, hormonal therapy, and radiotherapy.

Of 49 patients assigned to the HDC arm, 15 patients (31%) did not undergo the HDC, which was more than expected (up to 25%). Seven had relapsed before HDC, and seven refused it. When we compared the 69% (34/49) of patients in the HDC arm actually receiving HDC with the results of large studies (96% [264/274] in a Scandinavian study,[14] 90% [397/442] in a Dutch national phase III study,[15] 84% [214/254] in the ECOG study,[19] and 82% [123/150] in the German study[20]), fewer patients could complete HDC in the present study. In the US intergroup trial,[16] randomization was carried out after completion of the induction chemotherapy. This might have been a better option for the present trial.

In the present study, the effectiveness of HDC as consolidation was not confirmed in patients with high-risk postoperative breast cancer involving 10 or more axillary nodes. In the PEGASE 01 trial ($n = 314$) enrolling patients with eight or more positive axillary nodes, 3-year RFS was 71% and 55% ($P = 0.002$) for the HDC and STD arms, respectively.[18] Recently, Nitz *et al.* published the most successful results of HDC in the West German Study Group study.[22] In that study, tandem HDC was compared with dose-dense chemotherapy in 403 patients with at least nine positive nodes (mean, 17.6). Patients in the HDC arm received two cycles of standard-dose EC (epirubicin 90 mg/m^2 and cyclophosphamide 600 mg/m^2) at 2-week intervals followed by two cycles of HDC with epirubicin 90 mg/m^2, cyclophosphamide 3000 mg/m^2, and thiotepa 400 mg/m^2 every 21 days with autologous hematopoietic stem cell support. Patients in the control arm received dose-dense chemotherapy with four cycles of standard-dose EC followed by three cycles of cyclophosphamide 600 mg/m^2, methotrexate 40 mg/m^2, and 5-fluorouracil 600 mg/m^2 at 2-week intervals. With a median follow-up time of 48.6 months, 4-year EFS was 60% in the HDC arm and 44% in the control arm ($P = 0.00069$). The 4-year OS rates were 75% and 70% ($P = 0.02$), respectively. Although an early and rapidly cycled tandem HDC might be a promising approach to be prospectively examined, the efficacy of HDC in the treatment of high-risk primary breast cancer nonetheless remains inconclusive.

Retrospective subgroup analyses to find subsets with more benefit from HDC have been reported, but because of the limited sample size this could not be carried out in the present study. In the Dutch study, patients with HER2-negative disease benefited from HDC with a hazard ratio (HR) of 0.68 for RFS ($P = 0.002$) and 0.72 for OS ($P = 0.02$).[39] In the West German Study Group trial, retrospective subgroup analyses for triple negative patients showed that tandem HDC did significantly better than the control arm in terms of RFS (HR = 0.31) and OS (HR = 0.35, $P = 0.011$).[40]

In the present study, no treatment-related death occurred in either treatment arm. The ABMTR database reported that 3% of

patients treated with HDC died within 100 days after transplantation in stage II or III or inflammatory breast cancer.[34] Peters *et al.* reported a treatment-related mortality of 12% in the Duke series[7] and 7% in the HDC arm in the US intergroup trial.[16] Although the present trial was the first multi-institutional study using HDC for primary breast cancer patients in Japan, HDC could be safely used by the JCOG members.

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