Hypertension Research (2010) 33, 320–325

www.nature.com/hr

ORIGINAL ARTICLE

One-year effectiveness and safety of open-label losartan/hydrochlorothiazide combination therapy in Japanese patients with hypertension uncontrolled with ARBs or ACE inhibitors

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The long-term antihypertensive efficacy and safety of losartan/hydrochlorothiazide (HCTZ) combinations have not been appropriately evaluated in Japan. In this study, treated hypertensive patients taking angiotensin-receptor blocker (ARB) or angiotensin-converting enzyme inhibitor (ACEI) regimens not at blood pressure (BP) goals proposed by the Japanese Society of Hypertension (JSH) were switched to losartan/HCTZ combinations and followed for 1 year. Data analysis included 244 patients aged 64.5 ± 10.7 years, 56% male, 27% with diabetes mellitus and 36% with dyslipidemia. Pre-switching BP 157 ± 16/88 ± 10 mm Hg promptly decreased and maintained a steady state, reaching 132 ± 15/77 ± 9 mm Hg ($P<0.001$) 1 year later. After 1 year of treatment, 50% of patients cleared the goals of the JSH guideline for systolic BP and 79% for diastolic BP. Patients with maximal doses of ARBs tended to show larger decreases in BP (159 ± 11/90 ± 10 to 128 ± 10/75 ± 8 mm Hg, $P<0.001$, $n=32$). Clinical and laboratory adverse events were reported for 29 patients (11%), but serious abnormalities were not observed. In particular, plasma levels of uric acid (UA) were well-maintained for 1 year, and significant decreases in UA were observed in patients with higher levels of UA ($\geqslant 7.0$ mg dl^{-1}). Losartan/HCTZ combinations showed strong and steady hypotensive abilities and acceptable safety and tolerability in patients currently not at BP goals with regimens including ARBs or ACEIs in Japan.
Hypertension Research (2010) **33**, 320–325; doi:10.1038/hr.2009.230; published online 15 January 2010

Keywords: angiotensin-receptor blocker (ARB); Japanese; losartan/hydrochlorothiazide; uric acid

INTRODUCTION

Guidelines for hypertension treatment, including those of the Japanese Society of Hypertension (JSH), have recommended strict blood pressure (BP) control, with the aim of improving protection against cardiovascular and renal accidents.[1,2] However, considerable numbers of hypertensive patients have not achieved the recommended goals of BP in Japan.[3] The JSH guideline recommends angiotensin-receptor blockers (ARBs), angiotensin-converting enzyme inhibitors (ACEIs), Ca^{2+} channel blockers (CCB), β-blockers and diuretics as first-line drugs for hypertensive treatment.[1] The guideline also recommends appropriate combinations of the drugs, in particular low-dose (quarter to half dose) diuretics are recommended as an important candidate for satisfactory BP control.[1] However, the prescribing rate of diuretics was quite low (under 10%) in cases of monotherapy or combination therapy for hypertension in Japan.[4] The principal reason for reluctance to prescribe thiazide diuretics is the metabolic side effects of the drugs. However, low-dose thiazide diuretics retain their hypotensive abilities with minimal side effects.[5] Therefore, proper application of low-dose diuretics, particularly in combination therapies, is desirable in Japan to improve BP control.

A fixed dose combination of losartan (50 mg)/hydrochlorothiazide (HCTZ, 12.5 mg) (Preminent; Banyu/Merck, Tokyo, Japan) is the first combination of an ARB and a diuretic for hypertensive treatment in Japan, and is expected to be effective and safe from the pharmacological properties of both drugs. However, limited data were available on the combination drug in Japan, especially with regard to long-term treatment, large numbers of patients and its use in a clinical setting.[6–8] We organized a study group mainly consisting of clinical physicians in Miyazaki Prefecture in Japan (Preminent Assigned League in Miyazaki by Primary care physicians: PALM-1 study group), and evaluated the

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Received 21 October 2009; revised 6 December 2009; accepted 9 December 2009; published online 15 January 2010

efficacy and safety of the fixed combination of losartan/HCTZ for patients with essential hypertension for 1 year.

METHODS

Study subjects

This study was conducted at 43 centers for the PALM-1 study group (Appendix). Patients with essential hypertension (20–79 years old) were considered for screening and potential recruitment into the trial. They had visited the attending clinics from February 2007 to March 2008 and had not reached BP goals with antihypertensive therapy regimens, including ARBs or ACEIs, but not diuretics, over 1 month. Patients were excluded from the study if there was any evidence of secondary hypertension, renal failure (serum creatinine $\geqslant 2.0\,mg\,dl^{-1}$), severe liver dysfunction and symptomatic heart failure (New York Heart Association functional class-III or IV for dyspnea at exertion). Patients with concomitant use of two or more ARBs and/or ACEIs and any type of diuretics were also excluded.

Study protocol

The study was conducted in accordance with the principles of the declaration of Helsinki. The investigational protocol was approved by the ethics committee for human studies at the University of Miyazaki. Informed consent was obtained from all patients prior to recruitment.

This was an open-label, multicenter study consisting of a 3-month screening/baseline period and 1-year treatment period. Under antihypertensive treatment with regimens including ARBs or ACEIs, at least two BP measurements were conducted within 3 months of the baseline period to confirm baseline BP measurements were over the recommended BP goals of the JSH. The BP goals were 130/85 mm Hg for patients aged less than 65 years, 140/90 mm Hg for those aged 65 years or more, 130/80 mm Hg for patients with diabetes and/or chronic kidney disease and/or history of myocardial infarction, and 140/90 mm Hg for patients with a history of stroke.[1] After screening 311 patients, 266 entered the trial. Then only ARBs or ACEIs were switched to the fixed dose combination of losartan/HCTZ and patients were followed for 1 year. Changed prescriptions were kept for the initial 3 months and then, if needed, adjustments of antihypertensive drugs were allowed except for ARBs, ACEIs and diuretics. Symptoms, sitting BP, pulse rate and blood tests, including potassium, uric acid (UA), lipid profile, creatinine, glucose, hemoglobin-A1c (HbA1c, diabetic patients only), were evaluated every 3 months. Major complications were also evaluated. The criteria for diabetes and dyslipidemia were as follows: diabetes, using antiglycemic drugs or fasting blood glucose $\geqslant 126\,mg\,dl^{-1}$; dyslipidemia, using lipid-lowering drugs or total cholesterol $\geqslant 220\,mg\,dl^{-1}$ and/or high-density lipoprotein-cholesterol $<40\,mg\,dl^{-1}$, and/or triglyceride $\geqslant 150\,mg\,dl^{-1}$.

Statistical analysis

All data are expressed as mean ± s.d. The significance of differences was evaluated by one-factor analysis of variance with repeated measures on the time course of variables followed by Bonferroni/Dunn *post hoc* comparison tests. Comparisons of parameters among subgroups were made by unpaired Dunnet's C-test or analysis of variance followed by Scheffe's *post hoc* comparison test. *P*-value <0.05 was the criterion for statistical significance.

RESULTS

As indicated in Figure 1, 22 of the 266 enrolled patients dropped out within the first 3 months. The remaining 244 patients were considered as full analytical objects. Finally, 222 patients completed the entire trial and were used for evaluation of efficacy.

The baseline characteristics of the study population are summarized in Table 1. Patients' age was 64.5 ± 10.7 years, 56% were male and major complications included 27% of patients with diabetes, 36% with dyslipidemia and 18% with mild heart failure. Pre-prescribed ARBs or ACEIs were well distributed from among drugs on the market and, noteworthy, the average doses per day of the drugs were very close to the usual dosage of each drug (Table 1). ARBs or ACEIs were used as monotherapy for 93 patients (38%) and as combined therapy, mainly with CCB, for 151 patients (62%). Other pre-prescribed drugs were as follows and these drugs were not altered after introduction of the losartan/HCTZ combination: antiglycemic drugs for 38 of 266 patients (37 of 222), lipid-lowering drugs for 58 of 266 (53 of 222) and UA-lowering drugs for 14 of 266 (14 of 222).

The time course of BP in all patients is illustrated in Figure 2. Baseline BP 157 ± 16/88 ± 10 mm Hg significantly decreased to 134 ± 14/77 ± 9 mm Hg at 3 months ($P<0.001$) (fixed prescription period), and then steady levels were maintained throughout the remaining treatment period. The respective goals of BP were cleared by 50% of the patients for systolic BP and 79% of the patients for diastolic BP in the final assessment 1 year later. Interestingly, 32 of 222 patients who were switched from the maximum dose of ARBs showed a similar to larger decrease in BP

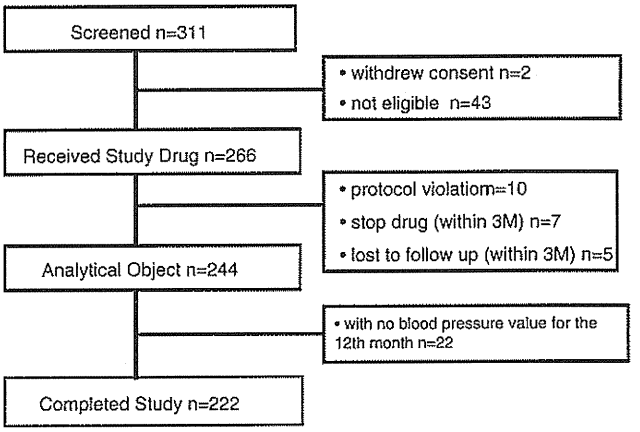


Figure 1 Patient disposition and reasons for exclusion.

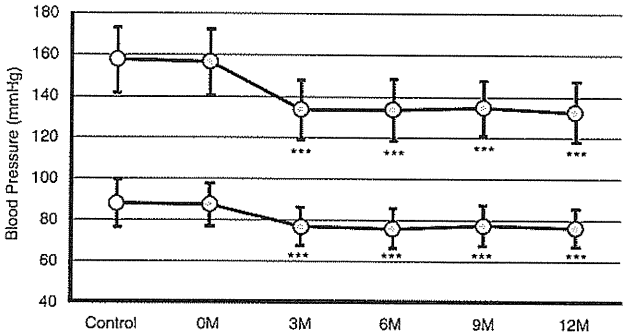
Table 1 Baseline characteristics (*n*=244)

Variable	*Value*	*Average doses (mg day^{-1})*
Age (years)	64.5 ± 10.7	
Male (*n*)	136 (56%)	
Body mass index (kg m^{-2})	25.2 ± 5.6	
Waist circumference (cm)	85.9 ± 8.6	
Obesity (*n*)	110 (48%)	
Diabetes (*n*)	66 (27%)	
Dyslipidemia (*n*)	88 (36%)	
Heart diseases (*n*)	43 (18%)	
Renal insufficiency (*n*)	8 (3%)	
Antihypertensives (n)		
One drug	93 (38%)	
Over two drugs	151 (62%)	
Pre-prescribed drugs (n)		
Valsartan	68 (28%)	88.8 ± 40.1
Candesartan	54 (22%)	8.4 ± 2.2
Losartan	34 (14%)	51.5 ± 8.6
Telmisartan	32 (13%)	40.3 ± 10.0
Olmesartan	31 (13%)	22.6 ± 8.6
ACE inhibitors	25 (10%)	

Abbreviation: ACE, angiotensin-converting enzyme.

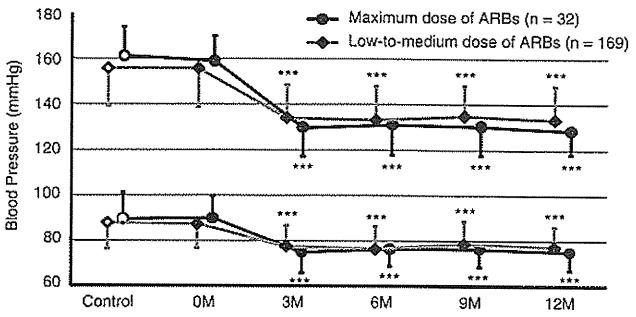
as compared with patients with low-to-medium dose of ARBs (Figure 3). There was a significant difference in the changes of BP from 3 months to 1 year between patients switched from low-to-medium dose of ARBs and maximum dose of ARBs (at 1 year: systolic BP, 23 ± 19 *vs.* 31 ± 13 mm Hg, P=0.005; diastolic BP, 10 ± 11 *vs.* 15 ± 10 mm Hg, P=0.027). As shown in Figure 4, similar and significant decreases in systolic and diastolic BP were achieved in all patients grouped based on pre-prescribed drugs at 1 year. Also there was no difference in BP changes among all ARBs and ACEI-receiving patients. The systolic and diastolic BPs at 0 and 12 month (changes of the BPs) for each drug were as follows: losartan, 154 ± 17 to 135 ± 10 mm Hg (−19 ± 17 mm Hg, P<0.001) and 87 ± 11 to 78 ± 8 mm Hg (−9 ± 10 mm Hg, P<0.001); candesartan, 156 ± 14 to 131 ± 14 mm Hg (−24 ± 17 mm Hg, P<0.001) and 87 ± 9 to 76 ± 9 mm Hg (−11 ± 10 mm Hg, P<0.001); valsartan, 160 ± 16 to 134 ± 13 mm Hg (−26 ± 18 mm Hg, P<0.001) and 89 ± 9 to 77 ± 8 mm Hg (−12 ± 10 mm Hg, P<0.001); telmisartan, 156 ± 17 to 132 ± 20 mm Hg (−24 ± 15 mm Hg, P<0.001) and 85 ± 12 to 75 ± 11 mm Hg (−10 ± 8 mm Hg, P<0.001); olmesartan, 153 ± 18 to 129 ± 14 mm Hg (−24 ± 24 mm Hg, P<0.001) and 88 ± 15 to 77 ± 10 mm Hg (−11 ± 15 mm Hg, P<0.001); and ACEIs, 159 ± 16 to 133 ± 19 mm Hg (−26 ± 20 mm Hg, P<0.001) and 87 ± 9 to 76 ± 12 mm Hg (−11 ± 12 mm Hg, P=0.001). There were very limited number of alterations in antihypertensive drugs after 3 months (8 of 222): two terminations of CCBs, one decrease of CCB, four introductions of low doses of CCBs for patients receiving low-to-medium dose of ARBs and one introduction of atenolol (12.5 mg) for a patient with maximum dose of ARBs.

To determine the difference in receptivity to losartan/HCTZ between specific backgrounds of the patients, we compared BP



	Control	0M	3M	6M	9M	12M
SBP :	157 ± 16	157 ± 16	134 ± 14	133 ± 15	134 ± 14	132 ± 15
DBP :	88 ± 12	88 ± 10	77 ± 9	76 ± 9	78 ± 10	77 ± 9
HR :	74 ± 11	74 ± 12	74 ± 13	74 ± 11	74 ± 12	73 ± 11

Figure 2 The time course of BP in all patients (n=222). ***P<0.001 compared with month 0. BP, blood pressure.



	Control	0M	3M	6M	9M	12M
Maximum dose of ARBs						
SBP :	161 ± 13	159 ± 11	130 ± 13	131 ± 13	130 ± 13	128 ± 10
DBP :	89 ± 12	90 ± 10	75 ± 9	76 ± 8	76 ± 8	75 ± 8
HR :	71 ± 11	70 ± 12	71 ± 12	72 ± 11	72 ± 11	72 ± 12
Low-to-medium dose of ARBs						
SBP :	156 ± 16	156 ± 17	134 ± 15	133 ± 15	135 ± 13	133 ± 15
DBP :	88 ± 11	87 ± 11	77 ± 9	76 ± 10	78 ± 10	77 ± 9
HR :	75 ± 11	74 ± 11	74 ± 13	74 ± 11	74 ± 12	74 ± 10

Figure 3 The time course of BP in patients switched from maximum dose (n=32) and low-to-medium dose (n=169) of ARBs. ***P<0.001 compared with month 0. ARB, angiotensin-receptor blocker; BP, blood pressure.

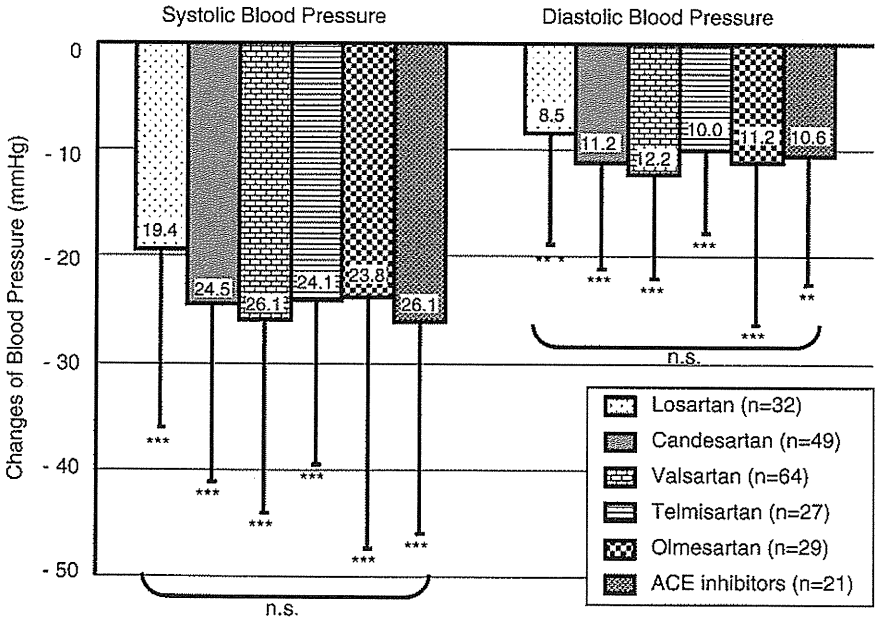


Figure 4 Decreases in BP after 12 months for each pre-prescribed drug. **P<0.01, ***P<0.001 compared with month 0. BP, blood pressure.

changes at 1 year for various subgroups. However, there was no difference among the subgroups and specific factors contributing to resistance against losartan/HCTZ were not detected. For example, if patients are grouped according presence (+) or absence (−) of diabetes (D) and obesity (O) (body mass index, $\geqslant 25\,kg\,m^{-2}$), decreases in systolic BP were 24 ± 18 mm Hg (D+/O+, $n=35$), 23 ± 17 mm Hg (D+/O−, $n=27$), 24 ± 15 mm Hg (D−/O+, $n=63$) and 25 ± 21 mm Hg (D−/O−, $n=97$). This indicates that the losartan/HCTZ combination is effective even for patients with diabetes and obesity.

Remarkable changes were not observed in metabolic parameters after 1 year of treatment with losartan/HCTZ. Figure 5 shows changes in UA levels in all patients (5.46 ± 1.43 to $5.62 \pm 1.43\,mg\,dl^{-1}$) and subgroups with high levels of UA at baseline and others. UA level was slightly increased in patients with relatively low levels of UA (UA $< 7.0\,mg\,dl^{-1}$, middle panel): 5.02 ± 1.11 to $5.37 \pm 1.34\,mg\,dl^{-1}$ ($P<0.001$). But, interestingly, UA level was significantly decreased in patients with high level of UA (UA $\geqslant 7.0\,mg\,dl^{-1}$, right panel): 7.66 ± 0.57 to $6.88 \pm 1.16\,mg\,dl^{-1}$ ($P=0.004$). Other changes (month 0 to 12) concerning parameters in blood tests are summarized in Table 2.

Adverse events were observed in 29 of 266 patients (10.9%) who received the losartan/HCTZ combination, including accidental events, and 16 (5.4%) discontinued the losartan/HCTZ combination, while the remaining 13 patients continued receiving the drug. Among the 16 patients who discontinued, 13 events (4.9%) were considered possibly, probably or definitely drug-related. Laboratory abnormalities were observed for 13 patients. The 13 drug-related adverse events included three cases of hypokalemia, two patients who complained of skin rash, one patient who suffered photosensitive dermatoses, worsening of diabetes in one patient and excessive BP depression in six patients. Four patients of 266 discontinued the losartan/HCTZ combination because of patient circumstances or requests, without adverse events. No death occurred during the study.

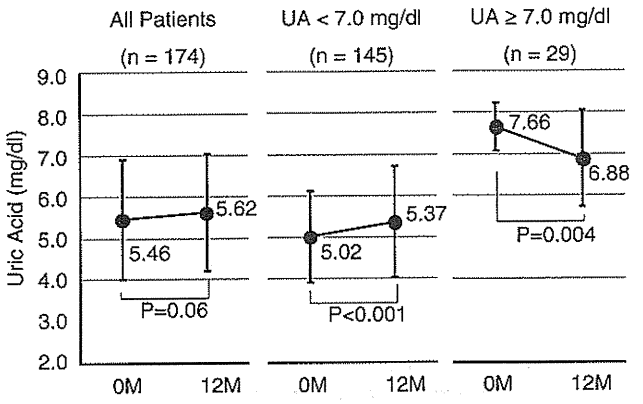


Figure 5 Changes in serum UA levels in all patients (left panel) and in those with high (middle panel) and low-to-medium levels (right panel) of UA. UA, uric acid.

Table 2 Changes of parameters in blood tests

	Month 0	*Month 12*	P-*value*
All patients			
Potassium ($mEq\,l^{-1}$)	4.13 ± 0.48	4.15 ± 0.52	0.67
Total cholesterol ($mg\,dl^{-1}$)	199 ± 34	191 ± 31	0.001
HDL-cholesterol ($mg\,dl^{-1}$)	56.4 ± 14.6	55.1 ± 13.6	0.075
Triglyceride ($mg\,dl^{-1}$)	147 ± 96	149 ± 96	0.74
Creatinine ($mg\,dl^{-1}$)	0.83 ± 0.29	0.88 ± 0.30	<0.001
Glucose ($mg\,dl^{-1}$)	118 ± 46	121 ± 52	0.24
Diabetic patients only (n=52)			
Glucose ($mg\,dl^{-1}$)	154 ± 62	155 ± 73	0.83
HbA1c (%)	6.45 ± 1.22	6.46 ± 1.15	0.91

Abbreviations: ACE, angiotensin-converting enzyme; HDL, high-density lipoprotein.

DISCUSSION

Only 42% of hypertensive patients reached the guideline BP goals in the J-HOME (Japan Home versus Office Blood Pressure Measurement Evaluation) study.[3] Mori *et al.*[4] reported that hypertensive patients attaining BP under 140/90 mm Hg by monotherapy were limited to 34.0% with ARBs and 40.3% with CCBs. Additionally, strict BP goals (130/80 mm Hg) are recommended for hypertensive patients with diabetes, chronic kidney disease and old myocardial infarction.[1] Addition of low-dose diuretics is recommended as a key combination therapy for better BP control in the JSH guideline.[1] However, the prescription rate of diuretics remains low in Japan, for example, 9.3% in the J-HOME study.[9] Additionally, combination therapy with diuretics seems to contribute to organ protection. Many large-scale clinical trials have shown organ-protective effects of losartan, and, importantly, the majority of patients in these trials concomitantly used diuretics, for example, 72% in the LIFE (Losartan Intervention For Endpoint) trial and 84% in the RENAAL (Reduction of Endpoints in NIDDM with the Angiotensin-II Antagonist Losartan) trial.[10,11] Therefore, an acceptable and safe way to introduce low doses of diuretics for hypertension therapy is desirable in Japan.

The losartan/HCTZ combination is composed of losartan, which displays superior activity under the activated renin–angiotensin system[12] and a thiazide-diuretic that activates renin–angiotensin system through a diuretic effect,[13] so this combination is expected to be efficient in BP lowering by the synergistic effect of both the drugs. In this study, BP was decreased by $23 \pm 17/11 \pm 10$ mm Hg at 3 months and $24 \pm 18/11 \pm 11$ mm Hg at 12 months after switching from ARBs or ACEIs alone to the losartan/HCTZ combination for patients who did not reach the BP goal with regimens including ARBs or ACEIs. Similar decreases in BP were observed with all types of pre-prescribed ARBs and ACEIs (Figure 4), and thus these strong and steady decreases in BP seem to depend on the HCTZ 'add-on' effect. Salt intake of the Japanese is relatively high,[14] and thus excess salt may suppress the renin–angiotensin system and disturb the ability of ARBs or ACEIs. In particular, this possibility seems high for patients whose BP was not satisfactorily suppressed by ARBs or ACEIs. Alternatively, HTCZ probably works well in that situation, and this possibility is indirectly supported by evidence that patients pre-using the maximum dose of ARBs showed larger decreases in BP than those using the low-to-medium dose of ARBs following introduction of the losartan/HCTZ combination (Figures 2 and 3). Also this synergistic effect is effective in a comprehensive range of patients; over 90% of patients showed meaningful reductions in diastolic BP ($\geqslant 10$ mm Hg) and 79% of patients reached the BP goals of the JSH guideline, and thus specific cases of diabetes or obesity resistant against losartan/HCTZ combination were not detected.

Diuretics such as HCTZ have been avoided in Japan for fear of their negative effects on metabolic parameters.[4] In particular, hypokalemia and increase in UA are associated with HCTZ. In combination with losartan, hypokalemia may be canceled by the anti-aldosterone effect

of ARBs and UA elevation may be enfeebled by the UA-decreasing ability of losartan. Losartan has a unique effect of stimulating UA excretion in urine by suppressing UA transporters URAT1 and URATv1, with a resulting decrease in the serum levels of UA.[15,16] In this study, these expectations were well achieved and potassium and UA levels were kept within normal ranges. Additionally, a significant decrease in UA was observed for patients with high levels of UA (Figure 5). Except for losartan, clinical doses of ARBs do not have suppressive properties on the UA transporters.[17] This property of losartan should be profitable in combination with HCTZ.

Another concern with HCTZ is worsening of glucose metabolism. A recent cohort study in Taiwan showed that diuretic or β-blocker monotherapy increased the risk of new-onset diabetes, but combination therapies composed of diuretics or β-blocker with ACEI or ARB did not. Conversely, there was a decrease in the risk of new-onset diabetes.[18] In this study, blood glucose and HbA1c levels were stable in patients with diabetes (Table 2), as was glucose level in all patients, and so the losartan/HCTZ combination appears to be safe for glucose metabolism. However, the sensitivity of glucose metabolism under diuretics use could be changed by gene variation,[19] and thus there may be small numbers of susceptible patients. In fact, one patient dropped out because of worsening of diabetes in this study. Therefore, careful monitoring of glucose metabolism is required.

Fixed dose combination drugs decrease the number of pills taken and may contribute to better adherence. Patients on a fixed-combination regimen showed better persistence after 1 year of antihypertensive treatment, namely 58% for combination therapy with ACEI plus diuretics in two pills, and 70% for one-pill fixed combination.[20] In this study, a limited number of patients, 44 of 266 (16.5%), dropped out despite the clinical setting, so this fixed combination could be beneficial in clinical use.

In summary, a fixed dose combination of losartan/HCTZ for 1 year of treatment in a clinical setting resulted in sufficient and steady BP decrease in a majority of Japanese hypertensive patients who had not been controlled with a regimen including ARBs or ACEIs. Also this combination showed acceptable safety and tolerability. A fixed dose combination of losartan/HCTZ is an available tool to introduce low-dose diuretics for treatment of uncontrolled hypertension in Japan.

ACKNOWLEDGEMENTS

This study was supported by foundation for multicenter clinical study of Japan Heart Foundation.

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APPENDIX

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Cancer Science
The official journal of the Japanese Cancer Association JCA

Prospective randomized phase II study determines the clinical usefulness of genetic biomarkers for sensitivity to primary chemotherapy with paclitaxel in breast cancer

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(Received May 17, 2010/Revised August 30, 2010/Accepted August 30, 2010/Accepted manuscript online September 3, 2010/Article first published online September 30, 2010)

In patients with breast cancer, taxane as well as anthracycline play central roles in systemic chemotherapy. By evaluating the pathological response, we can gauge sensitivity to primary chemotherapy. However, biomarkers that would predict a response to taxane have not yet been established. We conducted a prospective randomized trial to evaluate whether selecting patients using sensitivity testing based on the gene expression of the tumor might enhance the probability of the pathological response. Five genes were identified as biomarkers derived from a microarray of DNA gene profiles from microdisected breast tumors. In the experimental arm (B1), 12 cycles of weekly paclitaxel, 80 mg/m^2, were preoperatively given when the sensitivity test was positive and therefore judged to be sensitive to paclitaxel. When the test was negative, meaning insensitive to paclitaxel, four cycles of FEC100 were given (arm B2). In the control arm (A), paclitaxel was administered weekly without the use of the sensitivity test. A total of 92 patients were enrolled and 86 patients were analyzed. The pathological response rate (pRR) of each arm was 36.4% in B1 (expected sensitive to paclitaxel), 21.1% in A (control) and 12.5% in B2, respectively. Weekly paclitaxel-treated patients selected by the sensitivity test did not enhance the pRR. The study failed to validate sensitivity testing using five gene expressions for primary chemotherapy with paclitaxel in patients with breast cancer. However, this study suggests that a randomized phase II study is a robust tool for obtaining a rapid conclusion on the usefulness of biomarkers and could be the foundation for further large clinical trials. (*Cancer Sci* 2011; 102: 130–136)

Trastuzumab, a molecular targeted agent, has greatly improved the survival rate in patients with breast cancer.[1] Trastuzumab binds human epidermal growth factor receptor type 2 (HER2) and downregulates cell proliferation signaling. Trastuzumab enriches its activity by selecting patients with HER2-overexpressed breast cancer. Biomarkers can both maximize activity and minimize toxicities. Cytotoxic agents such as taxane or anthracycline also play a crucial role in systemic chemotherapy for breast cancer.[2] To date, no specific biomarker of cytotoxic chemotherapeutic agents has been established.

Primary chemotherapy with anthracycline and taxane is standard care for patients with early-stage breast cancers to obtain breast conservation and survival benefit.[3] Primary chemotherapy informs us of its sensitivity by evaluation of the pathological response. The probability of a pathological complete response (pCR) from a single administration of taxane is no more than 20%.[4] In our experience, primary treatment with paclitaxel weekly produced a 7% pCR with complete disappearance of intraductal lesions and a 30% pathological response with more than two-thirds reduction in invasive lesions.[5] Taxane induces microtubule bundling, formation of multipolar spindles, mitotic arrest and apoptosis. Resistance to taxane derives from overexpression of ATP-binding cassette (ABC) transporter, for example, P-glycoprotein,[6] somatic mutation of β-tubulin,[7] βIII-tubulin isoform[8] or low expression of tubulin-binding protein tau.[9] However, the clinical usefulness of these biomarkers has not been determined. The DNA microarray provides a unique molecular portrait or signature regarding clinical behavior and drug responsiveness.[10–14] The expression pattern of selected genes, if found to be related to the sensitivity of cytotoxic agents, could yield a biomarker to predict the clinical response and outcome. We have developed a sensitivity test using quantitative RT-PCR of five selected genes to predict the response to paclitaxel. Commonly, retrospective studies have been used to find predictive biomarkers, but their level of evidence is low. To our knowledge, there have been few randomized trials directly addressing biomarkers in a prospective fashion.

Therefore, we have conducted a prospective randomized trial on whether the selection of patients using a sensitivity test to predict paclitaxel based on the gene expression of the tumor might enhance the probability of the pathological response. The current study aimed to validate the genetic diagnosis to predict sensitivity in primary chemotherapy with paclitaxel in women with breast cancer.

Materials and Methods

Patients. Eligible patients were women with histologically confirmed invasive carcinomas of the breast with a tumor size 3 cm or more in stages IIA, IIB, IIIA or IIIB (T1-4, N0-1 and M0). All patients were younger than 70 years and had performance status (Eastern Cooperative Oncology Group performance status) 0 or 1; life expectancy 6 months or more; adequate organ function; white blood cell count 4.0×10^9/L or absolute neutrophil count 2.0×10^9/L; hemoglobin 9 g/dL; platelets 100×10^9/L; blood urea nitrogen (BUN) and serum creatinine within normal limits; aspartate transaminase (AST), alanine transaminase (ALT) twice the upper limit of normal; total bilirubin 1.5 mg/dL; and electrocardiography (ECG) within normal limits. Excluded patients were those with

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Name of the trial register: Validation of genetic diagnosis to predict sensitivity in primary systemic chemotherapy with paclitaxel in women with breast cancer. Registration number: C000000413, UMIN Clinical Trials Registry.

doi: 10.1111/j.1349-7006.2010.01740.x

Table 1. Five genes identified as biomarkers

Gene ID	Affy probe	GenBank	UniGene	Gene Symbol	Uni-title
03921	223235	NM022138	Hs.487200	SMOC2	Secreted protein, acidic, cystein-rich related modular calcium binding 2
05918	NA	BG928645	Hs.494395	C9orf121	Chromosome 9 open reading frame 121
06334	205009	NM003225	Hs.162807	TFF1	Trefoil factor 1
19403	224968	NM080667	Hs.264208	CCDC104	Coiled-coil domain containing 104
20850	229580	BX097190	Hs.7413	NA	Transcribed locus

NA, not applicable.

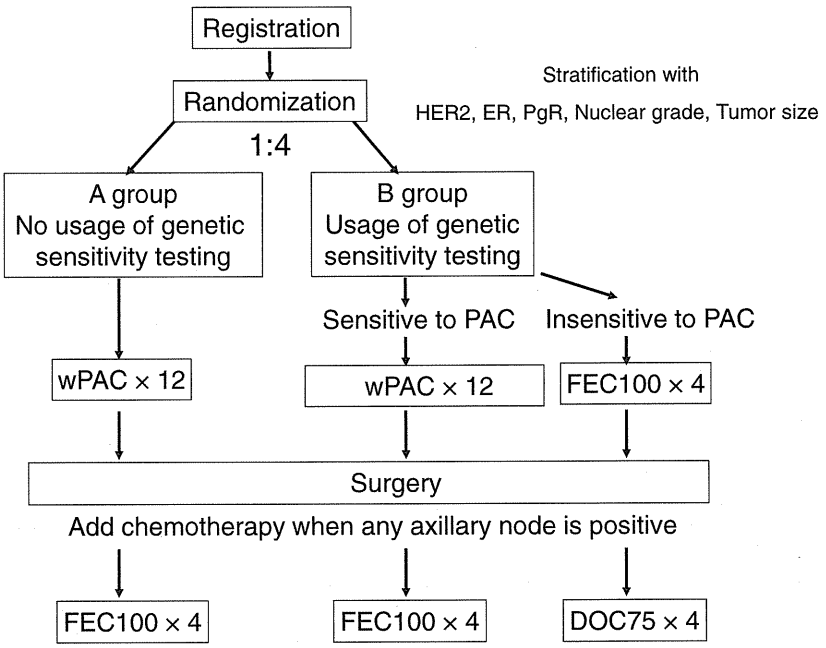

Fig. 1. Study design. Patients were stratified according to the status of human epidermal growth factor receptor type 2 (HER2), estrogen receptor (ER), progesteron receptor (PgR), nuclear grade and tumor size. Patients were randomly assigned to receive arm A or B with a ratio of 1:4. In arm A, patients received primary chemotherapy with paclitaxel without selection by genetic sensitivity testing. For patients in arm B, we used the genetic diagnosis for sensitivity to paclitaxel. In arm B1, patients diagnosed as sensitive to paclitaxel received paclitaxel. In arm B2, patients diagnosed as insensitive to paclitaxel received FEC100. When any axillary node was positive for cancer after curative breast surgery, additional chemotherapy was used. Patients pretreated with paclitaxel received FEC100. Patients pretreated with FEC100 received docetaxel. wPAC × 12, 12 cycles of weekly paclitaxel 80 mg/m^2; FEC100 × 4, four cycles of combination chemotherapy with fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2 and cycrophosphamide 500 mg/m^2 every 3 weeks; DOC75 × 4, four cycles of docetaxel 75 mg/m^2 every 3 weeks.

non-invasive or microinvasive breast cancer, stage IIIC or IV; inflammatory breast cancer; male gender; previous chemotherapy, hormone therapy or radiotherapy; active double cancer; serious complication with infection, cardiac disease, pulmonary fibrosis, interstitial pneumonitis, bleeding, hepatitis type B and its carrier; uncontrolled diabetes; heavy history of drug allergy, history of allergic reaction to drugs using the vehicle cremophor; pregnant, nursing or willing to become pregnant; or otherwise judged inadmissible by the investigators. The research ethics committee of Cancer Institute Hospital approved the study, and all patients gave written, informed consent.

Sensitivity testing. How the sensitivity testing was developed has been described in previous papers.[15,16] Basically, specimens were obtained by core needle biopsy before primary chemotherapy. To minimize the influence of stromal cells, pure populations of tumor cells were collected by laser captured microdissection. After RNA extraction, we performed gene expression profiling of 21 000 genes by DNA microarray to select the candidate genes. Surgically resected primary breast tumors were examined to determine the pathological response to chemotherapy. All clinical and genomic data were entered into an integrated database and analyzed to identify predictive factors. Differentially expressed genes were selected between the paclitaxel-resistant group and the paclitaxel-sensitive group. Then the expression of selected candidate genes was quantified by RT-PCR to confirm the array data and increase reliability. Furthermore, we narrowed the candidate genes down to establish a prediction system based on real-time RT-PCR. Finally, we identified a set of five genes predictive of patient response to paclitaxel in primary chemotherapy (Table 1). Before clinical application, the prediction system was validated retrospectively, revealing that in 51 patients the sensitivity testing using the expression of five genes produced 90% accuracy and a 9.8% error rate.

Study design. To validate the predictiveness of the pathological response by the sensitivity test in primary chemotherapy with paclitaxel, we conducted a prospective randomized trial, as shown in Fig. 1. Patients were stratified according to the status of HER2, estrogen receptor (ER), progesteron receptor (PgR), nuclear grade and tumor size. Participating patients were randomly assigned to receive arm A or B with a ratio of 1:4. For patients in arm A, we did not use the genetic diagnosis for sensitivity to paclitaxel, but they received primary chemotherapy with paclitaxel. For patients in arm B, we did use the genetic diagnosis for sensitivity to paclitaxel. When patients were diagnosed as sensitive to paclitaxel, they received primary chemotherapy with paclitaxel. Patients diagnosed as insensitive to paclitaxel received primary chemotherapy with FEC100.

Unless their disease progressed, patients were treated with 12 weeks of paclitaxel or four cycles of FEC100 and then underwent standard surgery. When any axillary node was positive for cancer, additional chemotherapy was used after surgery. Patients pretreated with paclitaxel received FEC100 and those pretreated with FEC100 (diagnosed as insensitive to paclitaxel) received docetaxel after surgery. In a partial resection of the breast, radiation was performed. If cancer was positive in four or more axillary nodes, prophylactic radiation was performed to the chest and regional nodes. Radiation was applied after completion of chemotherapy. In cases with positive estrogen receptor and/or progesterone receptor, appropriate endocrine

treatment of tamoxifen or aromatase inhibitors was used after completion of chemotherapy. Patients with HER2-overexpressed breast cancer received tri-weekly trastuzumab at a dose of 8 mg/kg followed by 6 mg/kg for 1 year after completion of surgery or postsurgical chemotherapy. Adjuvant trastuzumab was used subsequent to February 2008, which was the approval date in Japan.

Treatment. Paclitaxel was administrated at a dose of 80 mg/m^2 as an intravenous infusion over a period of 1 h every week for 12 weeks. Dexamethasone 10 mg, ranitidine 50 mg and granisetoron 3 mg were given intravenously 30 min before paclitaxel. Diphenhydramine 50 mg was given orally just before infusion. FEC100 consisting of fluorouracil (500 mg/m^2), epirubicin (100 mg/m^2) and cyclophosphamide (500 mg/m^2) was administered intravenously every 3 weeks for four cycles. Dexamethasone 20 mg and granisetoron 3 mg were given intravenously before FEC100. Docetaxel was intravenously administrated at a dose of 75 mg/m^2 for a 1 h infusion every 3 weeks for four cycles. Dexamethasone 8 mg was given as an intravenous infusion on day 1 followed by oral intake on days 2 and 3.

End-points. The primary end-point targeted improvement of the pathological response rate (pRR) as the percentage of patients with grade 2 and 3 as shown by sensitivity testing. The pathological response with grade 2 or 3 was defined as more than a two-third reduction in invasive lesions or complete disappearance of tumors, including intraductal lesions, respectively.[17] Secondary end-points examined the pathological complete response rate (probability of pathological response with grade 3), clinical response rate by the Response Evaluation Criteria in Solid Tumors guidelines (RECIST),[17] breast conservation rate, disappearance rate of axillary node metastasis, distant-metastasis-free survival, disease-free survival and overall survival. Adverse events and laboratory parameters were graded according to the National Cancer Institute, Common Toxicity Criteria, version 3.0.

Statistical analysis. Validity was defined as accuracy of the prediction system for sensitivity testing. Improvement of the pathological response was judged as high accuracy of the prediction system for sensitivity testing. The pathological response rates to paclitaxel in patients who were diagnosed as positive by sensitivity testing were compared with those in patients treated with paclitaxel who did not receive sensitivity testing. The difference in response rate in the two groups was assessed by the Fisher exact test for 2 × 2 contingency tables. The pathological response rate in the experimental arm was estimated as 80% compared with 30% in the control arm, which was calculated from 29% (15/51) of the pathological response rate in previous unpublished data. A sample size of 21 assessable patients in each arm (A and B1) was required to achieve 90% power with 5% error (two sided). A sample size of arm B (B1 + B2) required 72 (21 × 100/29) patients. The number of cases that dropped out for any reason including inadequate sampling was estimated as 15%. A total of 109 patients were required in the current study. Patients were randomly assigned to receive arm A or B with a ratio of 1:4. An interim analysis was planned when at least 10 pathological assessable patients were obtained in arm B1. Disease-free survival and overall survival were calculated by the Kaplan–Meier method.

Results

Patient characteristics. Ninety-two patients were registered and assessed between February 2006 and February 2009 at the Cancer Institute Hospital. Six patients had too few tumor specimens to evaluate sensitivity testing. Eighty-six patients were randomized. In two patients, we were not able to assess the pathological response in the resected breast tumors, because of progression during primary chemotherapy and a withdrawal of consent to additional post-surgical chemotherapy. A total of 85 patients were assessed for pathological response at surgery. The median follow-up time of patients was 40.0 months, and the range was 17.0–49.8 months. All patients were Japanese women. The demographic characteristics of the present study population are presented in Table 2. The median age was 52.5 years (range, 31–68). Median size of tumor estimated as an invasive lesion was 3.75 cm (range, 3.0–9.9). While 81% of patients were T2, 41% of patients had no clinical axillary lymph node metastasis. Histology showed papillotubular carcinoma (8%), solid-tubular carcinoma (24%) or scirrhous carcinoma (65%). In 24% of patients, we found nuclear grade 3. Estrogen receptor or PgR was positive in 71% or 47% of patients, respectively. Positive HER2 status was defined as immunohistological (Hercep test) score 3+ (>10%) or FISH positive (ratio >2.0). Twenty-one percent of patients were HER2 positive. Intrinsic subtypes were divided as follows. Luminal A was defined as negative HER2 status with ER positive and/or PgR positive. Luminal B was defined as positive HER2 status with ER positive and/or PgR positive. HER2 subtype was positive HER2 status with both ER and PgR negative. Triple negative was HER2 negative, ER negative and PgR negative. Luminal A, Luminal B, HER2 subtype or triple negative was 64%, 8%, 13% or 15%, respectively. The background of arms A and B (B1 + B2) was mostly balanced except for a slight tendency towards more patients with papillotubular carcinoma, HER2 positive or luminal B, and fewer patients with grade 3 in arm A. The background in arms A and B1 was different because of selection by sensitivity testing.

Pathological response and clinical outcome. Interim analysis was performed after 11 patients were assessable for pathological response in arm B1. As shown in Table 3, the patients in arm B1, diagnosed as sensitive to paclitaxel, demonstrated 36.4% (4/11) of the pathological response rate, whereas patients who did not use sensitivity testing of paclitaxel showed 21.1% (4/19). The difference between arms A and B1 was not significant ($P = 0.627$). Since the pathological response rate (36.4%) of the experimental arm (B1) was far below the expected rate of 80% despite achievement with 82% (89/109) of the planned accrual number, the committee decided to terminate the study. In arm B2, the patients who were treated with FEC100 judged as insensitive to paclitaxel showed 12.5% (7/56) of the response rate. A pathological complete response was seen in 3.6% (2/56) of FEC100 (B2), but no complete response in the paclitaxel arms (A1 or B1). Pathological metastasis in resected lymph nodes at surgery was absent in 27% (3/11) of arm B1, in which the mean number of pathological positive nodes was 3.8. In one out of four pathological responders with grade 2 and 3, all axillary nodes disappeared. The clinical response rate of the paclitaxel-sensitive group (B1) was not improved at 55% (6/11) as compared with 53% (10/19) of the control arm (A) or 54% (30/56) in patients who were treated with FEC100 (B2). The breast conservation rate was not improved at 36% in arm B1, compared with arm A (32%) or arm B2 (52%). Disease-free survival and overall survival at 3 years in all patients ($n = 86$) were 81.2% and 94.6%, respectively (Fig. 2). Disease-free survival at 3 years in arms A, B1 and B2 was 72.3%, 62.3% and 87.6%, respectively. Adverse events are summarized in Table 4. One patient, who dropped out after five cycles of preoperative paclitaxel, was excluded to evaluate toxicity. A total of 85 patients were assessed for toxicity. Grade 3 or 4 of adverse events in the preoperative paclitaxel (A + B1) or FEC100 (B2) was 1.0% and 8.1%, respectively. There was no difference in the profile of adverse events between arms A and B (data not shown). No unexpected adverse events were observed.

doi: 10.1111/j.1349-7006.2010.01740.x

Table 2. Patient characteristics

Sensitivity testing	A	B1	B2	Subtotal of patients in B (B1 + B2)	All patients
	Not performed	Performed			
		Sensitive to paclitaxel	Insensitive to paclitaxel		
Treatment	Paclitaxel	Paclitaxel	FEC100		
No. randomized patients	19	11	56	67	86
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Median age	50	57.0	52.0	53.0	52.5
T					
T2	14 (74)	8 (73)	48 (86)	56 (84)	70 (81)
T3	4 (21)	3 (27)	5 (9)	8 (12)	12 (14)
T4	1 (5)	0 (0)	3 (5)	3 (4)	4 (5)
Median size (cm)	3.6	4.2	3.6	3.8	3.75
Range (cm)	3.0–5.7	3.0–5.8	3.0–9.9	3.0–9.9	3.0–9.9
N					
0	7 (37)	4 (36)	24 (43)	28 (42)	35 (41)
1	11 (58)	6 (55)	31 (55)	37 (55)	48 (56)
2	1 (5)	1 (9)	1 (2)	2 (3)	3 (3)
Stage					
IIA	7 (37)	4 (36)	25 (45)	29 (43)	36 (42)
IIB	7 (37)	4 (36)	23 (41)	27 (40)	34 (40)
IIIA	4 (21)	3 (27)	6 (11)	9 (13)	13 (15)
IIIB	1 (5)	0 (0)	2 (3)	2 (3)	3 (3)
Histology					
Invasive ductal carcinoma					
Papillotubular carcinoma	3 (16)	0 (0)	4 (7)	4 (6)	7 (8)
Solid tubular carcinoma	4 (21)	6 (55)	11 (20)	17 (25)	21 (24)
Scirrhous carcinoma	11 (58)	5 (45)	40 (71)	45 (67)	56 (65)
Others	1 (5)	0 (0)	1 (2)	1 (1)	2 (2)
Nuclear grade					
1	10 (53)	1 (9)	34 (61)	35 (52)	45 (52)
2	5 (26)	3 (27)	11 (20)	14 (21)	19 (22)
3	4 (21)	7 (64)	10 (18)	17 (25)	21 (24)
Undetermined	0 (0)	0 (0)	1 (2)	1 (1)	1 (1)
Estrogen receptor					
Positive	12 (63)	2 (18)	47 (84)	49 (73)	61 (71)
Negative	7 (37)	9 (82)	9 (16)	18 (27)	25 (29)
Progesterone receptor					
Positive	10 (53)	0 (0)	30 (54)	30 (45)	40 (47)
Negative	9 (47)	11 (100)	26 (46)	37 (55)	46 (53)
HER2					
Positive (IHC 3+ or FISH+)	6 (32)	4 (36)	8 (14)	12 (18)	18 (21)
Negative	13 (68)	7 (64)	48 (86)	55 (82)	68 (79)
Intrinsic subtype					
Luminal A	10 (53)	2 (18)	43 (77)	45 (67)	55 (64)
Luminal B	3 (16)	0 (0)	4 (7)	4 (6)	7 (8)
HER2 subtype	3 (16)	4 (36)	4 (7)	8 (12)	11 (13)
Triple negative	3 (16)	5 (45)	5 (9)	10 (15)	13 (15)

FISH, fluorescence *in situ* hybridization; HER2, human epidermal growth factor receptor type 2; IHC, immunohistochemistry.

Discussion

The current study failed to validate the sensitivity of testing using the expression of five genes. However, we became aware of the importance of deciding how to incorporate a new biomarker into clinical practice. Evidence levels of a biomarker are commonly derived from retrospective studies,[18,19] which harbor strong bias due to differing backgrounds. A large cohort or meta-analysis is mandatory to establish usefulness. Prospective trials to evaluate biomarkers have rarely been reported. Simon and simon *et al.* have proposed a refined guideline system for biomarker studies.[20,21] The guideline indicates that level 1 evidence may permit reproducible positive results from high-quality retrospective studies using archived specimens in the prospective trials addressing therapeutic questions, but not biomarkers. However, a prospective trial that would directly address biomarkers is still the gold standard to achieve level 1 evidence. Designing randomized trials for biomarkers presents several challenges.[21] One involves the therapeutic question of accommodation of biomarkers, such as the Tailor X trial of the 21-gene classifiers. The other involves the biomarker question, such as microarray testing of the 70-gene classifier. However,

Table 3. Response and clinical outcome

Sensitivity testing	A	B1	B2	Subtotal of patients in B (B1 + B2) (n = 67)	All patients (n = 86)
	Not performed	Performed			
		Sensitive to paclitaxel	Insensitive to paclitaxel		
Treatment	Paclitaxel (n = 19)	Paclitaxel (n = 11)	FEC100 (n = 56)		
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Pathological response Grade 2 + Grade 3	4 (21.1)	4 (36.4)	7 (12.5)	11 (16.4)	15 (17.4)
Pathologically free metastasis in resected lymph nodes	7 (36.8)	3 (27)	26 (46.4)	29 (43.2)	36 (41.8)
Mean no. pathological positive nodes	3.9	3.8	2.2	2.5	2.8
Pathological disappearance of axillary nodes in pathological responders (grade 2 + 3)	3 (75)	1 (25)	5 (71)	6 (55)	9 (60)
Clinical response (RECIST) CR + PR	10 (53)	6 (55)	30 (54)	36 (54)	46 (54)
Breast conservation	6 (32)	4 (36)	29 (52)	33 (49)	39 (45)

RECIST, response evaluation criteria in solid tumors.

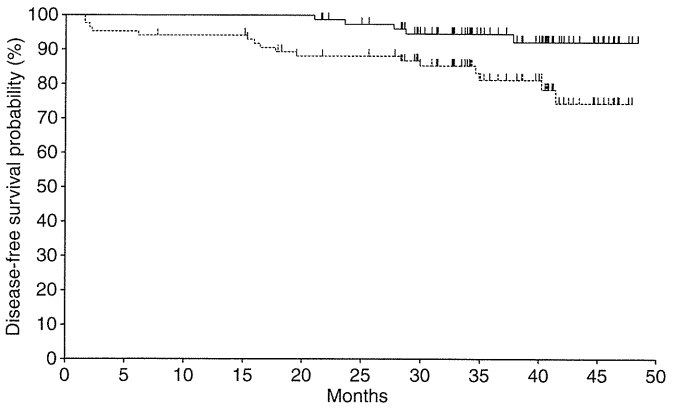

Fig. 2. Kaplan–Meier plot for disease-free survival (dashed line) and overall survival (solid line) in all randomized patients (n = 86).

these trials require a large number of patients to arrive at a definitive conclusion. It is difficult to conduct such a large trial for all possible biomarkers. A relatively smaller number of patients, approximately 100 like in this study, could be reasonable for evaluation in biomarker study design.

The current study failed to enrich responsive patients to treatment with paclitaxel. We expected that prediction of a pathological response would be more than 80% of sensitivity in the new testing. Unexpectedly, the pathological response of the experimental arm was as low as 36.4%. Since the pathological response rate, 21.1%, of the control arm was also lower than 30% as expected, performing the interim analysis in this study took a long time. We decided to terminate the study because we considered that the enrichment of response by sensitivity testing should be minimally more than 50% for clinically meaningful usage or further evaluation by a randomized large phase III study. We did not plan to address the specificity of gene testing, because the specificity could not be yielded from the data of arm B (B1 + B2). The reason was that patients in arm B2, who were judged as insensitive to paclitaxel, did not receive paclitaxel from an ethical point of view. However, we were able to examine the specificity of gene testing in arm A. In arm A (n = 19), 18 patients could be evaluated by gene testing, because of one sampling that contained no cancer cells. Twelve out of 15 patients who failed to obtain a pathological response exhibited as insensitive to paclitaxel by the gene testing. Therefore, the specificity resulted in 80% (12/15). One out of three patients who achieved a pathological response were revealed as sensitive to paclitaxel by the testing. The sensitivity of arm A resulted in 33.3% (1/3), which was similar to that of arm B1 (36.4%, 4/11). The present study aimed to examine whether the gene testing improved sensitivity, but not specificity. The number was too small to obtain a definitive result of specificity. The current study failed to show an enhanced response rate. However, if we conducted a phase II study with a single arm, we would not have been able to obtain such a clear conclusion as early as we did. Therefore, a small randomized study appears to be a robust tool in obtaining a rapid conclusion to evaluate the usefulness of biomarkers.

The methodology of this randomized trial might need further discussion. We wanted to determine whether the selection of patients by new testing could be useful. Thus, we considered that the selection by itself should be randomized. Namely, we compared the outcome for patients who were selected by testing with that of patients who were not selected. This is different from randomized trials that compare a new treatment with a standard therapy. Unbalanced randomization at a 1:4 ratio would minimize the number of patients in control arm A who were not selected by testing. Patients who wished to receive extensive, maximal primary chemotherapy did not enter this trial. One patient withdrew from this trial during her primary chemotherapy because she wanted to receive additional primary chemotherapy. Patients who wished to receive minimal chemotherapy were likely to participate in this trial. Patients with incomplete clearance of axillary tumors could receive additional chemotherapy after surgery. This ethical issue was discussed and approved by institutional review board.

In the current study, the clinical response rate and the conservation rate of the breast were 55% and 36%, respectively, with 27% of patients free from pathological metastasis in resected

doi: 10.1111/j.1349-7006.2010.01740.x

Table 4. Adverse events following preoperative chemotherapy

	Paclitaxel (n = 29)			FEC100 (n = 56)		
	Any grade	Grade 3	Grade 4	Any grade	Grade 3	Grade 4
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Anorexia	6 (21)	0	0	25 (45)	1 (2)	0
Fatigue	22 (76)	1 (3)	1 (3)	44 (79)	2 (4)	0
Nausea	7 (24)	0	0	36 (64)	0	0
Vomiting	2 (7)	0	0	23 (41)	4 (7)	0
Diarrhea	11 (38)	0	0	13 (23)	3 (5)	0
Constipation	15 (52)	0	0	31 (55)	1 (2)	0
Mucositis	0 (0)	0	0	13 (23)	1 (2)	0
Dysgeusia	2 (7)	0	0	4 (7)	0	0
Peripheral neuropathy	26 (90)	1 (3)	0	16 (29)	0	0
Alopecia	29 (100)	NA	NA	56 (100)	NA	NA
Hand-foot syndrome	0 (0)	0	0	2 (4)	0	0
Rash	3 (10)	0	0	1 (2)	0	0
Allergic reaction	1 (3)	0	0	0	0	0
Itching	1 (3)	0	0	0	0	0
Phlebitis	0 (0)	0	0	1 (2)	0	0
Myalgia	2 (7)	0	0	0	0	0
Infection	2 (7)	0	0	13 (23)	5 (9)	2 (4)
Febrile neutropenia	0 (0)	0	0	7 (13)	2 (4)	0
Leukopenia	19 (65)	1 (3)	0	53 (95)	28 (50)	15 (27)
Neutropenia	14 (48)	3 (10)	0	53 (95)	2 (4)	49 (88)
Anemia	15 (52)	0	0	37 (66)	2 (4)	0
Thrombocytopenia	0 (0)	0	0	3 (5)	1 (2)	0
AST elevation	15 (52)	0	0	23 (41)	0	0
ALT elevation	13 (45)	1 (3)	0	22 (39)	0	0
Total bilirubin elevation	3 (10)	0	0	3 (5)	0	0
Creatinine elevation	1 (3)	0	0	1 (2)	0	0
Hyperglycemia	3 (10)	0	0	14 (25)	0	0
All events	212 (27.0)	7 (0.9)	1 (0.1)	494 (32)	52 (3.6)	66 (4.5)

ALT, alanine transaminase; AST, aspartate transaminase; NA, not applicable.

lymph nodes. These results were not satisfactory. However, the study did not aim to improve breast conservation and clearance of axillary metastasis, but rather aimed to minimize exposure to cytotoxic chemotherapy for those sensitive to chemotherapy. Patients with axillary nodes involved were treated by adding adjuvant alternative chemotherapy with FEC100 or docetaxel. The results of disease-free survival and overall survival (81.2% and 94.6% at 3 years) were not assessable for further analysis. The safety profile of paclitaxel or FEC100 was similar to previous reports.[3–5] Both treatments were manageable.

In conclusion, the current study failed to validate sensitivity testing using five-gene expression for primary chemotherapy with paclitaxel in patients with breast cancer. However, a small prospective randomized study is useful for reaching a rapid conclusion on the usefulness of biomarkers. We consider that the present trial design is a prospective randomized phase II trial directly addressing the predictive biomarker question. The current compact trial could be a hallmark to proceed to further large clinical phase III trials.

Acknowledgments

This work was supported by funding from the Ministry of Economy, Trade and Industry. The authors thank the participating medical doctors in our institute: Kokoro Kobayashi, Shinichiro Taira, Chizuko Tsutsumi, Tsutomu Sugihara, Masafumi Oto, Mayuko Ito, Takayo Fukuda, Mari Hosonaga, Masaya Hattori, Hiroyoshi Miura, Taijiro Kosaka, Akiko Kuwayama, Rikako Hashimoto, Kenichi Inoue, Yoshie Nakayama, Yoko Chihara, Ippei Fukada, Natsue Uehiro, Aya Hirano, Yumi Miyagi, Seiichiro Nishimura, Kotaro Iijima, Kiyomi Kimura, Hidetomo Morizono, Takehiko Sakai, Reiko Iwasaki, Yoshimi Ide, Tomo Osako, Naoya Gomi, Keiko Nemoto, Akashi Toshiyasu and Masahiko Oguchi. The authors also thank the data managers: Chie Watanabe, Masumi Yamazaki, Chizuru Suizu, Michiko Yago, Kumiko Yamabe and Kaori Kobayashi.

Disclosure Statement

The authors have no conflict of interest.

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doi: 10.1111/j.1349-7006.2010.01740.x

Cancer Science
The official journal of the Japanese Cancer Association JCA

Phase I study of inotuzumab ozogamicin (CMC-544) in Japanese patients with follicular lymphoma pretreated with rituximab-based therapy

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(Received February 25, 2010/Revised April 12, 2010/Accepted April 18, 2010/Accepted manuscript online April 23, 2010/Article first published online May 19, 2010)

Inotuzumab ozogamicin (CMC-544), an antibody-targeted chemotherapeutic agent composed of an anti-CD22 antibody conjugated to calicheamicin, a potent cytotoxic antibiotic, specifically targets the CD22 antigen present in >90% of B-lymphoid malignancies, rendering it useful for treating patients with B-cell non-Hodgkin lymphoma (B-NHL). This phase I study evaluated the safety, tolerability, efficacy, and pharmacokinetics of inotuzumab ozogamicin in Japanese patients. Eligible patients had relapsed or refractory CD22-positive B-NHL without major organ dysfunction. Inotuzumab ozogamicin was administered intravenously once every 28 days (dose escalation: 1.3 and 1.8 mg/m^2). All 13 patients had follicular lymphoma, were previously treated with ≥1 rituximab-alone or rituximab-containing chemotherapy, and were enrolled into two dose cohorts (1.3 mg/m^2, three patients; 1.8 mg/m^2, 10 patients). No patient had dose-limiting toxicities, and the maximum tolerated dose, previously determined in non-Japanese patients (1.8 mg/m^2), was confirmed. Drug-related adverse events (AEs) included thrombocytopenia (100%), leukopenia (92%), lymphopenia (85%), neutropenia (85%), elevated AST (85%), anorexia (85%), and nausea (77%). Grade 3/4 drug-related AEs in ≥15% patients were thrombocytopenia (54%), lymphopenia (31%), neutropenia (31%), and leukopenia (15%). The AUC and C_{max} of inotuzumab ozogamicin increased dose-dependently with pharmacokinetic profiles similar to non-Japanese. Seven patients had complete response (CR, 54%) including unconfirmed CR, four patients had partial response (31%), and two patients had stable disease (15%). The overall response rate was 85% (11/13). Inotuzumab ozogamicin was well tolerated at doses up to 1.8 mg/m^2 and showed preliminary evidence of activity in relapsed or refractory follicular lymphoma pretreated with rituximab-containing therapy, warranting further investigations. This trial was registered in ClinicalTrials.gov (NCT00717925). (*Cancer Sci* 2010; 101: 1840–1845)

The successful use of monoclonal antibodies (mAbs) in the treatment of human diseases has been growing steadily in the past decade. Rituximab, a human-mouse chimeric anti-CD20, unconjugated antibody, was approved in 1997 in the USA as the first mAb for antilymphoma therapy. It is now most commonly used in combination with chemotherapy for first and subsequent lines of therapy in B-cell non-Hodgkin lymphoma (B-NHL), such as diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL).[1–6] However, a subgroup of patients does not respond, and early relapses occur in patients with initial response, thus indicating rituximab resistance. This indicates a clear unmet need to explore alternative antibodies non-cross resistant to rituximab as a therapy for B-NHL. One alternative is inotuzumab ozogamicin (CMC-544), an antibody-targeted chemotherapy agent that specifically targets CD22. Inotuzumab ozogamicin is composed of a recombinant engineered humanized IgG4 anti-CD22 antibody G544 conjugated to calicheamicin, a potent cytotoxic antibiotic derivative.[7]

CD22 is a potential therapeutic target for B-NHL because it is expressed in >90% of B-NHL cells.[8] In addition, CD22 is expressed in mature B cells, but not in their precursor or memory B cells, which may potentially minimize the adverse effect of CD22-targeted treatment on long-term immune function. Moreover, when antibodies bind to the CD22 antigen, the antigen is internalized, that is it is not shed into the extracellular environment.[9]

Both inotuzumab ozogamicin and unconjugated calicheamicin showed potent cytotoxic activity *in vitro* against CD22-positive B cells in preclinical studies.[7] In addition, the unconjugated form of inotuzumab ozogamicin, G544, did not demonstrate any antitumor activity in preclinical studies.[7] Inotuzumab ozogamicin inhibited the growth and the establishment of B-cell lymphomas and induced the regression of large B-cell lymphomas in mouse xenograft models.[7] Furthermore, in preclinical models of disseminated B-NHL in which rituximab was ineffective, treatment with inotuzumab ozogamicin lead to a significant tumor regression and an improvement in survival.[10] This potent cytotoxic activity in preclinical murine models of B-cell lymphomas in which rituximab had failed as a therapeutic agent[11] establishes support for the clinical investigation of inotuzumab ozogamicin for the treatment of CD22-positive B-NHL.

A phase I dose escalation study was previously conducted in the USA and the European Union in patients with relapsed or refractory B-NHL (both FL and DLBCL).[12] In this study, intravenous administration of the drug demonstrated clinical activity in patients with relapsed or refractory B-NHL with clinically manageable thrombocytopenia as the main toxicity. The maximum tolerated dose (MTD) in this non-Japanese patient population was determined to be 1.8 mg/m^2 once every 4 weeks.

The objectives of the present study were to assess the safety, toleralility, efficacy, and pharmacokinetics of inotuzumab ozogamicin in Japanese patients with relapsed or refractory B-NHL who had received prior treatment with rituximab.

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doi: 10.1111/j.1349-7006.2010.01601.x

Materials and Methods

Study design. The present trial was an open-label multicenter phase I study in which inotuzumab ozogamicin was administered intravenously (IV) as a single agent to patients with CD22-positive B-NHL once every 28 days (±2 days, 1 cycle) for at least four doses provided that the drug was well tolerated with no evidence of progressive disease (PD). The protocol was approved by the Institutional Review Board of each participating institution, and it conformed to the provisions of the Declaration of Helsinki in 1995 (as revised in Tokyo, 2004). All the patients gave written informed consent.

Patients. Patients were eligible for enrollment if they had a diagnosis of CD22-positive B-NHL, according to the World Health Organization (WHO) classification, version 3.[13] Patients were included if they had progressed after at least one prior chemotherapy regimen for indolent B-NHL, or after one or two chemotherapy regiments, which included anthracyline or anthraquinone for aggressive B-NHL. Other inclusion criteria were age ≥20 and <75 years, a performance status of one or better on the Eastern Cooperative Oncology Group Scale, life expectancy ≥12 weeks, an absolute neutrophil count (ANC) $\geq 1.5 \times 10^9$/L and platelet count $\geq 100 \times 10^9$/L, serum creatinine ≤1.5 × upper limit of normal (ULN), urine protein-to-creatinine ratio of ≤0.2, total bilirubin ≤1.5 × ULN, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤2.5 × ULN, and at least one measurable lesion ≥1.5 cm in at least one dimension by computer tomography (CT) at inclusion, in an area of no prior radiation therapy, or clear progression in an area that had been previously irradiated.

Dose escalation and toxicity criteria. Dose escalation decisions were based on the toxicities observed in the first 28 days after the administration of the first dose. Patients (three and 10 patients per cohort) could receive more than the four planned doses of inotuzumab ozogamicin if they experienced at least stable disease and tolerated treatment. The starting dose was 1.3 mg/m^2 administered IV once every 28 days, and dose escalation was performed up to the MTD of 1.8 mg/m^2 administered IV once every 28 days. Both the starting dose and the MTD were based on information from a previous clinical trial.[12] The dose escalation in subsequent cohorts was based on the toxicity assessed in the first 28 days after the first dose. Dose escalation continued until three or more patients in a cohort experienced a dose-limiting toxicity (DLT).

A DLT was defined as any of the following that were at least possibly related to inotuzumab ozogamicin during the first 28 days after the first dose: any grade 3 or 4 (National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTC], version 3.0) nonhematologic toxicity (except grade 3 alopecia, nausea, or vomiting unless the patient was receiving optimal medical therapy); febrile neutropenia (grade 4 ANC ≥3-day duration and temperature ≥38.0°C); grade 4 ANC ≥7-day duration; grade 4 thrombocytopenia ≥3-day duration, or any bleeding episode requiring platelet transfusion; or delayed recovery (to grade 1 or baseline, except alopecia or grade 2 nausea or vomiting unless the patient was receiving optimal medical therapy) from a toxicity related to inotuzumab ozogamicin that delayed the initiation of the next dose by more than 3 weeks. Patients who experienced a DLT had the subsequent doses of inotuzumab ozogamicin reduced by one dose level, the maximum allowed dose reduction per patient. Patients who experienced toxicities other than DLTs could receive additional doses of inotuzumab ozogamicin at the same dose if they met the following criteria: recoveries to ≤grade 1 (nonhematologic), or baseline toxicity except alopecia; ANC $\geq 1.5 \times 10^9$/L; platelet count $\geq 75 \times 10^9$/L; serum creatinine ≤1.5 × ULN, and urine protein-to-creatinine ratio of ≤0.2. The maximum number of doses of inotuzumab ozogamicin was 8 for 1.3 mg/m^2 and 7 for 1.8 mg/m^2.

Pharmacokinetics. Timed blood samples for pharmacokinetic analysis were collected for cycles 1–3 at 0 (pre-dose), 1, 4 (cycles 1 and 3 only), 24, 48, 120, 168, 216, 336, and 504 h relative to the start of infusion for each dosing period and at predose only for cycle 4. If the patient received four doses, then the sample had to be drawn before cycle 5. The serum concentrations of inotuzumab ozogamicin and total calicheamicin were determined using a validated enzyme-linked immunosorbent assay.

The noncompartmental pharmacokinetic parameters of inotuzumab ozogamicin and total calicheamicin were estimated using the WinNonlin (version 4.1) program. The parameters which were determined included the following: end-of-infusion peak concentration (C_{max}), area under the concentration-time curve (AUC), clearance (CL), apparent steady-state volume of distribution (V_{ss}), and the terminal-phase elimination half-life ($t_{1/2}$).

Safety. An AE was considered to be treatment emergent if its onset occurred between the first and the last dose, plus a lag of 28 days provided the following criteria were met: (i) the AE was not present before the start of the first dose and did not occur in the patient as a chronic condition; (ii) the AE was present before the start of the first dose or was part of the patient's medical history, but the severity or frequency increased after the start of the first dose.

Efficacy. Patients were evaluable for efficacy if they received ≥2 doses of inotuzumab ozogamicin, had a baseline tumor CT scan and had undergone at least one tumor assessment for response after baseline assessment. In addition, patients with documented PD prior to receiving two doses of inotuzumab ozogamicin were considered evaluable for efficacy. Tumor response was assessed according to the International Workshop Response Criteria for Non-Hodgkin Lymphoma.[14] The overall response rate (ORR) was defined as the percentage of patients meeting the criteria for complete response (CR), unconfirmed complete response (CRu), or partial response (PR). Stable disease (SD) was measured from the start of the treatment until the criteria for PD were met, taking as the reference the smallest measurements recorded since the initiation of treatment.

Statistical analysis. The sample size for this study was determined by clinical rather than statistical considerations. The probabilities of detecting at least one AE of grade ≥3 with six patients receiving inotuzumab ozogamicin were 0.469, 0.822, and 0.984 when the true rates were 0.10, 0.25, and 0.50, respectively. The probabilities of detecting at least one such event in 10 patients receiving treatment were 0.651, 0.944, and 0.999, respectively.

With cohort sizes of three to six patients, if the true underlying rates of DLT were 0.1, 0.2, 0.3, 0.4, and 0.5, there would be a 0.985, 0.905, 0.754, 0.558, and 0.359 chance, respectively, of escalating to the next full dose. The ORR was estimated using an exact confidence interval (CI) approach.

Results

Patients. From March 2007 to July 2008, a total of 13 patients were enrolled in the study; three patients enrolled in the 1.3 mg/m^2 dose cohort and 10 patients in the 1.8 mg/m^2 dose cohort. The summary of demographic and other baseline characteristics for all patients is presented in Table 1. There were seven males and six females, all with a median age of 49 years (range, 43–72 years). All 13 patients had FL. The median number of prior treatment regimens was 1 (range, 1–13). All 13 patients had previous rituximab treatment (monotherapy or in combination with chemotherapy). Patients were categorized in low (38.5%), intermediate (42%), and high (15%) risk groups according to Follicular Lymphoma International Prognostic Index (FLIPI).[15]

Table 1. Demographic and baseline characteristics, safety population

Characteristics	Inotuzumab ozogamicin treatment		
	1.3 mg/m^2 (n = 3)	1.8 mg/m^2 (n = 10)	Total (n = 13)
Median age, years (range)	57 (51–66)	48 (43–72)	49 (43–72)
Sex, n (%)			
Female	2 (67)	4 (40)	6 (46)
Male	1 (33)	6 (60)	7 (54)
ECOG performance status, n (%)			
0	3 (100)	10 (100)	13 (100)
Primary diagnosis, n (%)			
Follicular lymphoma	3 (100)	10 (100)	13 (100)
FLIPI risk groups, n (%)			
Low	2 (67)	3 (30)	5 (39)
Intermediate	1 (33)	5 (50)	6 (46)
High	0	2 (20)	2 (15)
Number of prior chemo-/immunotherapy regimens, n (%)			
1	2 (67)	6 (60)	8 (62)
2	0	0	0
3	0	1 (10)	1 (8)
≥4	1 (33)	3 (30)	4 (31)

ECOG, Eastern Cooperative Oncology Group; FLIPI, Follicular Lymphoma International Prognostic Index.

Safety. In dose escalation, no patients had DLTs, and the MTD previously determined in non-Japanese patients (1.8 mg/m^2) was confirmed for Japanese patients in this study. The most common drug-related AEs were thrombocytopenia (100% patients); leukopenia (92%); neutropenia, elevated AST, anorexia, and lymphopenia (85%, each); elevated blood fibrinogen (69%); nausea (77%); elevated ALT, elevated alkaline phosphatase, and decreased hemoglobin (54%, each); malaise, elevated blood bilirubin, and headache (46%, each; Table 2(a)).

A summary of drug-related grade 3 or higher AEs is shown in Table 2(b). At least one drug-related grade ≥3 AEs was reported in nine of the 13 (69%) patients. Drug-related grade ≥3 AEs were thrombocytopenia (7 patients, 54%), lymphopenia and neutropenia (4, 31% each), leukopenia (2, 15%), and elevated blood bilirubin and hypokalemia (1, 8% each). Although neither lymphopenia nor leukopenia was reported for the 1.3 mg/m^2 cohort, the overall incidence of drug-related grade ≥3 AEs was comparable between the two cohorts. There were no patients who died during the study.

A total of four patients experienced dose delays, one (33%) patient in the 1.3 mg/m^2 cohort and three (30%) patients in the 1.8 mg/m^2 cohort (Table 3). Each had one delay. The AEs leading to dose delays were neutropenia (3 patients, 23%) and thrombocytopenia (2, 15%). Two (20%) patients in the 1.8 mg/m^2 cohort had one dose reduction (Table 4). Adverse events (AEs) leading to the dose reduction were thrombocytopenia and pleural effusion (1 patient, 8% each). There were no dose reductions in the 1.3 mg/m^2 cohort.

Seven patients discontinued treatment due to AEs: one patient because of grade 2 rash, one patient because of grade 2 urticaria, and five patients because of AEs that required treatment delays of >3 weeks (two patients with prolonged thrombocytopenia, one patient with prolonged thrombocytopenia and neutropenia, one patient with neutropenia and elevated alkaline phosphatase, and one patient with prolonged neutropenia and elevated total bilirubin).

Pharmacokinetics. Pharmacokinetic data after the first dosing were obtained for all 13 patients. The two patients who received 1.8 mg/m^2 inotuzumab ozogamicin and had a dose reduction after cycle 1 were excluded from pharmacokinetic assessments for cycle 2 and thereafter. The mean ± SD serum concentrations of inotuzumab ozogamicin and total calicheamicin *versus* time

Table 2. Inotuzumab ozogamicin-related adverse events, (a) all grades in ≥4 patients (b) grades ≥3

Adverse event, n (%)	Inotuzumab ozogamicin treatment		
	1.3 mg/m^2 (n = 3)	1.8 mg/m^2 (n = 10)	Total (n = 13)
(a) all grades in ≥4 patients			
Thrombocytopenia	3 (100)	10 (100)	13 (100)
Leukopenia	3 (100)	9 (90)	12 (92)
Lymphopenia	3 (100)	8 (80)	11 (85)
Neutropenia	3 (100)	8 (80)	11 (85)
Aspartate aminotransferase increased	3 (100)	8 (80)	11 (85)
Anorexia	3 (100)	8 (80)	11 (85)
Nausea	3 (100)	7 (70)	10 (77)
Blood fibrinogen increased	2 (67)	7 (70)	9 (69)
Alanine aminotransferase increased	1 (33)	6 (60)	7 (54)
Blood alkaline phosphatase increased	1 (33)	6 (60)	7 (54)
Hemoglobin decreased	1 (33)	6 (60)	7 (54)
Malaise	3 (100)	3 (30)	6 (46)
Blood bilirubin increased	2 (67)	4 (40)	6 (46)
Headache	2 (67)	4 (40)	6 (46)
Constipation	1 (33)	4 (40)	5 (39)
Influenza	1 (33)	4 (40)	5 (39)
Blood lactate dehydrogenase increased	2 (67)	3 (30)	5 (39)
Fibrin D dimer increased	0	5 (50)	5 (39)
Hyperglycemia	1 (33)	4 (40)	5 (39)
Stomach discomfort	1 (33)	3 (30)	4 (31)
Fatigue	0	4 (40)	4 (31)
Hypercholesterolemia	1 (33)	3 (30)	4 (31)
Hypokalemia	2 (67)	2 (20)	4 (31)
Somnolence	2 (67)	2 (20)	4 (31)
Epistaxis	0	4 (40)	4 (31)
Rash	1 (33)	3 (30)	4 (31)
(b) grades ≥3			
Thrombocytopenia	2 (67)	5 (50)	7 (54)
Lymphopenia	0	4 (40)	4 (31)
Neutropenia	1 (33)	3 (30)	4 (31)
Leukopenia	0	2 (20)	2 (15)
Blood bilirubin increased	1 (33)	0	1 (8)
Hypokalemia	1 (33)	0	1 (8)

Table 3. Number (%) of patients reporting adverse events leading to dose delays, safety population

Parameter, n (%)	Inotuzumab ozogamicin treatment		
	1.3 mg/m^2 (n = 3)	1.8 mg/m^2 (n = 10)	Total (n = 13)
No. of patients with dose delays			
No dose delays	2 (67)	7 (70)	9 (69)
One or more dose delays	1 (33)	3 (30)	4 (31)
No. of dose delays per patient*			
One	1 (100)	3 (100)	4 (31)
Any adverse event leading to dose delay†	1 (33)	3 (30)	4 (31)
Neutropenia	1 (33)	2 (20)	3 (23)
Thrombocytopenia	1 (33)	1 (10)	2 (15)

*Percentages are based on number of patients with ≥1 inotuzumab ozogamicin dose delay in each treatment group. †Totals at a higher level are not necessarily the sum of those at the lower levels since a patient was able to report two or more different adverse events within the higher level category.

doi: 10.1111/j.1349-7006.2010.01601.x

Table 4. Number (%) of patients reporting adverse events leading to dose reduction, safety population

Parameter, *n* (%)	Inotuzumab ozogamicin treatment		
	1.3 mg/m^2 (*n* = 3)	1.8 mg/m^2 (*n* = 10)	Total (*n* = 13)
No. of patients with dose reductions			
No dose reductions	3 (100)	8 (80)	11 (85)
One or more dose reductions	0	2 (20)	2 (15)
No. of dose reductions per patient*			
One	0	2 (100)	2 (15)
Any adverse event leading to dose reduction†	0	2 (20)	2 (15)
Thrombocytopenia	0	1 (10)	1 (8)
Pleural effusion	0	1 (10)	1 (8)

*Percentages are based on number of patients with ≥1 dose reduction in each treatment group. †Totals at a higher level are not necessarily the sum of those at the lower levels since a patient was able to report two or more different adverse events within the higher level category.

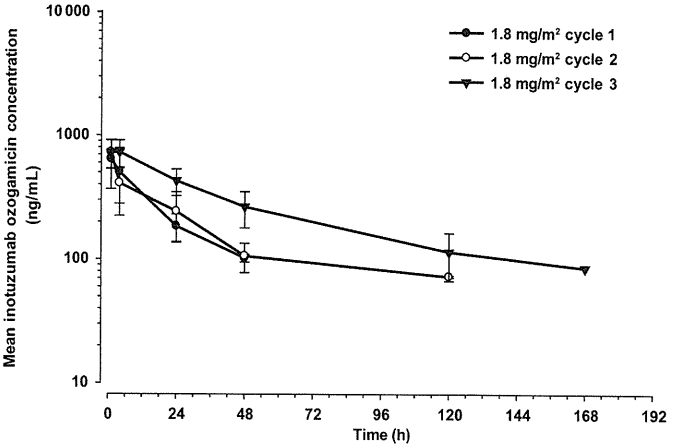

Fig. 1. Mean (SD) serum concentrations of inotuzumab ozogamicin after 1.8 mg/m^2 infusion of inotuzumab ozogamicin once every 4 weeks. Closed circle, cycle 1 (day 1, *n* = 10); open circle, cycle 2 (day 29, *n* = 8); closed triangle, cycle 3 (day 57, *n* = 5).

for patients who received 1.8 mg/m^2 are shown in Figures 1 and 2, respectively. The peak concentration of inotuzumab ozogamicin was generally observed at or shortly after the termination of infusion with moderate intersubject variability. The peak total calicheamicin concentrations were observed typically within 4 h after the start of inotuzumab ozogamicin infusion with small intersubject variability.

The mean pharmacokinetic parameters for inotuzumab ozogamicin and total calicheamicin are shown in Tables 5 and 6, respectively. The AUC of inotuzumab ozogamicin tended to increase with increased dose and period. The $t_{1/2}$ was prolonged with repeated treatment cycles. These were reflected by substantial decreases in clearances.

The mean total calicheamicin C_{max} appeared to increase with dose. The AUC of total calicheamicin increased with increased dose and period. No antibodies to inotuzumab ozogamicin were detectable in patients' serum during the course of the study. The pharmacokinetics data indicate that the disposition of inotuzumab ozogamicin and total calicheamicin following IV treatment was nonlinear with dose or number of doses.

Efficacy. The best tumor response is presented in Table 7. Antitumor activity was observed at both dose levels. In the 1.3 mg/m^2 cohort, two out of three patients had CR, and one patient had CRu for an ORR of 100% (95% CI, 29–100%). In the 1.8 mg/m^2 cohort, one out of 10 patients had CR, three patients had CRu, and four patients had PR for an ORR of 80% (95% CI, 44–98%).

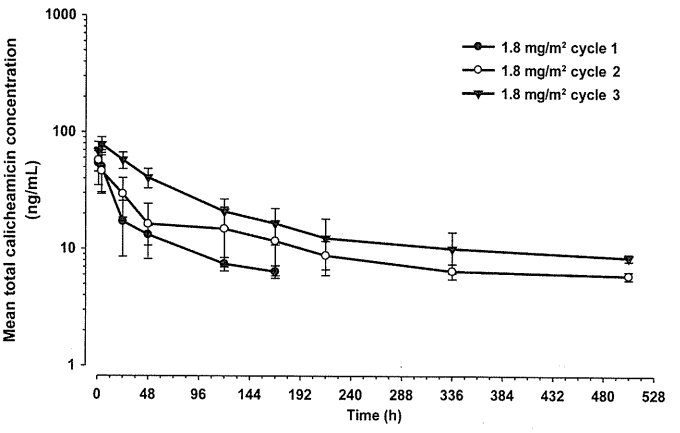

Fig. 2. Mean (SD) serum concentrations of total calicheamicin after 1.8 mg/m^2 infusion of inotuzumab ozogamicin once every 4 weeks. Closed circle, cycle 1 (day 1, *n* = 10); open circle, cycle 2 (day 29, *n* = 8); closed triangle, cycle 3 (day 57, *n* = 5).

Discussion

To improve the clinical outcome of patients with B-NHL who were pretreated with rituximab or rituximab-containing regimens, a number of new agents including antibodies, small mole-

Table 5. Serum pharmacokinetic parameters of inotuzumab ozogamicin

Dose (Once/4 weeks)	Treatment Day (*n*)	Number of cycles	C_{max} (ng/mL) (%)	$t_{1/2}$ (h) (%)	AUC (ng h/mL) (%)	CL (L/h) (%)	V_{ss} (L) (%)
1.3 mg/m^2	1 (3)	1	463 (8)	NC	NC	NC	NC
	29 (3)	2	610 (17)	29.7 (30)	24166 (29)	0.08 (32)	3.27 (11)
	57 (3)	3	524 (18)	43.6 (18)	31642 (21)	0.06 (22)	3.79 (12)
1.8 mg/m^2	1 (10)	1	657 (41)	13.0 (30)	14266 (32)	0.24 (40)	4.06 (21)
	29 (8)	2	727 (27)	35.8 (43)	34518 (46)	0.11 (54)	4.40 (20)
	57 (5)	3	763 (20)	44.0 (32)	39677 (41)	0.09 (56)	4.89 (19)

Data are expressed as mean, and percent coefficient of variance is expressed in parentheses. AUC, total area under the concentration-time curve; CL, clearance; C_{max}, peak concentration; NC, not calculated; $t_{1/2}$, terminal-phase elimination half-life (0.693/λz); V_{ss}, steady-state volume of distribution.

Table 6. Serum pharmacokinetic parameters of total calicheamicin

Dose (Once/4 weeks)	Treatment Day (*n*)	Number of cycles	C_{max} (ng/mL) (%)	$t_{1/2}$ (h) (%)	AUC (ng h/mL) (%)	CL (L/h) (%)	V_{ss} (L) (%)
1.3 mg/m^2	1 (3)	1	44.6 (17)	17.0 (39)	987 (44)	2.35 (58)	49.44 (13)
	29 (3)	2	52.4 (22)	150.6 (45)	5754 (40)	0.38 (48)	62.86 (30)
	57 (3)	3	56.6 (26)	216.3 (55)	8060 (37)	0.27 (43)	60.17 (25)
1.8 mg/m^2	1 (10)	1	59.0 (31)	49.6 (77)	2329 (51)	1.61 (54)	72.3 (28)
	29 (8)	2	59.4 (15)	162.4 (34)	7100 (48)	0.54 (62)	89.18 (41)
	57 (5)	3	78.2 (15)	172.7 (48)	9225 (32)	0.37 (44)	68.37 (26)

Data are expressed as mean, and percent coefficient of variance is expressed in parentheses. AUC, total area under the concentration-time curve; CL, clearance; C_{max}, peak concentration; NC, not calculated; $t_{1/2}$, terminal-phase elimination half-life (0.693/λz); V_{ss}, steady-state volume of distribution.

Table 7. The best tumor response during treatment: number (%) of patients in efficacy population

Best tumor response	Inotuzumab ozogamicin treatment		
	1.3 mg/m^2 (*n* = 3)	1.8 mg/m^2 (*n* = 10)	Total (*n* = 13)
CR, CRu	3 (100)	4 (40)	7 (54)
PR	0	4 (40)	4 (31)
OR	3 (100)	8 (80)	11 (85)
SD	0	2 (20)	2 (15)

CR, complete response; CRu, unconfirmed complete response; OR, overall response (CR + CRu + PR); PR, partial response; SD, stable disease.

cule, targeted agents, and chemotherapeutic drugs have been developed. However, new treatment modalities with improved toxicity profiles and better responses are needed. Inotuzumab ozogamicin (CMC-544), an antibody-targeted chemotherapy agent, has demonstrated an acceptable toxicity profile and high activity against relapsed or refractory patients with FL who were pretreated with rituximab or rituximab-containing treatment.

In a recent phase I, multicenter, open-label, dose escalation study of inotuzumab ozogamicin administered IV as a single agent in the USA and the European Union, inotuzumab ozogamicin was found to be reasonably well-tolerated with the MTD of 1.8 mg/m^2 administered every 4 weeks and with the major toxicity of grade 3 or greater thrombocytopenia, which was manageable with careful monitoring and platelet transfusion. Response rates of 69% in patients with FL and 33% in patients with DLBCL in the expanded cohort of this trial were observed.[12]

In the present phase I dose escalation study in Japanese patients with relapsed or refractory FL, who were pretreated with rituximab, the MTD of inotuzumab ozogamicin was determined to be 1.8 mg/m^2 administered once every 28 days, a value that was the same as that observed for non-Japanese patients.

Most common inotuzumab ozogamicin related adverse events were thrombocytopenia, leukopenia, lymphopenia, neutropenia, elevated AST, anorexia, and nausea, a finding that was very similar to the non-Japanese study. Adverse events (AEs) leading to dose delays were neutropenia and thrombocytopenia.

The pharmacokinetic profiles of inotuzumab ozogamicin and total calicheamicin indicated that disposition was non-linear and was associated with increases in drug exposure with increasing dose or number of doses. The pharmacokinetic profiles of inotuzumab ozogamicin and total calicheamicin in Japanese patients were similar to the values for non-Japanese patients. The study population was very limited, thus no definite conclusion can be made for Japanese patients. However, nonlinearities in drug disposition are known for antibodies[16] and had been observed previously for gemtuzumab ozogamicin.[17] Saturable binding with target antigen is thought to influence antibody disposition, potentially leading to nonlinear distribution and elimination.

Potent antitumor activity for inotuzumab ozogamicin was observed at both the 1.3 and 1.8 mg/m^2 dose levels. In the 1.3 mg/m^2 cohort, all three patients had CR or CRu for an ORR of 100%. In the 1.8 mg/m^2 cohort, one out of 10 patients had CR, three patients had CRu, and four patients had PR for an ORR of 80%. Although the number of patients was limited, our preliminary ORR was greater in comparison to other reported antibody-based agents in the treatment of patients with FL and prior exposure to rituximab-containing regimens. For example, in a recent phase I/II study, veltuzumab, a humanized second-generation anti-CD20 monoclonal antibody, was reported to have an ORR of 44%.[18] In another phase I/II, single-agent, dose escalation study, galiximab, an anti-CD80 antibody, demonstrated an ORR of only 11%.[19] Fludarabine phosphate, one of the most effective drugs in the treatment of indolent B-NHL, had an ORR of 65%, when administered as a single agent.[20]

The FLIPI scores in this study were good predictors of favorable outcome. Of the five patients who had low scores (low risk) two demonstrated CR, two had CRu, and one had PR. Of the six patients who had intermediate scores, one had CR, two had CRu, one had PR, and two had SD. The two patients with high FLIPI scores demonstrated only PR.

In conclusion, the results from this phase I study suggest that inotuzumab ozogamicin is safe, well tolerated, and shows promising efficacy in Japanese patients with relapsed or refractory FL pretreated with rituximab-containing therapy. In addition, pharmacokinetics and efficacy in this study are comparable with those in preceding studies in non-Japanese patients. These results therefore warrant further investigation of inotuzumab ozogamicin in relapsed or refractory B-NHL.

Acknowledgments

The authors thank the patients, doctors, nurses and staff members who participated in this multicenter trial for their excellent cooperation. Participating institutions of this phase I study: Nagoya Daini Red Cross Hospital, National Cancer Center Hospital, and Cancer Institute Hospital. Protocol Committee members: Drs Michinori Ogura, Kensei Tobinai, and Kiyohiko Hatake. The authors also thank Joseph Ramcharan, PhD, and Tricia Gooljarsingh, PhD, for writing assistance that was funded by Pfizer, Inc.

Disclosure Statement

This study was funded by Wyeth which was acquired by Pfizer, Inc., in October 2009. Dr. Junko Ohata was an employee of Wyeth K.K. at the time of the study. Dr. Chiho Ono is an employee of Wyeth K.K. No other potential conflict of interest relevant to the article is reported.

doi: 10.1111/j.1349-7006.2010.01601.x

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J Med Syst
DOI 10.1007/s10916-011-9688-z

ORIGINAL PAPER

Development of Fundamental Infrastructure for Nationwide EHR in Japan

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Received: 4 November 2010 / Accepted: 15 March 2011

Abstract The movement of create medical information systems that is now taking place involves both progress in EMR (Electronic Medical Records)—computerization of records at hospitals and clinics, and also in EHR (Electronic Health Records) in which information is shared with individual regions. However, the geographical coming and going of people in modern society is extremely active. Naturally the places these people move to are not necessarily within the same region. For this reason, even if the basic unit for the health care supply system is in practical terms limited to the local level, if services are restricted to only one region, many persons may be unable to receive the benefits of health care cooperation. In this study, we constructed a mechanism for a medical cooperation system which links the EHR systems of individual regions and is able to create a one-patient, one-record system on the national level. In this paper, we will provide a report of this mechanism and of the 4-year operational trial.

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Published online: 01 April 2011

Keywords Nation level EHR · Dolphin Project · XML data mapping · Directory service

Introduction

The movement of create medical information systems that is now taking place involves both progress in EMR (Electronic Medical Records) computerization of records at hospitals and clinics, and also in EHR (Electronic Health Records) in which information is shared with individual regions. A variety of trials have been carried out worldwide for this purpose, primarily in developed countries, and informatics is also receiving attention as an effective means of improving the efficiency of medical services in newly industrialized and developing countries as well. For example under the leadership of the state, Canada and England have invested at least 1.6 billion U.S. dollars [1] and 20 billion U.S. dollars [2] respectively. It is said that the United States will invest 20 billion U.S. dollars in switching to electronic medical documents. For EHR, many successful examples in sharing medical information within regions continue to be reported from around the world, and several EHR projects have been carried out in Japan as well [3].

However the geographical coming and going of people in modern society is extremely active. In the United States, 35 million people change their place of residence each year [4], and it is said that on average a person in Japan moves 5 times in his or her life [5]. Naturally the places these people move to are not necessarily within the same region. For this reason, even if the basic unit for the health care supply system is in practical terms limited to the local level, if services are restricted to only one region, many persons may be unable to receive the benefits of health care