tance in these tumors.[30] As the amounts of each clinical specimen were limited, we would like to perform further analyses in future studies should sufficient amounts of specimens become available.

Recent studies indicated that multiple resistance factors can be induced simultaneously in a single cancer. For example, Qi et al.[31] reported the simultaneous occurrence of *Met* mutation and activation of the EGFR pathway by ligand overexpression, similar to T790M mutation and HGF overexpression in EGFR mutant lung cancer, which caused resistance to Met-TKIs in gastric cancer. Katayama et al.[32] also reported that *ALK* gene amplification and gatekeeper mutation in *ALK* occurred simultaneously and conferred resistance to ALK inhibitors in EML4-ALK lung cancer. In this study, T790M secondary mutation and the high HGF expression level were simultaneously detected at high incidence (50%) in tumors with acquired resistance. Irreversible EGFR-TKIs were thought to have potential to control acquired resistance caused by T790M secondary mutation, but clinical responses were rarely observed in clinical trials.[33,34] We recently found that HGF induces resistance to not only reversible EGFR-TKIs but also irreversible EGFR-TKIs by activating the MET/PI3K/Akt pathway in *EGFR* mutant lung cancer cells with or without T790M secondary mutation.[26] Taken together, these observations suggest that HGF would be simultaneously expressed with T790M secondary mutation in tumors with acquired resistance and reduce the sensitivity to irreversible EGFR-TKIs in *EGFR* mutant lung cancer patients.

MET amplification has been detected in ~20% of tumors with acquired resistance to EGFR-TKIs in *EGFR* mutant lung cancer,[13,16,17] while the incidence reported in Japanese patients is rare.[14,18] Here, we detected *MET* amplification in two tumors (9%) with acquired resistance, suggesting that *MET* amplification can be detected in a significant proportion of tumors with acquired resistance even in Japanese patients. One case with high-level HGF expression and *MET* amplification (KZ-1) was treated with gefitinib and PFS was 254 days. The other case with low HGF and *MET* amplification (SG4) was treated with erlotinib and PFS was 60 days (Table 3). Although it is not possible to make definitive conclusions based on the data from only these two cases, the shorter PFS in the former case tentatively supports the observation that HGF accelerates expansion of preexisting clones with MET amplification.[16] Notably, simultaneous expression of these two factors was also detected in one tumor with intrinsic resistance (nonresponder). However, the mechanism by which HGF is induced in *EGFR* mutant lung cancer is still not well defined. Further examinations are warranted to elucidate the interaction between HGF expression and *MET* amplification in *EGFR* mutant lung cancer.

Among 68 resistant tumors, high-level HGF expression, T790M secondary mutation, and *MET* amplification were not detected in one tumor with acquired resistance and 31 tumors with intrinsic resistance, indicating the involvement of other mechanisms of resistance in these tumors. *EGFR* D761Y secondary mutation in exon 20 was detected in two tumors from the same patient.[24] *EGFR* D761Y mutation was originally identified in recurrent brain metastasis and was shown to induce intermediate-grade resistance to EGFR-TKIs.[35] In addition, rare secondary mutations (other than T790M and D761Y) or a preexisting resistance mutation in a minority of clones may also be involved in intrinsic resistance. Moreover, it was recently reported that a subpopulation of cancer cells that transiently exhibit a distinct phenotype characterized by engagement of IGF-1R activity, hypersensitivity to HDAC inhibition, and altered chromatin showed an intrinsic ability to tolerate exposure to EGFR-TKI.[36] Minor secondary mutations, a preexisting resistance mutation in a minority of clones, or chromatin-mediated drug resistance mechanisms may be involved in resistant tumors without high HGF expression, T790M secondary mutation, and *MET* amplification.

To overcome the HGF-induced resistance to EGFR-TKI in *EGFR* mutant lung cancer, double blockade of the EGFR pathway and HGF-MET pathway is therefore theoretically necessary.[14,16,27] To inhibit mutant EGFR with or without T790M secondary mutation, EGFR mutant-specific inhibitors were developed in addition to irreversible EGFR-TKIs.[37] To inhibit HGF-MET signaling, several inhibitors, including anti-HGF antibody, NK4 (natural antagonist of MET), and MET-TKIs, were developed.[16,25–27] Further studies are essential to determine optimal combined therapy with best efficacy and safety. In addition, a prospective study is required to determine whether immunohistochemical detection of HGF would be sufficiently reliable to identify patients with HGF-induced resistance to EGFR-TKIs. As levels of HGF in peripheral blood are correlated with clinical outcome to EGFR-TKIs in patients with non-small cell lung cancer,[38,39] such noninvasive methods may facilitate individual therapy for overcoming HGF-induced resistance to EGFR-TKIs in *EGFR* mutant lung cancer patients.

Recent studies indicated at least three important roles of HGF in EGFR-TKI resistance in *EGFR* mutant lung cancer. First, HGF induces resistance to reversible EGFR-TKIs, gefitinib, and erlotinib, by restoring MET/Gab1/PI3K/Akt pathways.[14,16] Second, HGF accelerates expansion of preexisting *MET*-amplified cancer cells and facilitates MET amplification-mediated resistance during EGFR-TKI treatment.[16] Third, after acquiring resistance to reversible EGFR-TKIs, HGF induces resistance of lung cancer cells with T790M secondary mutation to irreversible EGFR-TKIs.[24] Here, we detected high-level HGF expression frequently in tumors with intrinsic and acquired resistance to EGFR-TKIs in *EGFR* mutant lung cancer in Japanese patients. These findings indicate the value of HGF as a therapeutic target for EGFR-TKI-resistant *EGFR* mutant lung cancer. Therefore, combined therapy with EGFR-TKIs and HGF-MET inhibitors in patients with HGF-induced resistance may improve the clinical outcome of *EGFR* mutant lung cancer.

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Epidermal Growth Factor Receptor Mutation Status in Circulating Free DNA in Serum

From IPASS, a Phase III Study of Gefitinib or Carboplatin/Paclitaxel in Non-small Cell Lung Cancer

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Introduction: In IPASS (IRESSA Pan-Asia Study), clinically selected patients with pulmonary adenocarcinoma received first-line gefitinib or carboplatin/paclitaxel. This preplanned, exploratory analysis was conducted to increase understanding of the use of surrogate samples, such as serum, versus tumor biopsy samples for determining *EGFR* mutation status in the Japanese cohort (n = 233).

Methods: *EGFR* mutations were assessed using tumor tissue-derived DNA (n = 91) and circulating free (cf) DNA from pretreatment serum samples (n = 194).

Results: Fewer patients were *EGFR* mutation positive when assessed using pretreatment cfDNA (23.7%) versus tumor tissue-derived DNA (61.5%). cfDNA results identified no false positives but a high rate of false negatives (56.9%). There was a significant interaction between cfDNA *EGFR* mutation status and treatment for progression-free survival (PFS) (p = 0.045). PFS was significantly longer and objective response rate (ORR) higher with gefitinib than carboplatin/paclitaxel in the cfDNA *EGFR* mutation-positive subgroup (PFS: hazard ratio [HR], 0.29; 95% confidence interval [CI], 0.14–0.60; $p < 0.001$; ORR: odds ratio [OR], 1.71; 95% CI, 0.48–6.09; 75.0% versus 63.6%; p = 0.40). There was a slight numerical advantage in PFS and ORR for gefitinib over carboplatin/paclitaxel in the cfDNA *EGFR* mutation-negative subgroup, likely due to the high rate of false negatives within this subgroup.

Conclusions: These results merit further investigation to determine whether alternative sources of tumor DNA, such as cfDNA in serum, could be used for determining *EGFR* mutation status in future; currently, where a sample is available, analysis of tumor material is recommended.

Key Words: EGFR, Mutation, Gefitinib, NSCLC, Serum.

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The epidermal growth factor receptor (EGFR) superfamily has been implicated in the regulation of tumor cell biology and, as such, has emerged as a therapeutic target.[1] In 2004, mutations in the *EGFR* were reported to be associated with sensitivity to EGFR tyrosine kinase inhibitors (EGFR-TKIs).[2–4] The presence of such mutations in tumor tissue is associated with a number of clinical factors including Asian origin, female sex, adenocarcinoma histology, and a never-smoking history, and these factors have additionally been correlated with response to gefitinib (IRESSA, AstraZeneca, Macclesfield, UK), an EGFR-TKI.[5]

The IRESSA Pan-Asia Study (IPASS) compared gefitinib with carboplatin/paclitaxel as first-line treatment in 1217 never-smokers/light ex-smokers with advanced adenocarcinoma of the lung in East Asia.[6] Subgroup analysis of patients with *EGFR* mutations ($n = 261$) detected in DNA derived from tumor tissue samples demonstrated significantly longer progression-free survival (PFS) with gefitinib versus carboplatin/paclitaxel (hazard ratio [HR], 0.48; 95% confidence interval [CI], 0.36–0.64; $p < 0.001$).[6] In the *EGFR* mutation-negative (M−) subgroup ($n = 176$), PFS was significantly longer with carboplatin/paclitaxel versus gefitinib (HR, 2.85; 95% CI, 2.05–3.98; $p < 0.001$). Objective response rates (ORR) were 71.2% versus 47.3% ($p < 0.001$) and 1.1% versus 23.5% ($p = 0.001$) with gefitinib versus carboplatin/paclitaxel in *EGFR* M+ and M− patients, respectively.

The difficulties of collecting sufficient tumor tissue for biomarker analyses have stimulated interest in analyses using surrogate samples, such as serum and plasma samples, which frequently contain circulating free (cf) DNA derived from tumor tissues. Previous studies in relatively few patients had detected *EGFR* mutations in cfDNA in serum or plasma samples and suggested that using such methodology to predict response to gefitinib was worthy of further evaluation.[7–12] However, most of these studies were retrospective.

Herein, we report the evaluation of *EGFR* mutations in cfDNA from serum samples of patients in the IPASS study recruited in Japan. This preplanned, exploratory analysis was conducted to increase the understanding of the use of surrogate samples, such as serum, versus tumor biopsy samples for determining *EGFR* mutation status.

MATERIALS AND METHODS

Study Design and Patients

Full details of the IPASS study design (ClinicalTrials.gov identifier NCT00322452) have been published previously.[6] Planned objectives of this substudy of IPASS were evaluations of efficacy between the gefitinib and carboplatin/paclitaxel treatment groups by cfDNA *EGFR* mutation status from pretreatment serum samples and evaluation of the concordance between *EGFR* mutation status in pretreatment cfDNA versus tumor. Comparison of *EGFR* mutation status in pretreatment versus postprogression serum samples was also performed; however, not all patients with a pretreatment sample had a postprogression sample, which limited the comparison. In addition, comparisons with postprogression serum and pretreatment pleural effusion samples are reported in Supplemental Digital Content 1 (Methods http://links.lww.com/JTO/A152). Preplanned analysis of the Japanese subset of the IPASS population was performed to meet Japanese regulatory requirements.

All patients provided written informed consent. Provision of samples for biomarker research was optional and involved separate consent procedures for tumor and serum sampling. An independent ethics committee at each participating institution approved the study protocol. The study was conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonisation Guidelines for Good Clinical Practice, applicable regulatory requirements, and AstraZeneca's policy on bioethics.

Biomarker Analyses

Sample collection and DNA extraction are described in Supplemental Digital Content 1 (Methods http://links.lww.com/JTO/A152). *EGFR* mutations were detected using the DxS *EGFR* Mutation Test Kit for Research Use Only (DxS, Manchester, UK), which combines Amplification Refractory Mutation System (ARMS) (allele-specific polymerase chain reaction [PCR]) with the Scorpions real-time PCR technology.[13,14] Modified run conditions and cutoffs (delta Ct values [dCt]) used to define M+ samples for cfDNA derived from serum and pleural effusion samples were as follows: 50 cycles of PCR were carried out and the dCt for exon 19 deletions was 12, L858R was 14, and T790M was 8 (for tumor DNA, 40 cycles of PCR were carried out and the dCt cutoffs were 9, 11, and 8, respectively). In analyses of tumor DNA, all 29 mutations detected by the kit were assayed (19 deletions in exon 19, L858R, T790M, L861Q, G719X [S, A, or C], S768I, and 3 insertions in exon 20); whereas for serum and pleural effusion samples, the 21 most common mutations (19 deletions in exon 19, L858R, and T790M) were assayed (to make the best use of limited cfDNA yield). Samples were tested in duplicate, and only if both replicates were positive for at least one of the mutations was the sample defined as M+. Patients without a tumor sample evaluable for mutation analysis and samples which were not successfully analyzed were classified as *EGFR* mutation unknown. Biomarker samples were assayed blinded to clinical outcome and randomized treatment.

Statistical Analyses

Serum samples were collected for patients recruited in Japan and who consented to this optional analysis. Analyses of efficacy end points comparing treatment groups in the Japanese subset (intent-to-treat [ITT] population) were assessed as described previously for the overall IPASS population.[6] However, for the analyses in the cfDNA M+ and M− subgroups, the prespecified covariates of World Health Organization (WHO) performance status (PS), smoking history, and sex could not be included as covariates because of the small number of patients who had a WHO PS 2, were ex-smokers, or were males; therefore, models without covariates were used. Because of the lack of power to detect treatment differences, the result of the Japanese subset should be interpreted with caution, taking into account the associated variability and overlap in plausible range of effects (CIs). Analyses comparing treatment groups were performed for PFS (by Cox proportional hazards model) and ORR (by logistic regression model) in subgroups defined by cfDNA *EGFR* mutation status. A test for interaction between cfDNA *EGFR* mutation status (M+ or M−) and treatment was used to assess whether the PFS treatment effect was statistically different between subgroups.

Comparison of pretreatment cfDNA versus tumor *EGFR* mutations was based on the 21 mutations analyzed for cfDNA using patients with known mutation status (M+ or M−) in both samples. The sensitivity, specificity, positive

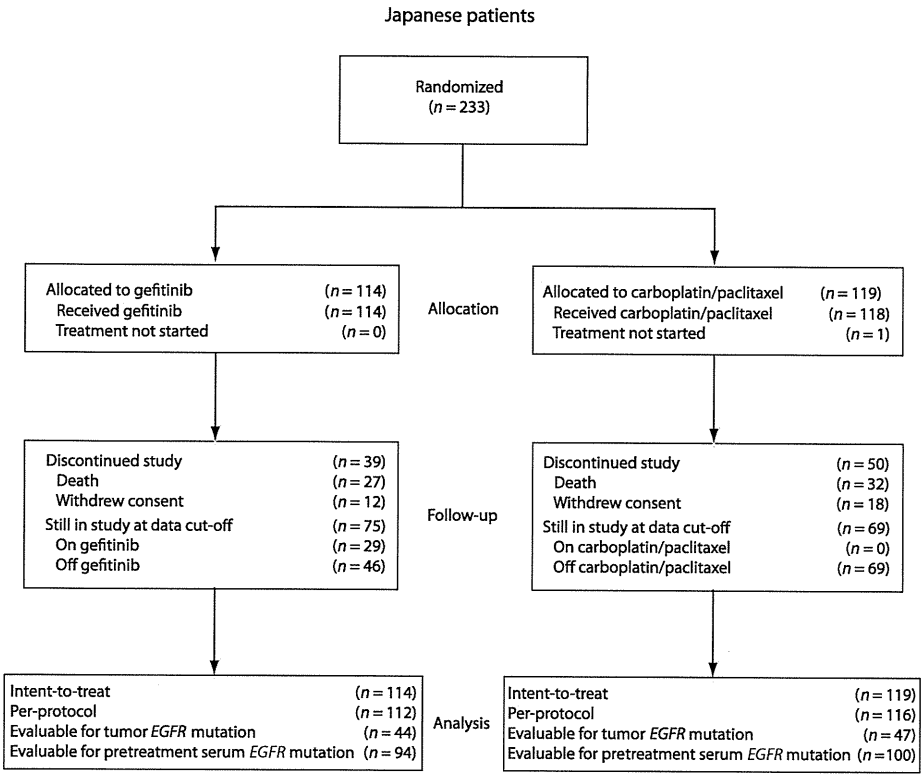

FIGURE 1. CONSORT diagram representing patient disposition (including number of patients with tumor tissue or serum evaluable for *EGFR* mutation status). *EGFR*, epidermal growth factor receptor.

and negative predictive values and their exact 95% CIs, and the kappa coefficient and 95% CI, for *EGFR* mutation status in serum samples, were evaluated assuming that the *EGFR* mutation status in tumor tissue was a true reflection of tumor biology. The proportion of concordance between cfDNA and tumor was calculated on a similar basis by excluding patients judged as unknown using either cfDNA or tumor samples.

RESULTS

Patients

In total, 233 patients from Japan were randomized to study treatment (19.1% of the overall IPASS population). Preplanned evaluations of efficacy, quality of life, and safety for the overall Japanese study population have been previously presented[15,16] and are summarized in Supplemental Digital Content 2 (Results http://links.lww.com/JTO/A153) and 3 (Figure http://links.lww.com/JTO/A154). The patient disposition for the Japanese subset of IPASS is shown in Figure 1.

EGFR Mutation Status

An evaluable DNA sample for *EGFR* mutation status derived from tumor tissue was available for 91 patients; of these, 56 (61.5%) patients were *EGFR* M+, with a lower proportion of *EGFR* M+ patients in the gefitinib group compared with the carboplatin/paclitaxel group (52.3% [23/44] versus 70.2% [33/47]) (Figure 2). A total of 194 patients provided a pretreatment serum sample for mutation analysis; all were evaluable. Of these, 46 (23.7%) patients were cfDNA *EGFR* M+ (25.5% [24/94] and 22.0% [22/100] in the gefitinib and carboplatin/paclitaxel groups, respectively) (Figure 2). Data from pretreatment pleural effusion (9 patients) and postprogression serum analyses (144 patients) are presented in Supplemental Digital Content 2 (Results http://links.lww.com/JTO/A153) and 4 (Table http://links.lww.com/JTO/A155).

Demographic and Baseline Characteristics of Patients with Known *EGFR* Mutation Status

Key demographic and baseline characteristics for patients with known (i.e., evaluable) cfDNA or tumor *EGFR* mutation status were generally consistent with the overall Japanese study population (Table 1).

Pretreatment cfDNA *EGFR* Mutation Status and Clinical Outcomes

The subset of patients with known cfDNA *EGFR* mutation status could be assumed to be representative of the overall Japanese study population (and therefore the overall study population) as shown by similar PFS and ORR results (Table 1).

A significant interaction between cfDNA *EGFR* mutation status and treatment was evident for PFS (interaction test $p = 0.045$). PFS was significantly longer with gefitinib than carboplatin/paclitaxel in the cfDNA *EGFR* M+ subgroup (HR, 0.29; 95% CI, 0.14–0.60; $p < 0.001$) (Figure 3*A*). In the cfDNA *EGFR* M− subgroup, there were no significant differences for PFS with gefitinib compared with carboplatin/paclitaxel (HR, 0.88; 95% CI, 0.61–1.28; $p = 0.50$) (Figure 3*B*). However, the HR was not constant over time. We

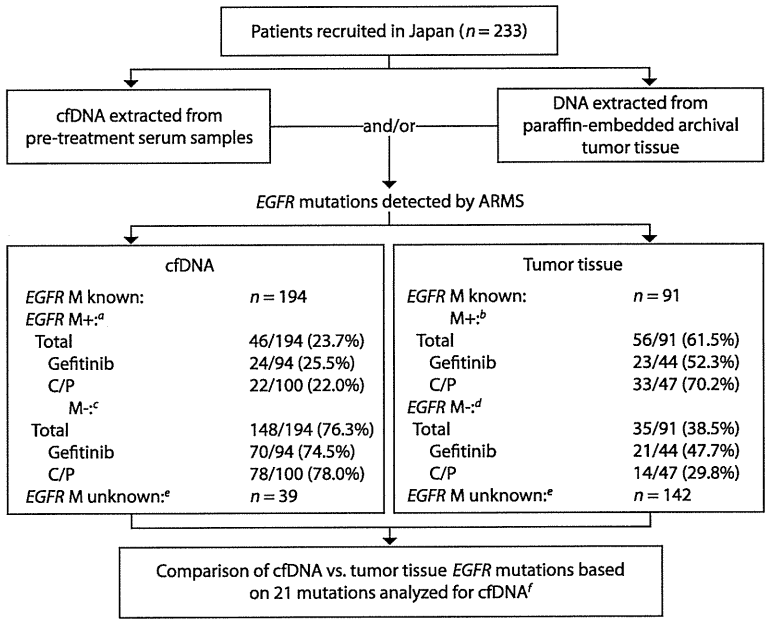

FIGURE 2. Flow and results of *EGFR* mutation analysis. [a]Sample positive for ≥1 of 21 mutations tested; detected 19 deletions in exon 19, L858R, and T790M. [b]Sample positive for ≥1 of 29 mutations tested; detected 19 deletions in exon 19, L858R, T790M, L861Q, G719S, G719A, G719C, S768I; 3 insertions in exon 20. [c]Sample negative for all 21 mutations tested. [d]Sample negative for all 29 mutations tested. [e]Unknown *EGFR* mutations: no sample available or failed analysis. [f]86 patients had known mutation status by both tumor tissue and cfDNA. C/P, carboplatin/paclitaxel; *EGFR*, epidermal growth factor receptor; M, mutation; M+, mutation-positive; M−, mutation-negative.

TABLE 1. Patient Demographics, Baseline Characteristics, and Efficacy (PFS and ORR) for Patients with Samples (cfDNA or Tumor) Evaluable for *EGFR* Mutation Status Compared with the Overall Japanese[a] Study Population (Japanese ITT Population)

	Evaluable for *EGFR* Mutation Status (cfDNA) (n = 194)[b]	Evaluable for *EGFR* Mutation Status (Tumor) (n = 91)[b]	Overall Japanese Study Population (n = 233)
Demography, n (%)			
Female	172 (88.7)	84 (92.3)	204 (87.6)
WHO PS 0/1	185 (95.4)	89 (97.8)	223 (95.7)
Never-smoker	177 (91.2)	83 (91.2)	212 (91.0)
Stage IIIB	66 (34.0)	27 (29.7)	73 (31.3)
Age <65 yr	97 (50.0)	45 (49.5)	121 (51.9)
Efficacy			
PFS HR[c] (95% CI)	0.68 (0.49–0.95)	1.08 (0.68–1.72)	0.69 (0.51–0.94)
ORR OR[d] (95% CI)	1.45 (0.80–2.61)	0.99 (0.41–2.40)[e]	1.34 (0.78–2.30)

[a] Refers to the country of recruitment and not necessarily to racial origin.
[b] Includes both mutation-positive and mutation-negative samples.
[c] HR <1 indicates a difference in favor of gefitinib.
[d] OR >1 indicates a greater chance of response on gefitinib.
[e] These results should be interpreted with caution as the logistic regression model did not converge.
cfDNA, circulating free DNA; CI, confidence interval; *EGFR*, epidermal growth factor receptor; HR, hazard ratio; ITT, intent-to-treat; OR, odds ratio; ORR, objective response rate; PFS, progression-free survival; PS, performance status; WHO, World Health Organization.

believe that this result was due to the high rate of false negative results as described later (i.e., this group included both tumor *EGFR* M+ and M− patients).

In the cfDNA M+ subgroup, ORR was not significantly different in the gefitinib group compared with carboplatin/paclitaxel treatment (75.0% [18/24] and 63.6% [14/22], respectively; odds ratio [OR], 1.71; 95% CI, 0.48–6.09; p = 0.40). In the cfDNA M− subgroup, there were no significant differences in ORR with gefitinib compared with carboplatin/paclitaxel (27.1% [19/70] and 21.8% [17/78], respectively; OR, 1.34; 95% CI, 0.63–2.84; p = 0.45) (Figure 4). Again, this subgroup included both tumor *EGFR* M+ and M− patients as described later.

The results for clinical outcome by *EGFR* mutation status (M+, M−) for the Japanese subset of patients with known tumor *EGFR* mutation status (n = 91) are included in Supplemental Digital Content 2 (Results http://links.lww.com/JTO/A153).

Comparison of *EGFR* Mutation Status in Pretreatment cfDNA and Tumor Tissue

A total of 108 patients had a known mutation result by cfDNA but not by tumor; 5 patients had a known mutation

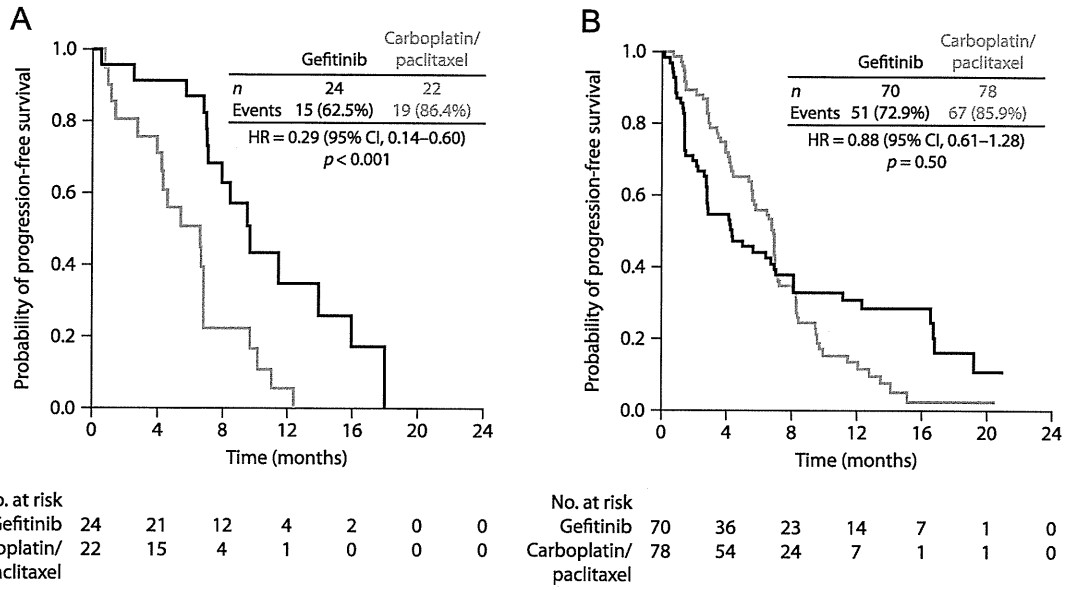

FIGURE 3. Kaplan-Meier curves of progression-free survival in cfDNA *EGFR* mutation-positive (*A*) and cfDNA *EGFR* mutation-negative (*B*) patients in the Japanese subset of IPASS. HR <1 indicates a difference in favor of gefitinib. CI, confidence interval; cfDNA, circulating free DNA; *EGFR*, epidermal growth factor receptor; HR, hazard ratio.

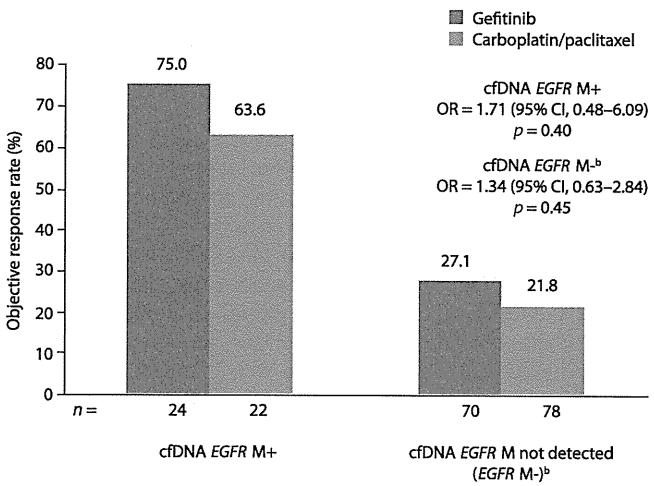

FIGURE 4. Objective response rates by treatment and by cfDNA (serum) *EGFR* mutation status (Japanese ITT population[a]). [a]Refers to the country of recruitment and not necessarily to racial origin. [b]There was a high rate of false-negative results, i.e., this group included both tumor *EGFR* M+ and M− patients. OR >1 implies a greater chance of response on gefitinib. OR, CI, and *p* values from logistic regression. cfDNA, circulating free DNA; CI, confidence interval; *EGFR*, epidermal growth factor receptor; ITT, intent-to-treat; M+, mutation-positive; M−, mutation-negative; OR, odds ratio.

result by tumor but not cfDNA (no serum sample provided); and 86 patients had a known mutation status by both tumor and cfDNA.

Of the 86 patients who had a known tumor and cfDNA mutation status, no false positives were identified (i.e., no samples were tumor M− but cfDNA M+). All 22 patients

TABLE 2. Comparison of *EGFR* Mutation Status in cfDNA and Tumor Samples in 86 Patients with a Known *EGFR* Mutation Status Using Both Methods (Japanese[a] ITT Population)

	Mutation Status (Tumor Tissue), *n*		
	M+	M−	Total
Mutation status (cfDNA), *n*			
M+	22	0	22
M−	29	35	64
Total	51	35	86

Sensitivity = 43.1% (22 cfDNA M+ out of 51 tumor M+).[b]
Specificity = 100% (all 35 tumor M- were cfDNA M−).[b]
Positive predictive value = 100% (all 22 cfDNA M+ were tumor M+).[b]
Negative predictive value = 54.7% (35 tumor M- out of 64 cfDNA M−).[b]
Concordance = 66.3% (cfDNA and tumor results agreed in 57 of 86 cases).[b,c]

[a] Refers to the country of recruitment and not necessarily to racial origin.
[b] Those with a known *EGFR* mutation status using both methods.
[c] Kappa coefficient 0.38 (95% CI, 0.24–0.53).
cfDNA, circulating free DNA; CI, confidence interval; *EGFR*, epidermal growth factor receptor; ITT, intent-to-treat; M+, mutation positive; M−, mutation negative.

identified as cfDNA *EGFR* M+ were tumor *EGFR* M+, i.e., the positive predictive value was 100% (all samples that were cfDNA M+ were tumor M+) and the specificity was 100% (all samples that were tumor M− were cfDNA M−) (Table 2). However, the rate of false negatives was high: 29/51 (56.9%) of patients identified as tumor *EGFR* M+ were cfDNA *EGFR* M− (Table 2).

EGFR Mutation Types in Pretreatment cfDNA and Tumor Tissue

Of the patients classified as *EGFR* M+ at pretreatment by both tumor and cfDNA, all had the same mutation type in

TABLE 3. *EGFR* Mutations in Pretreatment cfDNA vs. Tumor Samples (Japanese[a] ITT Population)

	Tumor *EGFR* Mutation[b]						
cfDNA *EGFR* Mutation	Exon 19 Deletions Only	Exon 20 T790M Only	Exon 21 L858R Only	Exon 20 T790M and Exon 21 L858R	Negative	Unknown	Total
Exon 19 deletions only	11	0	0	0	0	15	26
Exon 20 T790M only	0	0	0	1	0	1	2
Exon 21 L858R only	0	0	10	0	0	8	18
Exon 20 T790M and exon 21 L858R	0	0	0	0	0	0	0
Negative	18	0	11	0	35	84	148
Unknown	2	1	0	0	2	34	39
Total	31	1	21	1	37	142	233

The categories are mutually exclusive. The categories "Exon 19 deletions and exon 20 T790M" and "Exon 19 deletions and exon 21 L858R" were 0 for both tumor and cfDNA and have been omitted from the table.

[a] Refers to the country of recruitment and not necessarily to racial origin.

[b] Mutations that were tested in tumor tissue samples but not serum included: exon 20 insertion, exon 21 L861Q, exon 18 G719X, and exon 20 S768I. Two patients with tumor samples had these mutations (1 with exon 20 insertion and 1 with exon 21 L861Q). These patients were excluded from the comparative analysis of mutation detection by sample type.

cfDNA, circulating free DNA; *EGFR*, epidermal growth factor receptor; ITT, intent-to-treat.

tumor and cfDNA except one patient who had exon 20 T790M and exon 21 L858R by tumor but exon 20 T790M only by cfDNA (Table 3).

DISCUSSION

The feasibility of using cfDNA to detect *EGFR* mutations was assessed in the Japanese subset of patients from the IPASS study. The proportion of patients identified as *EGFR* M+ was lower when assessed in cfDNA (23.7%) compared with tumor tissue (61.5%). Although cfDNA results identified no false positives, a high rate of false negatives (56.9%) was observed, with more than half of the tumor M+ patients not detected by cfDNA testing (of patients with evaluable mutation status from both cfDNA and tumor). Further research into appropriate methods and analysis needs to be performed before it could be accepted as an option in the diagnostic or screening setting. If larger patient series confirmed the absence of false-positive results and demonstrated an improvement or lowering of false-negative results, serum testing may prove useful for patients for whom tumor samples are not available.

Testing of biopsied tumor tissue remains the current recommended method for *EGFR* mutation analysis.[8] However, tumor tissue is often difficult to obtain, particularly from patients with advanced non-small cell lung cancer (NSCLC), and a lack of tumor cells in a given sample and subsequently failure on pathological examination can make *EGFR* mutation analysis very difficult. The increased recognition of the relevance of mutation testing to treatment selection may stimulate efforts to better obtain tissue for *EGFR* mutation testing in the future. In the meantime, detection of *EGFR* mutation status in cfDNA derived from serum/plasma may allow patients without diagnostic tumor material the opportunity to benefit from personalized treatment and also has a use in the clinical trial setting where tumor material is not always available.

Although minimally invasive, the use of serum as a nontumor surrogate sample may be limited by the amount of cfDNA available in the sample, meaning that some positive samples are not detected. In addition, some patients may not have cf tumor DNA as their tumors may not be releasing this material into the bloodstream, giving rise to false-negative results. Because of the limited yields of cfDNA obtained from serum, two changes (in addition to duplicate tests) were made to the *EGFR* mutation ARMS kit used to detect *EGFR* mutations in this study: an increase in the number of PCR cycles and an alteration of the cutoffs used to define M+ samples (dCt values). Further analysis is underway to investigate whether these conditions are the most appropriate and whether less stringent settings could result in more true positives (fewer false negatives) while retaining no false positives.

There have been several reports on the detection of cfDNA *EGFR* mutation status using different methods. A significant correlation between cfDNA *EGFR* mutation status and clinical response to gefitinib was found in two previous small studies that assessed cfDNA *EGFR* mutation status using the ARMS method of detection, a highly sensitive (1% sensitive) targeted technique to detect specific known *EGFR* mutations.[9,11] Other screening techniques detect all *EGFR* mutations, known and novel variants, by PCR amplification followed by sequencing, pyrosequencing, or melt analysis (10–30% sensitivity).[8] However, although these methods are widely used for *EGFR* mutation analysis of DNA derived from tumor tissue, not all of these methods have demonstrated utility for *EGFR* mutation analysis of cfDNA. In a small study that used DNA sequencing to detect *EGFR* mutations in serum, mutations were more frequently observed in patients experiencing partial response or stable disease compared with those whose disease progressed, although the difference did not reach statistical significance.[10] No statistically significant association between cfDNA *EGFR* mutation status and PFS by multivariate analysis (HR, 1.48; 95% CI, 0.93–2.36; $p = 0.09$) was found in the study by Rosell et al.[12] which assessed *EGFR* mutations by PCR-based methods in the presence of a protein nucleic acid (PNA) clamp in the cfDNA extracted from serum of 164 patients

treated with erlotinib. In another study that used denaturing high-performance liquid chromatography to analyze for mutations in exons 19 and 21 from matched plasma and tumor samples, patients with plasma *EGFR* mutations had significantly higher ORR and prolonged PFS.[7] The present study using ARMS demonstrated that the treatment effect for the Japanese cfDNA *EGFR* M+ subgroup followed the same pattern as the tumor *EGFR* M+ subgroup of the overall IPASS population (i.e., PFS HR significantly in favor of gefitinib and higher ORR with gefitinib versus carboplatin/paclitaxel).[6] There was a significant interaction between cfDNA *EGFR* mutation status and treatment for PFS.

Any variance in concordance rates for mutation results between pretreatment serum versus tumor tissue (66.3% in our study and between 58 and 93% in previously reported studies)[7,9–11] may be attributed to different methods of extraction, detection, run conditions, the size and yield of the DNA fragments, and the fact that cfDNA may not be present in the circulation of all patients with NSCLC. For example, targeted sequences amplified by ARMS are short, at 100–150 bp, leading to decreased assay failure rates (particularly from formalin-fixed paraffin-embedded material or fragments of cfDNA) compared with sequencing methods, which tend to involve the amplification of longer target sequences of 150–250 bp or above.[8,13,14,17,18]

In patients who were cfDNA *EGFR* M− in this study, no significant difference for PFS was seen with gefitinib compared with carboplatin/paclitaxel; however, the HR was not constant over time (as was observed for the overall Japanese study population). These results should be interpreted with caution as there was a high rate of false negatives, and this subgroup is likely to include tumor *EGFR* M+ and M− patients.

In conclusion, these results merit further investigation to determine whether alternative samples, including serum or plasma, may be considered for determining *EGFR* mutation status in future, particularly in cases where diagnostic tumor material is not available. Currently, analysis of tumor material is the recommended method for determining *EGFR* mutation status.

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PHASE I STUDIES

Figitumumab combined with carboplatin and paclitaxel in treatment-naïve Japanese patients with advanced non-small cell lung cancer

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Summary *Objectives* The insulin-like growth factor (IGF) signaling pathway has been implicated in the pathogenesis of numerous tumor types, including non-small cell lung cancer (NSCLC). Figitumumab is a fully human IgG2 monoclonal antibody against IGF-1 receptor (IGF-1R). *Methods* This phase I, open-label, dose-escalation study (ClinicalTrials.gov: NCT00603538) assessed the safety and tolerability of figitumumab (6, 10 and 20 mg/kg) in combination with carboplatin (area under the curve: 6 mg·min/mL) and paclitaxel (200 mg/m^2) in Japanese patients (N=19) with chemotherapy-naïve, advanced NSCLC. Treatments were administered intravenously on day 1 of a 21-day cycle for four to six cycles. Pharmacokinetics, biomarkers, and antitumor activity were also evaluated. *Results* Figitumumab in combination with carboplatin and paclitaxel was well tolerated at doses up to 20 mg/kg; no dose-limiting toxicities were observed at this dose level. When given in combination, figitumumab plasma exposure increased in an approximately dose-proportional manner. The approximate 2-fold accumulation following repeated administration supported the 21-day regimen as appropriate for figitumumab administration. Serum total IGF-1 and IGF binding protein-3 concentrations increased following figitumumab dosing, but a clear dose-dependent relationship was not demonstrated. Seven of 18 evaluable patients experienced a partial response. *Conclusions* Figitumumab 20 mg/kg in combination with carboplatin and paclitaxel was well tolerated in chemotherapy-naïve Japanese patients with NSCLC. Further analysis of biomarker data is necessary for the development of figitumumab therapy.

Keywords Carboplatin · Figitumumab · Non-small cell lung cancer · Paclitaxel

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Introduction

The insulin-like growth factor (IGF) signaling pathway comprises IGF ligands (IGF-1 and IGF-2), IGF binding proteins (IGFBP1–6) which regulate ligand bioavailability, and IGF receptors (IGF-1R and IGF-2R) [1–3]. IGF signaling has been implicated in the development of a variety of tumors, including breast, colorectal, prostate, and lung cancers [2, 3]. IGF-1R is a receptor tyrosine kinase involved in the regulation of various biological processes, including cell growth, proliferation, and inhibition of apoptosis. In non-small cell lung cancer (NSCLC), IGF-1R is frequently over-expressed in tumor tissue and also mediates the proliferation of lung cancer cell lines [3–6].

Figitumumab (CP-751,871; Pfizer Inc, La Jolla, USA), a fully human IgG2 monoclonal antibody (mAb) against IGF-1R, is one of several agents currently in development which target the IGF pathway [7]. Figitumumab monotherapy has been well tolerated in phase I studies of patients with refractory solid tumors or multiple myeloma [8–12]. The safety and efficacy of figitumumab in combination

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with carboplatin and paclitaxel were investigated previously in a Western phase Ib/II study in patients with chemotherapy-naïve, locally advanced or metastatic NSCLC [13]. Results suggested that figitumumab in combination with chemotherapy was safe and effective in this patient population.

The aim of this phase I, open-label, dose-escalation study was to assess the safety and tolerability of figitumumab in combination with carboplatin and paclitaxel in Japanese chemotherapy-naïve patients with advanced NSCLC. Secondary objectives were to evaluate pharmacokinetics, biomarkers, and antitumor activity.

Materials and methods

Study population

Patients eligible for inclusion in the study were aged 20–74 years, had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 and had previously untreated, measurable, stage IIIB/IV NSCLC. All patients had adequate organ function assessed by hemoglobin (≥10 g/dL), platelet (≥100 000 cells/μL), and absolute neutrophil (≥2,000 cells/μL) counts; serum creatinine (≤1.5 mg/dL), albumin (≤3.0 g/dL), total bilirubin (≤1.8 mg/dL), and alanine aminotranferase and aspartate aminotransferase (≤80 IU/L) levels; circulating glycosylated hemoglobin (HbA_{1c}) <7% and fasting plasma glucose levels <126 mg/dL.

Exclusion criteria included prior anticancer therapy for advanced NSCLC, presence of symptomatic brain metastases or central nervous system metastases, history of active malignancy other than NSCLC within the previous 5 years (skin cancer other than malignant melanoma and in situ cervical, gastric, and colorectal cancers were permitted), treatment for pleural effusions and/or pericardial effusions, gastrointestinal bleeding within the previous 3 months, treatment with systemic corticosteroids within the previous 2 weeks, or neuropathy ≥grade 2 within the past 2 weeks. Subjects with diabetes and significant cardiac disease, including myocardial infarction, angina, uncompensated congestive heart failure, and serious cardiac ventricular arrhythmia, and uncontrolled hypertension within the past 6 months were also excluded.

The study protocol was approved by the Institutional Review Board at the National Cancer Center, Tokyo, Japan, and the study conformed to the provisions of the Declaration of Helsinki (1996). All patients provided written, informed consent.

Study design and dosing

This was a phase I, single-center, open-label, dose-escalation study to evaluate the safety and tolerability of figitumumab in combination with carboplatin (area under the curve [AUC] 6 mg·min/mL) and paclitaxel (200 mg/m^2).

Treatments were administered intravenously on day 1 of a 21-day cycle for four to six cycles, unless disease progression or unacceptable toxicity was observed. Carboplatin was administered following completion of the paclitaxel infusion, and figitumumab was administered following completion of the carboplatin infusion. A standard 3+3 dose-escalation scheme was used to escalate the dose of figitumumab. The first cohort of patients received figitumumab 6 mg/kg, and the second and third cohorts received figitumumab at doses of 10 mg/kg and 20 mg/kg, respectively. To minimize the risk of hypersensitivity, patients received prophylactic anti-allergy medication prior to paclitaxel administration, per the prescribing information for paclitaxel. The 20 mg/kg dose was judged effective and tolerable in phase I/II studies in Western patients [8–11, 13] and therefore no dose-expansion cohort was enrolled in this study.

Dose-limiting toxicities (DLTs) were figitumumab-related grade 3 or 4 toxicities assessed during the first treatment cycle according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v3.0. DLTs included: grade 4 neutropenia lasting ≥7 days or complicated by fever (body temperature >38.0°C); and grade 4 thrombocytopenia or grade ≥3 thrombocytopenia necessitating a blood transfusion. Grade ≥3 non-hematologic adverse events (AEs; including gastrointestinal events, hyperglycemia, and/or fatigue despite the use of adequate medical intervention), and other clinically significant treatment-related AEs identified by the investigator, were also considered as DLTs.

Assessments and analyses

All patients who received at least one dose of figitumumab, carboplatin or paclitaxel were assessed for safety. AEs were graded according to the NCI CTCAE v3.0. Laboratory tests were performed at regular intervals throughout the study (including measurement of hematology, blood chemistry, coagulation, and urinalysis parameters). Vital signs and electrocardiograms (ECGs) were also assessed during screening and at regular intervals throughout the study. Blood samples for the measurement of circulating total IGF-1 and IGFBP3 by radio-immunoassay were collected prior to chemotherapy dosing and 168 h post-figitumumab infusion in cycles 1–4, prior to dosing in cycles 5 and 6, and at the end of the study. Circulating levels of human growth hormone (hGH) and insulin (fasting) were assessed at screening, day 1 (prior to dosing with study medication), and day 22 of each cycle, and at the end of the study. Glycosylated hemoglobin (HbA_{1c}) levels were assessed at screening only. Circulating glucose (fasting) and other

blood chemistry parameters were assessed on days 8 and 15 of each cycle. Tumor assessments were performed at baseline, during cycles 2, 4, and 6, and at end of study treatment. Objective response was determined according to Response Evaluation Criteria In Solid Tumors (RECIST v1.0) [14].

Pharmacokinetic profiles of figitumumab were obtained during cycles 1 and 4: blood samples were collected prior to figitumumab infusion, and 1, 24, 72, and 168 h after figitumumab infusion. In other cycles, samples were collected prior to figitumumab infusion, and 1 h after figitumumab infusion. An additional pharmacokinetic blood sample was collected at the end of the study. Plasma concentrations of figitumumab were determined by a validated enzyme-linked immunosorbent assay. Briefly, an IGF-1-soluble receptor was utilized to capture figitumumab. Figitumumab bound to the receptor was detected using a biotinylated mouse anti-human IgG2, followed by Streptavidin–Horseradish Peroxidase conjugate, and visualized using SureBlue™ peroxidase substrate. The lower limit of quantitation for the assay was 120 ng/mL. Pharmacokinetic parameters, which were calculated using non-compartmental methods, included C_{max} (maximum observed plasma concentration after the end of figitumumab infusion), $AUC_{(0-day22)}$ (area under the plasma concentration–time curve from time zero to day 22 [the nominal time of the pre-dose sample for the next cycle]), AUC_{tau} (AUC from time zero to tau [the actual time of the pre-dose sample for the next cycle]), and $t_{½}$ (apparent disposition half-life). The accumulation ratio was calculated as: cycle 4 AUC_{tau}/cycle 1 AUC_{tau}.

Due to the exploratory nature of this study, enrollment was dependent upon the observed safety profile and confirmatory inferential analyses were not planned. Descriptive statistics were used to summarize patient characteristics, safety, antitumor activity, and pharmacokinetic parameters. Analyses of the relationships between antitumor activity and circulating levels of total IGF-1, IGFBP3, hGH, and insulin were conducted. Summary statistical data are shown; statistical associations between biomarker levels and clinical outcome were not investigated due to the exploratory nature of these analyses and the small patient numbers.

Results

Baseline characteristics

Nineteen patients were enrolled across three figitumumab dose levels: 6 mg/kg, n=6; 10 mg/kg, n=7; 20 mg/kg, n=6. All patients were Japanese, and demographic and baseline characteristics are summarized in Table 1. Most patients (n=15; 78.9%) presented with stage IV NSCLC. Fourteen patients (73.7%) had adenocarcinoma, four patients (21.1%) had squamous cell carcinoma, and one patient had their histology classified as 'NSCLC not otherwise specified'.

Eighteen patients completed the first treatment cycle. One patient in the 10-mg/kg cohort discontinued the study due to a serious paclitaxel-related AE (hypersensitivity); this patient did not receive figitumumab. The median number of treatment cycles started for the 6-, 10-, and 20-mg/kg figitumumab dose levels was 4 (range 2–6), 4 (range 1–6), and 4 (range 3–6), respectively.

Safety and tolerability

DLTs were experienced by one patient at the figitumumab 6-mg/kg dose level (grade 4 thrombocytopenia) and by two patients at the 10-mg/kg dose level (one patient had grade 4 hyperuricemia, grade 3 hypermagnesemia, grade 3 hyponatremia, and grade 3 hyperkalemia, and another patient had grade 4 thrombocytopenia). No DLTs occurred in

Table 1 Patient characteristics at baseline

Characteristic	Figitumumab dose level[a]		
	6 mg/kg	10 mg/kg	20 mg/kg
Enrolled patients, *n*	6	7	6
Median age (years)	54	40	63
Range	45–69	21–61	37–74
Gender, *n*			
Male	3	4	5
Female	3	3	1
ECOG performance status, *n*			
0	5	7	4
1	1	0	2
NSCLC histologic subtype, *n*			
Adenocarcinoma	4	6	4
Squamous cell carcinoma	1	1	2
Not otherwise specified	1	0	0
Disease stage, *n*			
IIIB	0	1	3
IV	6	6	3
Smoking history, *n*			
Never smoker	4	3	0
Smoker	2	2	2
Ex-smoker	0	2	4

AUC area under the curve, *ECOG* Eastern Cooperative Oncology Group, *NSCLC* non-small cell lung cancer

[a] Figitumumab in combination with carboplatin (AUC 6 mg·min/mL) and paclitaxel (200 mg/m^2)

patients treated at the highest figitumumab dose level of 20 mg/kg (Table 2).

The most common all-causality, non-hematologic AEs of all grades across all dose levels and cycles were peripheral sensory neuropathy (n=16 [84.2%]), anorexia (n=14 [73.7%]), and diarrhea (n=9 [47.4%]). Grade 3 and 4 treatment-related hematologic and non-hematologic AEs occurring at each figitumumab dose level are presented in Table 3. The only grade ≥3 non-hematologic AEs to occur in two or more patients across all dose levels and cycles were hyponatremia/blood sodium decrease (grade 3, n=3 [15.8%]), anorexia (grade 3, n=2 [10.5%]), and hyperuricemia (grade 4, n=2 [10.5%]); hyperuricemia was the only grade 4 non-hematologic AE reported. There were no cases of grade 3 or 4 hypoglycemia or hyperglycemia. Most grade 3 or 4 non-hematologic AEs were observed during cycle 1, and figitumumab dose level did not appear to influence the frequency of grade 3 or 4 AEs.

Grade 3 treatment-related hematologic AEs (across all dose levels and cycles) were neutropenia (n=5 [26.3%]), leukopenia (n=4 [21.1%]), anemia (n=2 [10.5%]), and thrombocytopenia (n=1 [5.3%]), and grade 4 treatment-related hematologic AEs were neutropenia (n=11 [57.9%]) and thrombocytopenia (n=3 [15.8%]; Table 3). No treatment-related deaths occurred at any figitumumab dose level.

Pharmacokinetics

When given in combination with carboplatin and paclitaxel, plasma concentrations of figitumumab declined in a multi-exponential manner (Fig. 1a, b). Both C_{max} and AUC within the first cycle increased in an approximately dose-proportional manner (Table 4). As indicated by the accumulation ratio, repeated administration of figitumumab every 21 days resulted in moderate accumulation (an approximate 2-fold increase in plasma exposure in the limited number of patients with sufficient data in cycle 4; Table 4). The $t_{½}$ of figitumumab was at least 248 h (more than 10 days) for all three dose levels, and achieved the $t_{½}$ of endogenous IgG2 (approximately 21 days) in some patients.

Biomarkers

Serum total IGFBP3, IGF-1 and hGH concentrations increased following figitumumab dosing compared with baseline values for each dose level (Fig. 2a–c). However, a clear dose-dependent relationship was not demonstrated, and the levels of insulin levels were not changed clearly prior and after figitumumab administration (data not shown).

Antitumor activity

Of 18 evaluable patients, seven experienced a partial response (PR; 38.9%). One PR was observed at the figitumumab 6-mg/kg dose level, and three PRs were observed at each of the 10- and 20-mg/kg dose levels. PRs were observed in patients with adenocarcinoma (five of 14 patients [35.7%]) and in patients with squamous cell carcinoma (two of four patients [50.0%]). No patient had a complete response. Stable disease (SD) was observed in eight patients (44.4%; three at each of the 6- and 20-mg/kg dose levels, and two at the 10-mg/kg dose level), and progressive disease (PD) was observed in two patients (11.1%; both at the 6-mg/kg dose level).

Relationship between antitumor activity and biomarkers

Serum total IGFBP3 and IGF-1 concentration–time profiles were stratified by best response (PR, SD, and PD, respectively) to evaluate the relationship between concentrations of biomarkers and clinical response. No clear

Table 2 Planned dose levels and observed DLTs

Dose level	Figitumumab[a]	Paclitaxel (mg/m^2)	Carboplatin (mg·min/mL)	N	DLTs
1	6 mg/kg	200	6	6	Grade 4 thrombocytopenia (n=1)
2	10 mg/kg	200	6	7	Grade 3: hyperkalemia, hypermagnesemia, hyponatremia; grade 4 hyperuricemia (n=1) Grade 4 thrombocytopenia (n=1)
3	20 mg/kg[b]	200	6	6	None

DLT dose-limiting toxicity

[a] If none of the three patients in the 6 mg/kg cohort experienced a DLT during cycle 1, subjects were enrolled onto the next dose level. If one DLT was observed, the cohort was to be expanded to six patients. If none of the three or two or less of the six patients experienced a DLT, then dose escalation was to be continued and three patients were enrolled to the 10 mg/kg cohort. In a similar manner depending on observed DLTs, the 10 mg/kg cohort could be expanded to six patients and dose escalation continued to a 20 mg/kg cohort of six patients. If two or more of the three, or three or more of the six patients experienced a DLT, dose escalation would be stopped

[b] Six patients dosed, and 20 mg/kg deemed tolerable if two or fewer of the six patients experienced a DLT

Table 3 Treatment-related AEs with maximum CTC grade ≥3, in cycle 1 and all cycles. The numbers of patients are shown for grades 3 and 4 AEs separately, and for all grades

	Figitumumab dose level																	
	6 mg/kg (n=6)						10 mg/kg (n=7)						20 mg/kg (n=6)					
	Cycle 1			All cycles			Cycle 1			All cycles			Cycle 1			All cycles		
	All	G3	G4	All	G3	G4	All	G3	G4	All	G3	G4	All	G3	G4	All	G3	G4
AEs, hematologic																		
Anemia	4	0	0	5	0	0	3	0	0	3	1	0	4	0	0	5	1	0
Leukopenia	6	0	0	6	1	0	6	1	0	6	1	0	5	0	0	6	2	0
Neutropenia	6	2[a]	3	6	0	5	6	1	2	6	3	2	5	2	1	6	2	4
Thrombocytopenia	4	0	1	5	0	1	5	0	1	5	0	1	4	1	0	6	1	1
AEs, non-hematologic																		
Anorexia	2	1	0	3	2	0	5	0	0	6	0	0	5	0	0	5	0	0
Diarrhea	3	0	0	3	0	0	1	0	0	2	1	0	3	0	0	4	0	0
Hyperkalemia	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0
Hypermagnesemia	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0
Hypersensitivity	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0
Hyperuricemia	0	0	0	0	0	0	2	0	1	2	0	1	2	0	1	2	0	1
Hyponatremia	2	1	0	2	1	0	1	1	0	1	1	0	1	0	0	3	1	0
Peripheral sensory neuropathy	3	0	0	5	0	0	4	0	0	5	0	0	2	0	0	6	1	0
Vomiting	1	0	0	2	1	0	2	0	0	2	0	0	1	0	0	1	0	0

AE adverse event, *All* all grades, *CTC* Common Terminology Criteria, *G* grade

[a] Two patients with grade 3 neutropenia during cycle 1 experienced worsening of symptoms to grade 4 after cycle 2

differences were observed in the IGFBP3 concentration–time profiles according to clinical response, or when baseline IGFBP3 concentration was stratified by best response (Fig. 3a, b). However, the serum total IGF-1 concentration–time profile in patients with PR as their best response was higher than the profiles in both SD and PD patients (Fig. 3c). Higher baseline serum total IGF-1 concentrations were also observed for patients with PR compared with patients with SD/PD as best response (Fig. 3d).

Discussion

Figitumumab in combination with carboplatin and paclitaxel was well tolerated at doses up to 20 mg/kg in chemotherapy-naïve Japanese patients with advanced NSCLC in this phase I study. No DLTs were observed at the highest figitumumab dose level of 20 mg/kg. In addition, no grade 3 or 4 AEs appeared to show dose dependency, and there was no apparent tendency towards cumulative toxicity.

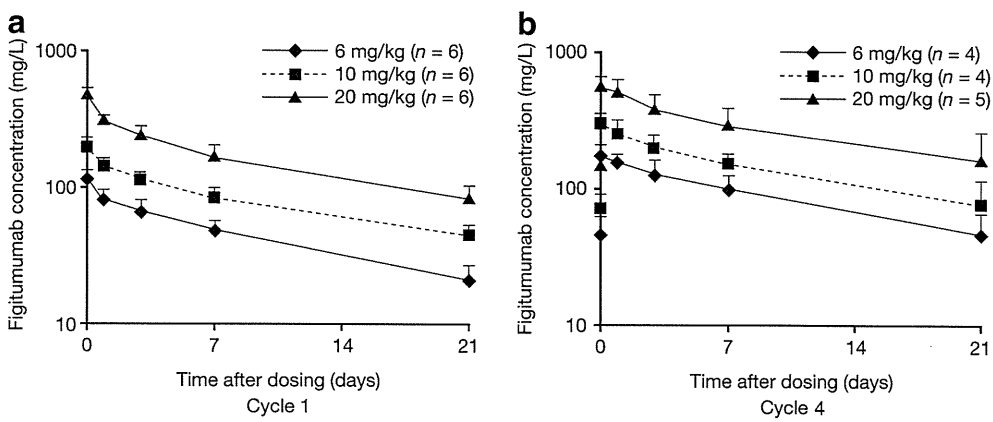

Fig. 1 Concentration–time profiles of plasma figitumumab. Data shown are mean + standard deviation

Table 4 Plasma pharmacokinetic parameters (mean ± SD) of figitumumab given in combination with carboplatin and paclitaxel

Figitumumab dose level (mg/kg)	Cycle 1				Cycle 4			Accumulation ratio
	n	C_{max} (mg/L)	$AUC_{(0-day22)}$ (mg·h/L)	$t_{½}$ (h)	*n*	C_{max} (mg/L)	AUC_{tau} (mg·h/L)	
6	6	113±16	22,400±4,050	264[a]	4	178±35	39,000, 66,000[b]	1.7, 2.6[b]
10	6	197±33	36,700±10,400	301[a]	4	294±61	96,100, 96,800[b]	2.2, 2.2[b]
20	6	485±59	82,700±11,200	248[a]	5	550±89	116,000, 190,000[b]	1.6, 2.1[b]

$AUC_{(0-day22)}$ area under the plasma concentration–time curve from time zero to day 22, AUC_{tau} AUC from time zero to tau (the actual time of the pre-dose sample for the next cycle), C_{max} maximum observed plasma concentration after the end of figitumumab infusion, *SD* standard deviation, $t_{1/2}$ apparent disposition half-life

[a] n=4 at 6 mg/kg, n=1 at 10 mg/kg, and n=4 at 20 mg/kg (sampling was not sufficient to capture terminal disposition phase in other patients)

[b] n=2

No cases of grade 3 or 4 hyperglycemia (treatment-related or all-causality) were reported in the present study (grade 2 hyperglycemia was reported in one patient). Hyperglycemia has been reported in other studies of figitumumab and in studies of other IGF-1R-targeted mAbs [8–11, 13, 15–17]. Hyperglycemia may be a characteristic of the anti-IGF-1R class of compounds; however, its mechanism is unknown.

Incidences of grade 3 and 4 treatment-related neutropenia and thrombocytopenia in the figitumumab arm of the larger Western phase II randomized study of figitumumab in combination with paclitaxel and carboplatin in chemotherapy-naïve NSCLC were 28% and 7%, respectively, compared with 84% and 21% in the current trial [13]. Similar ethnic differences in the incidence of neutropenia have also been observed in a Japanese–US common-arm analysis of carboplatin plus paclitaxel in advanced NSCLC, and were suggested to be related to differences in allelic distribution of genes associated with DNA repair and paclitaxel disposition [18]. However, such an ethnic difference was not observed for thrombocytopenia [18]. Further studies would be required to determine whether there are pharmacogenetic or other reasons for ethnic differences in the incidence of thrombocytopenia in patients treated with figitumumab.

In line with previous phase I studies [8–11], figitumumab plasma exposure increased in an approximately dose-proportional manner and concentrations declined in a multi-exponential manner when figitumumab 6–20 mg/kg was given in combination with carboplatin and paclitaxel in the present study. The approximate 2-fold accumulation following repeated administration supported the 21-day regimen as appropriate for figitumumab administration in Japanese patients; similar accumulation was reported in Western studies [8–11]. No relationship between ethnicity and pharmacokinetics was expected, since figitumumab (as a mAb) extravasates mainly by convection and is eliminated by catabolism and/or target-mediated clearance.

High serum total IGF-1 and low IGFBP3 levels have been associated with higher incidence of NSCLC [19]. Hepatic IGF-1 production is stimulated by hGH, and hGH production is regulated by IGF-1 through negative feedback [20]. A previous phase I study demonstrated that single-agent figitumumab (20 mg/kg) altered the endocrine feedback mechanisms regulating hGH [8]. In the present study, both hGH and serum total IGF-1 concentrations appeared to increase following dosing with study medication, and this suggests blockade of IGF-1R with loss of IGF-1 regulatory feedback at the pituitary. In a phase I

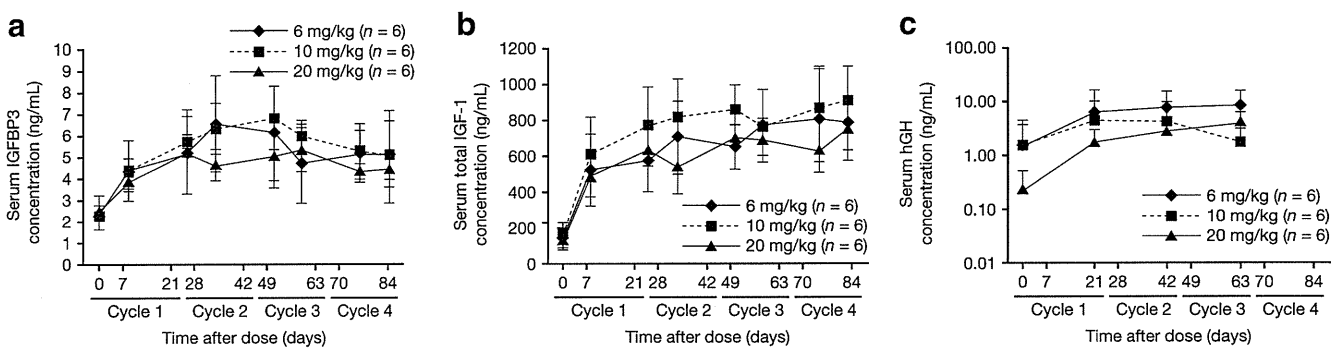

Fig. 2 Circulating biomarker concentrations during study treatment: concentration–time profile of serum IGFBP3 (panel **a**), serum total IGF-1 (panel **b**), and serum hGH (panel **c**). Data shown are mean ± standard deviation (panel **a** and panel **b**) and mean + standard deviation (panel **c**)

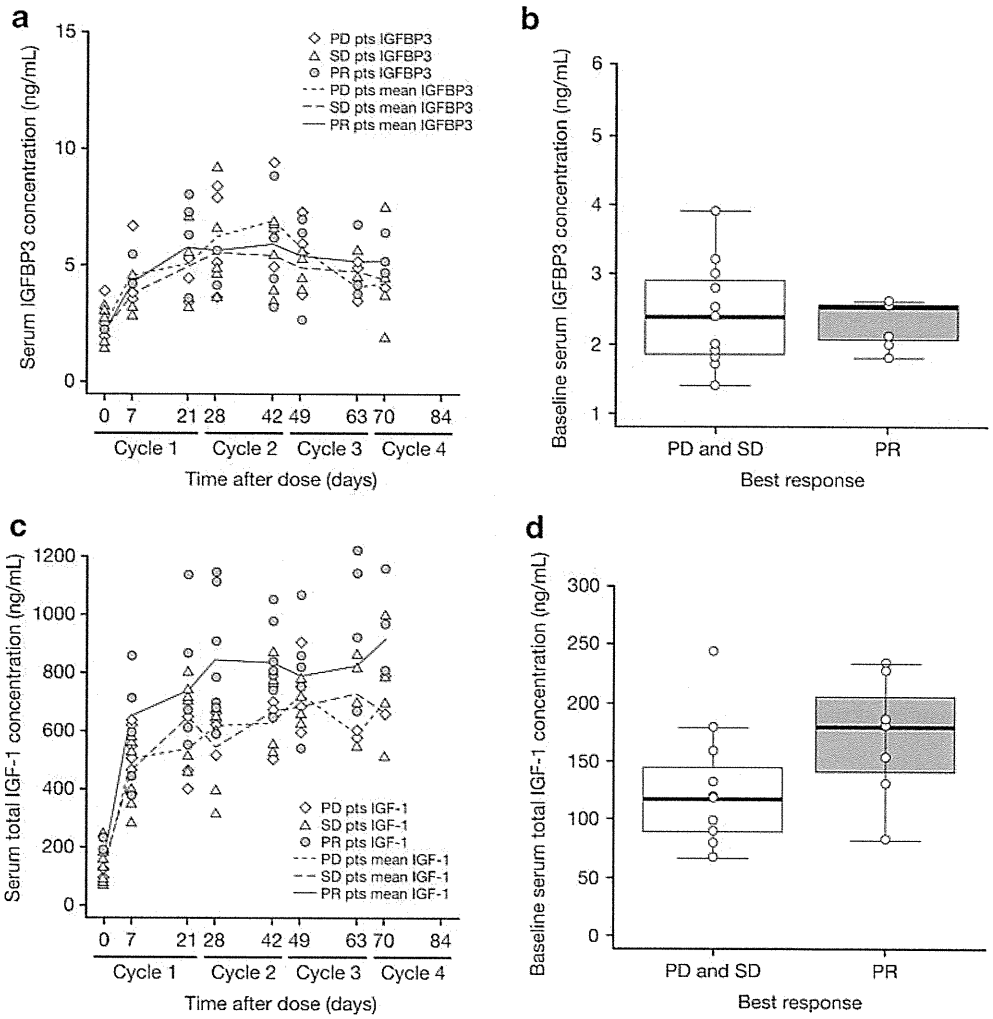

Fig. 3 Relationship between biomarkers and clinical response: serum IGFBP3 concentration–time profiles by best response (panel **a**); baseline serum IGFBP3 concentrations by best response—boxes represent median, 25%, and 75% percentiles (panel **b**); serum total IGF-1 concentration–time profiles by best response (panel **c**); baseline serum total IGF-1 concentrations by best response—boxes represent median, 25%, and 75% percentiles (panel **d**). *Pts* patients

study in myeloma patients and in the Western phase II trial in NSCLC patients described above, dose-dependent sustained elevations of serum IGF-1 and IGFBP3 concentrations were observed following administration of figitumumab [10, 21], indicating dose-dependent blockade of IGF-1R by figitumumab. However, a similar dose-dependent relationship between figitumumab and circulating IGF-1 and IGFBP3 concentrations was not clearly demonstrated in the current phase I study. Large inter-individual variability in serum total IGF-1 and IGFBP3 concentrations is known to occur naturally, and the differences between the studies may reflect the small number of patients included in each dose cohort level in the current study. Alternatively, the lack of a dose-dependent elevation of IGF-1 noted in this study may be related to the Japanese patients in this study having lower body mass index (BMI) compared with patients in other figitumumab studies. This possibility is supported by a report which suggests a relationship between BMI and IGF-1 levels [22].

Closure of the phase III studies of figitumumab in NSCLC (ADVIGO [ADVancing IGF-IR in Oncology] 1016 and 1018) due to potential futility of the combination regimens (figitumumab with paclitaxel plus carboplatin, and with erlotinib, respectively) has underscored the need to identify patients most likely to benefit from anti-IGF-1R therapy [23]. Studies have indicated that baseline levels of circulating free IGF-1 may be a positive biomarker for clinical response to figitumumab [13, 21, 23]. In the present study, serum total IGFBP3 and IGF-1 concentration–time profiles were stratified by best response as part of an exploratory analysis of the relationship between biomarker levels and antitumor activity. No clear differences were observed in the IGFBP3 concentration–time profiles according to clinical response, or when baseline IGFBP3 concentration was stratified by best response. However, the serum total IGF-1 concentration–time profile in patients with PR as their best response was higher than the profiles in both SD and PD patients. Additionally, baseline serum total IGF-1 concentration appeared higher in patients with PR compared with patients having SD/PD as their best response. Although the relationship between outcome and biomarkers was not examined statistically due to the exploratory nature of these investigations and the small number of patients, these observations suggest that serum

total IGF-1 concentrations prior to the start of treatment may also be a positive biomarker for response.

In summary, figitumumab 20 mg/kg in combination with carboplatin and paclitaxel was well tolerated in chemotherapy-naïve Japanese patients with advanced NSCLC. Serum total IGF-1 is a potential biomarker for clinical response to figitumumab and requires further investigation.

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Quantity of supporting information None.

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PHASE I STUDIES

Safety and pharmacokinetic study of *nab*-paclitaxel plus carboplatin in chemotherapy-naïve patients with advanced non–small cell lung cancer

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Summary *Background* Nanoparticle albumin-bound paclitaxel (*nab*-paclitaxel) is a Cremophor EL–free formulation of paclitaxel newly designed to avoid solvent-related toxicities. We have evaluated the safety, tolerability, pharmacokinetics, and tumor response profile of weekly *nab*-paclitaxel (100 mg/m^2) infusion together with administration of carboplatin at an area under the curve (AUC) of 6 every 3 weeks in Japanese patients with advanced non–small cell lung cancer (NSCLC). *Methods* *Nab*-paclitaxel (100 mg/m^2) was administered without steroid or antihistamine premedication as a 30-min intravenous infusion once a week in combination with carboplatin at an AUC of 6 on day 1 of repeated 21-day cycles. The pharmacokinetics of both drugs were analyzed, and both adverse events and treatment response were monitored. *Results* Eighteen patients were enrolled in the study. The most frequent treatment-related toxicities of grade 3 or 4 were neutropenia (67%), leukopenia (50%), and anemia (22%). No severe hypersensitivity reactions were observed despite the lack of premedication, and no unexpected or new toxicities were detected. Pharmacokinetics analysis did not reveal any substantial drug-drug interactions. Seven partial responses were observed among the 18 evaluable patients, yielding a treatment response rate of 38.9%. *Conclusions* The combination of *nab*-paclitaxel (100 mg/m^2) administered weekly and carboplatin at an AUC of 6 every 3 weeks was well tolerated in Japanese patients with advanced NSCLC. This combination therapy also showed promising antitumor activity and was not associated with relevant pharmacokinetic interactions.

Keywords *Nab*-paclitaxel · Carboplatin · Non–small cell lung cancer · Pharmacokinetics · Safety

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Introduction

Lung cancer is the leading cause of death related to cancer worldwide, with non–small cell lung cancer (NSCLC) accounting for 85% of lung cancer cases [1]. Platinum-based chemotherapy is the mainstay of first-line treatment for advanced NSCLC on the basis of the moderate improvement in survival and quality of life it confers compared with best supportive care alone [2]. Given the safety and efficacy limitations of current therapeutic options, however, new chemotherapeutic agents and combi-

nation regimens are needed to further ameliorate symptoms and increase antitumor activity in a manner that is both convenient and safe in patients with advanced NSCLC.

The most commonly used taxane combination regimen for treatment of advanced NSCLC is carboplatin plus solvent-based paclitaxel. Paclitaxel is highly hydrophobic, and first-generation formulations include Cremophor EL (polyoxyethylated castor oil) and an ethanol vehicle to allow parenteral administration [3]. Given that Cremophor EL causes leaching of the plasticizers from standard intravenous tubing and is also associated with hypersensitivity reactions, administration of solvent-based paclitaxel requires a long infusion period (typically 3 h), the use of special non–polyvinyl chloride infusion systems and in-line filtration, and premedication with corticosteroids, diphenhydramine, and an H_2 histamine receptor antagonist to minimize the incidence of potentially life-threatening hypersensitivity [4, 5]. Severe and sometimes fatal hypersensitivity reactions sometimes still occur, however, even after administration of these premedications [6].

Nanoparticle albumin-bound paclitaxel (*nab*-paclitaxel, Abraxane) was developed for delivery of paclitaxel as a suspension of albumin particles in saline, allowing a shorter infusion time and use of a standard infusion set [7]. This new Cremophor EL–free formulation does not require steroid and antihistamine premedication to prevent hypersensitivity reactions. Furthermore, preclinical studies have suggested that this formulation may improve drug delivery into tumors [8]. In phase I trials, *nab*-paclitaxel has been administered safely at doses higher than labeled doses for solvent-based paclitaxel [7]. A phase III trial in patients with advanced breast cancer showed that administration of *nab*-paclitaxel every 3 weeks (q3w) resulted in a significantly higher response rate (33 versus 19%, $P<0.001$) and longer time to tumor progression (5.8 versus 4.2 months, $P<0.006$) compared with q3w solvent-based paclitaxel [9]. A phase II study of *nab*-paclitaxel at 260 mg/m^2 q3w in chemotherapy-naïve patients with advanced NSCLC also revealed single-agent antitumor activity with a response rate of 16% [10]. Furthermore, weekly administration of *nab*-paclitaxel (125 mg/m^2) yielded an increased response rate of 30% in 40 individuals with advanced NSCLC who had not received prior chemotherapy [11]. More recently, a dose-finding phase II study demonstrated that *nab*-paclitaxel administered weekly was associated with less serious adverse events when administered q3w, with significant reductions in the incidence of peripheral neuropathy, myalgia, arthralgia, and alopecia [12]. In the phase II study, weekly administration of *nab*-paclitaxel at 100 mg/m^2 combined with carboplatin (area under the curve [AUC], 6) yielded a response rate of 48% and median progression-free survival of 6.2 months as first-line treatment for advanced NSCLC [12]. Given the promising efficacy and excellent safety of *nab*-paclitaxel, the combination of weekly *nab*-paclitaxel with carboplatin warrants further investigation. To date, however, pharmacokinetic data for such treatment have been limited. The primary objective in the present study was to evaluate the safety of weekly *nab*-paclitaxel (100 mg/m^2) administered in combination with q3w carboplatin at an AUC of 6 in Japanese advanced NSCLC without prior systemic chemotherapy. The secondary objectives were to determine the pharmacokinetics of paclitaxel after *nab*-paclitaxel administration on cycle 1 days 1 (with carboplatin) and 15 (without carboplatin).

Patients and methods

Patients

Eligible patients were 18 years of age or older with histologically or cytologically confirmed NSCLC of stage IIIB or IV. They were required to be naïve with regard to chemotherapy for metastatic disease. The eligibility criteria also included adequate bone marrow, hepatic, and renal function, an Eastern Cooperative Oncology Group performance status of 0 or 1, a life expectancy of >12 weeks, and radiologically documented measurable disease. Individuals were excluded if they had evidence of active brain metastasis or preexisting peripheral neuropathy of grade ≥2 defined according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v3.0, or if they had received radiotherapy in the previous 4 weeks. Patients with any other clinically serious concurrent illness were also excluded.

The study followed the ethical principles in the Declaration of Helsinki, and the study protocol was approved by the institutional review board of each participating center. All patients received information regarding the nature and purpose of the study, and they provided written informed consent before study-related procedures were performed.

Treatment

The study was conducted to evaluate the safety, tolerability, pharmacokinetics, and tumor response profile of weekly *nab*-paclitaxel at 100 mg/m^2 and q3w carboplatin at an AUC of 6 in Japanese patients with advanced NSCLC. Carboplatin was administered at an AUC of 6 calculated according to the Calvert formula on day 1 of a 21-day cycle. *Nab*-paclitaxel (100 mg/m^2) was administered by a 30-min intravenous infusion on days 1, 8, and 15 of each cycle without steroid or antihistamine premedication to prevent a hypersensitivity reaction. On days of carboplatin dosing, patients received the serotonin/5-hydroxytryptamine receptor 3 (5-HT_3) antagonist as antiemetic therapy. A