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ORIGINAL ARTICLE

Phase 1 study of the investigational, oral angiogenesis inhibitor motesanib in Japanese patients with advanced solid tumors

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Abstract
Purpose The aim of this study was to investigate the safety and pharmacokinetics of motesanib (AMG 706), a small-molecule antagonist of vascular endothelial growth factor receptors 1, 2, and 3, platelet-derived growth factor receptor, and c-Kit in Japanese patients with advanced solid tumors.
Methods Patients were administered motesanib orally once daily (QD) at doses of 50, 100, and 125 mg QD. The total study duration for each patient consisted of three cycles of 28 days per cycle. The primary endpoints were the incidence of dose-limiting toxicities (DLTs), estimation of the maximum tolerated dose (MTD), and assessment of pharmacokinetic parameters of motesanib.
Results Fifteen patients were enrolled and received motesanib. No DLTs were observed and, therefore, the MTD was not reached. Motesanib had acceptable toxicity at doses up to 125 mg QD. The pharmacokinetics of motesanib appears to be dose proportional. No objective responses per RECIST were observed. However, all 15 patients achieved stable disease, and five patients had durable (>24 weeks) stable disease.
Conclusions The results of this study demonstrate that motesanib is tolerable in Japanese patients at doses up to 125 mg QD.

Keywords Motesanib · Advanced solid tumors · Pharmacokinetics · Maximum tolerated dose

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Introduction

Cancer is the leading cause of death in Japan [1]. Despite the use of surgery, chemotherapy, radiation therapy, and other treatments, more than 325,000 Japanese are estimated to die of cancer each year [1] and, consequently, attention has focused on the development of novel treatments for cancer. In particular, because solid tumors are dependent on the development of vascular networks for continued growth and development, there has been interest in inhibition of angiogenesis (the process by which new blood vessels develop) as an anticancer therapy [2–4]. Antiangiogenic agents have been shown to have antitumor activity in preclinical models of human cancer [5–7] and to have clinical activity in the treatment of advanced solid tumors [8–11].

Vascular endothelial growth factor (VEGF) is among the most potent proangiogenic factors [12, 13]. The effects of VEGF on the vasculature are mediated by activation of its receptors, the receptor tyrosine kinases VEGFR1 (Flt-1) and VEGFR2 (KDR), with most of the proangiogenic effects of VEGF being mediated by VEGFR2 [12]. In addition to its effects on cellular proliferation, activation of the platelet-derived growth factor receptor (PDGFR) may also

contribute to angiogenesis by both increasing VEGF expression and promoting migration of endothelial cells [14–16].

Motesanib (AMG 706) is an orally administered small-molecule antagonist of VEGFR1, 2, 3; PDGFR, and c-Kit, and is currently in development for the treatment of solid tumors [17]. Furthermore, in a phase 1 study conducted in the US, motesanib was shown to have promising antitumor activity and acceptable toxicity [18]. The aims of this study were to investigate the safety (including the maximum tolerated dose [MTD]), pharmacokinetics, antitumor activity, and pharmacodynamics of motesanib in Japanese patients with advanced solid tumors.

Patients and methods

Patients

Eligible patients were aged 20–74 and had histologically or cytologically documented advanced solid tumors that were refractory to standard therapy or for which no standard therapy was available. Additional inclusion criteria were an Eastern Cooperative Oncology Group performance status of ≤ 2, absolute neutrophil count $\geq 1.5 \times 10^9$/l, platelet count $\geq 100 \times 10^9$/l, hemoglobin ≥ 9.5 g/dl, serum creatinine ≤ 1.5 mg/dl, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 80 IU/l, alkaline phosphatase $\leq 1{,}000$ IU/l, and total bilirubin ≤ 1.8 mg/dl. Patients were excluded from the study if they had hematologic malignancy; central nervous system metastases requiring therapy or with symptoms; non-small-cell lung cancer with squamous cell histology in the hilar regions; active multiple primary cancer; history of bleeding, diathesis, or hypercoagulopathy; history of arterial thrombosis; history of cardiovascular disease including myocardial infarction, congestive heart failure, uncontrolled hypertension (diastolic >85 mm Hg, systolic >145 mm Hg), and arrhythmia; concurrent interstitial pneumonitis, pulmonary fibrosis, hemoptysis, diabetes or poorly controlled diabetes; or pleural effusion or ascites requiring drainage. Patients were also excluded if they had received previous treatment with small-molecule VEGF receptor inhibitors; received chemotherapy, radiation therapy, or surgery within 4 weeks of study day 1; received antibody treatment within 12 weeks of study day 1; or received anticoagulation therapy within 7 days of study day 1. This protocol was approved by the Institutional Review Board of National Cancer Center (Tokyo, Japan). All patients provided written informed consent.

Study design

This was a phase 1, single-center, open-label, sequential dose-escalation study conducted in Japan. The primary endpoints were the incidence of dose-limiting toxicities (DLTs; defined later), estimation of the MTD, and assessment of pharmacokinetic parameters of motesanib following oral administration. Secondary endpoints included the incidence of adverse events, changes in clinically significant laboratory markers, measurement of pharmacodynamic parameters (including markers of angiogenesis), and assessment of tumor response and serum tumor markers. Patients were hospitalized at least from one day before motesanib administration and discharged after day 1 (cycle 2). After being discharged, during cycles 2 and 3, patients visited the clinic every week (during cycle 2) or every 2 weeks (during cycle 3), at which time clinical and laboratory evaluations were performed.

Based on the results of a previously reported clinical study, the starting dose of motesanib (Amgen Inc., Thousand Oaks, CA) was 50 mg. Planned motesanib dosages were 50, 100, 125, and 150 mg. Intrapatient dose escalation was not allowed. The total study duration for each patient consisted of three cycles of 28 days per cycle. The first cycle consisted of dosing on day 1, no dosing on day 2, daily dosing from day 3 through day 21 (19 days of consecutive dosing) and 1 week off. During the first cycle, the first motesanib dose was followed by a 48-h PK assay and measurements of urinary motesanib. During the second and third cycles, dosing will occur daily for 28 consecutive days. Dosing in this study was intended to continue for three cycles but was discontinued if disease progression was observed. If a DLT developed, administration was interrupted, and thereafter the decision to discontinue or restart was made by the investigator after the toxicity had resolved.

Three patients were initially enrolled in each cohort. If no patients experienced a DLT at the initial dose level, patients could be enrolled in the subsequent cohort. If a DLT occurred, an additional three patients could be enrolled at the current dose level to assess safety. If one patient experienced a DLT, the next dose level could be enrolled; if two or more patients experienced a DLT the next appropriate dose (increased or decreased) was determined by the principal investigator, Amgen, Inc., the study medical expert, and the study efficacy and safety evaluation committee. Additional patients could be enrolled in each cohort to further evaluate safety. Treatment with motesanib continued for up to 12 weeks (i.e., three treatment cycles). However, patients could choose to continue receiving motesanib until disease progression or unacceptable toxicity occurred.

Motesanib was also withheld when ANC was $<0.5 \times 10^9$/l. Treatment with motesanib could resume at the level of the immediately preceding dose cohort at the discretion of the investigator. Patients in the 50 mg QD cohort could restart at a dose of 25 mg QD. Patients who

required >2 weeks to recover from a DLT, experienced a second DLT, or who had cardiotoxicity meeting the DLT criteria were withdrawn from the study.

Dose-limiting toxicities and maximum tolerated dose

In this study, DLTs were defined as any treatment-related grade 3 or 4 nausea, diarrhea, or vomiting despite maximum supportive care; grade 3 or 4 neutropenia with fever >38.5°C; grade 3 fatigue, persistent for ≥7 days; grade 4 hypertension; or AST or ALT >300 IU/l; any other grade 3 or 4 non-hematologic toxicity; or any other grade 4 hematologic toxicity occurring during the first cycle. The MTD was defined as the highest dose level at which the incidence of DLTs was <33% of patients enrolled in the cohort.

Pharmacokinetics

Intensive pharmacokinetic analysis was performed for all patients on days 1 and 21 of the first treatment cycle. Plasma samples were taken predose and at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 24, and 48 h postdose (the 48-h collection was performed on day 1 only). Aliquots of plasma were prepared for analysis using solid phase extraction and motesanib concentrations were measured by a liquid chromatography–tandem mass spectrometry method using d3-motesanib as an internal standard. Two versions of a validated method were utilized having calibration curve ranges of 0.200–100 ng/ml for the 50 mg QD cohort and 0.500–100 ng/ml for other cohorts. Pharmacokinetic parameters of motesanib including observed maximum plasma concentration (C_{max}), time of maximum observed plasma concentration (t_{max}), area under the concentration versus time curve (AUC), and terminal elimination half-life ($t_{1/2,z}$) were calculated by non-compartmental analysis using WinNonlin software (Version 4.1e, Pharsight Corporation, Mountain View, CA).

Safety

Adverse events and their relationship to treatment were recorded throughout the study. Adverse events were classified according to the Common Terminology Criteria for Adverse Events (version 3.0). Urinalysis and assessments of hematologic function, coagulation, and clinical chemistry were performed up to four times each cycle.

Efficacy

Tumors were measured by magnetic resonance imaging or computed tomography at a maximum of 4 weeks before study day 1 and at approximately 8-week intervals during the study. Tumor responses were evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST) [19].

Exploratory analysis of markers of angiogenesis

Serum samples for measurement of markers of angiogenesis were collected on study days 1 and 22 and at the end of the study. Serum concentrations of placental growth factor (PlGF), basic fibroblast growth factor (bFGF), VEGF, soluble KDR (sKDR), Flt-1, and soluble c-Kit were measured using a multiplexed sandwich immunoassay technique (Meso Scale Discovery (Gaithersburg, MD)).

Statistical analyses

Descriptive statistics are provided for each endpoint by cohort. The safety analysis population consisted of all patients who received at least one dose of motesanib. Dose–response relationships of the changes in biomarkers were analyzed by the regression models using F-test. Analyses were performed using SAS (Version 8.2, SAS Institute Inc., Cary, NC).

Results

Patient characteristics

A total of 15 patients were screened for eligibility between September 2004 and May 2005; all were subsequently enrolled in the study and received at least one dose of motesanib. Baseline demographic and clinical characteristics are summarized in Table 1. Of the patients enrolled, 9 (60%) were women; the median age was 55 years, and the median weight was 52 kg. Thirteen patients had received prior chemotherapy and two had received prior radiotherapy. All 15 patients discontinued treatment with motesanib. Fourteen patients discontinued motesanib treatment early due to disease progression and one patient discontinued due to an adverse event. No patients died during the study. Patients received motesanib for a median of 77 days (range 19–583).

Dose escalation, dose-limiting toxicities, and maximum tolerated dose

No dose-limiting toxicities were observed in patients enrolled in the 50 mg QD cohort, or the 100 mg QD cohort, or in the initial three patients enrolled in the 125 mg QD cohort. The 125 mg QD dose was previously established as the MTD in a similarly designed study conducted in the US [20]. Consequently, it was decided not to exceed a dose of 125 mg QD in this study. To further assess safety at this

Table 1 Baseline demographic and clinical characteristics

	Motesanib dose cohort			All patients ($n = 15$)
	50 mg QD ($n = 3$)	100 mg QD ($n = 3$)	125 mg QD ($n = 9$)	
Women, *n* (%)	2 (67)	1 (33)	6 (67)	9 (60)
Median age, years (range)	65 (45–71)	68 (46–69)	51 (32–72)	55 (32–72)
Median weight, kg (range)	37 (32–65)	48 (47–61)	55 (36–76)	52 (32–76)
Eastern Cooperative Oncology Group score, *n* (%)				
0	2 (67)	1 (33)	5 (56)	8 (53)
1	1 (33)	2 (67)	4 (44)	7 (47)
Tumor type, *n*				
Sarcoma	0	1	3	4
Gastrointestinal stromal tumor	0	0	3	3
Colorectal	1	1	0	2
Bile duct	1	0	0	1
Hilar cholangiocarcinoma	0	0	1	1
Lung	0	1	1	2
Stomach	1	0	0	1
Thymoma	0	0	1	1
Prior chemotherapy, *n*				
0	0	0	2	2
1	0	1	1	2
2	2	1	3	6
≥3	1	1	3	5
Prior radiotherapy, *n*				
0	3	2	8	13
2	0	1	1	2

dose level, six additional patients were enrolled in the 125 mg QD cohort. Again, no DLTs occurred. The 150 mg QD cohort was not evaluated, since the recommended dose was decided based on the results from the previous study conducted in the US [20] and this study. Therefore, the MTD was not reached in this study.

Safety

The most frequently reported treatment-emergent adverse events (with a patient incidence of at least 10%) during cycle 1 by preferred term were hypertension (47%), protein urine present (33%), blood urine present (20%), constipation (20%), cough (20%), fatigue (20%), headache (20%), white blood cell count decreased (20%), alanine aminotransferase increased (13%), aspartate aminotransferase increased (13%), blood triglycerides increased (13%), diarrhea (13%), dry skin (13%), eyelid edema (13%), hypoesthesia (13%), nausea (13%), pyrexia (13%), rash (13%), stomach discomfort (13%), and vomiting (13%) (Table 2).

All 15 patients enrolled in the study experienced treatment-related adverse events (Table 3). The most frequently occurring motesanib-related adverse events were proteinuria ($n = 10$, 67%), hypertension ($n = 9$, 60%), fatigue ($n = 7$, 47%), headache ($n = 6$, 40%), hematuria ($n = 5$, 33%), and diarrhea ($n = 5$, 33%). Two patients in the 100 mg QD dose cohort experienced grade 3 motesanib-related hypertension. In one patient motesanib treatment was interrupted due to hypertension, which resolved within 15 days after antihypertensive treatment started. In all other instances, hypertension was managed by administration of antihypertensive therapy alone. No patient enrolled in the study experienced either a grade 4 adverse event or a serious motesanib-related adverse event. One patient in the 50 mg QD cohort withdrew from the study due to grade 3 anorexia that was considered to be unrelated to motesanib treatment.

Pharmacokinetics

Evaluable plasma samples for pharmacokinetic analysis were available from all 15 patients during cycle 1. Motesanib was rapidly absorbed following single-dose oral administration, with a median t_{max} of between 0.25 and 1.0 h across the three dose cohorts. Similar t_{max} values were obtained following multiple-dose administration of motesanib

Table 2 Treatment-emergent adverse events occurring in at least 10% of patients (cycle 1)

Adverse event	Motesanib dose cohort			All patients ($n = 15$)
	50 mg QD ($n = 3$)	100 mg QD ($n = 3$)	125 mg QD ($n = 9$)	
Number of patients reporting adverse events, *n*	3	3	7	13
Hypertension	0	3	4	7
Protein urine present	2	1	2	5
Blood urine present	0	1	2	3
Constipation	0	1	2	3
Cough	0	1	2	3
Fatigue	1	1	1	3
Headache	0	1	2	3
White blood cell count decreased	0	1	2	3
Alanine aminotransferase increased	0	1	1	2
Aspartate aminotransferase increased	0	1	1	2
Blood triglycerides increased	0	1	1	2
Diarrhea	1	0	1	2
Dry skin	0	1	1	2
Eyelid edema	0	1	1	2
Hypoesthesia	0	0	2	2
Nausea	0	1	1	2
Pyrexia	0	0	2	2
Rash	0	0	2	2
Stomach discomfort	2	0	0	2
Vomiting	1	1	0	2

(i.e., day 21). Pharmacokinetic parameters of motesanib on days 1 and 21 are summarized in Table 4 and mean plasma motesanib concentration versus time profiles are shown in Fig. 1. The median of $t_{1/2}$ values ranged from 6.0 to 7.3 h after single-dose administration and from 3.8 to 4.8 h after multiple-dose administration, whereas these values did not appear to be dose dependent. The mean C_{max}, and $AUC_{0\text{-}24}$, values were approximately proportional to dose. However, these values were similar or slightly lower on day 21 than on day 1, indicating that there was no accumulation after daily administration.

Efficacy

All 15 patients were evaluable for antitumor response. No complete or partial responses per RECIST were observed (Table 5). All evaluable patients achieved stable disease. Five patients had durable (>24 weeks) stable disease: one patient with non-small-cell lung cancer (duration: 465 days), one patient with a gastrointestinal stromal tumor (duration: 175 days), one patient with a thymoma (duration: 252 days), one patient with malignant hemangiopericytoma (duration: 175 days) and one patient with alveolar soft part sarcoma (duration 539 days). All patients with durable stable disease were enrolled in the 125 mg QD dose cohort. Motesanib showed promising antitumor activity (all stable disease) in this study. The response duration for alveolar soft part sarcoma and non-small cell lung cancer patients were 539 and 465 days, respectively.

Analysis of proangiogenic markers

Several angiogenic markers such as VEGF, PlGF, bFGF, sFlt-1, sKDR, and c-Kit were determined in association with motesanib exposure. As shown in Fig. 2, change in sKDR from baseline was inversely related to dose and showed statistical significance ($R^2 = 0.275$; $p = 0.045$). In addition, changes in c-Kit from baseline was inversely related to dose and showed statistical significance ($R^2 = 0.449$; $p = 0.006$). However; changes in VEGF, PlGF, bFGF, and sFlt-1 did not show a statistically significant ($p > 0.05$) correlation with motesanib exposure (data not shown).

Discussion

Inhibition of angiogenesis has recently emerged as an effective therapy for solid tumors. Motesanib is an orally administered small-molecule antagonist inhibitor of VEGFR1, 2, 3; PDGFR, and c-Kit and has demonstrated antiangiogenic

Table 3 Patient incidence of motesanib-related adverse events occurring in more than 10% of patients

Adverse event	Motesanib dose cohort			All patients ($n = 15$)
	50 mg QD ($n = 3$)	100 mg QD ($n = 3$)	125 mg QD ($n = 9$)	
Incidence of motesanib-related adverse events, *n*	3	3	9	15
Proteinuria	3	2	5	10
Hypertension	0	3	6	9
Fatigue	2	1	4	7
Headache	0	2	4	6
Hematuria	1	2	2	5
Diarrhea	1	0	4	5
Alanine aminotransferase increased	0	1	2	3
Dry skin	0	1	2	3
Nausea	0	1	2	3
Stomach discomfort	2	0	1	3
Vomiting	1	1	1	3
White blood cell count decreased	0	1	2	3
Aspartate aminotransferase increased	0	1	1	2
Blood alkaline phosphatase increased	0	0	2	2
Blood creatinine phosphokinase MB increased	1	1	0	2
Blood triglycerides increased	0	1	1	2
Cough	0	1	1	2
Eosinophil count increased	0	0	2	2
Eyelid edema	0	1	1	2
Edema	0	0	2	2
Pleural effusion	0	1	1	2
Rash	0	0	2	2
Weight decreased	1	0	1	2

and antitumor activity in preclinical models of human cancer [17] and acceptable toxicity and promising clinical efficacy in a phase 1 study conducted in the US [18].

The aim of this study was to investigate the safety, pharmacokinetics, and antitumor efficacy of motesanib in Japanese patients with advanced solid tumors.

No DLTs occurred in this study and, therefore, the MTD was not reached. We confirmed the tolerance of the 125 mg QD dose recommended in the US [18].

The safety profile of motesanib in this population of Japanese patients was similar to that observed in the US study [18].

Adverse events were typically mild to moderate in severity, and all of toxicities were acceptable at all motesanib doses tested in this study.

The most frequently occurring non-hematologic toxicity in cycle 1 was hypertension. There were two patients with grade 3 hypertension in level 2. The median time to onset of hypertension was 9 days (cycle 1) after treatment initiation. Hypertension increased in frequency as well as in severity at high dose or with multiple doses of motesanib. However, hypertension was typically manageable with antihypertensive therapy medications including calcium blocker.

The incidence of hypertension in this study was similar to that observed in the motesanib phase 1 study conducted in the US [18] as well as the incidence rate noted in studies of other VEGF inhibitors [20, 21]. Hypertension has been observed during treatment with other VEGF inhibitors and is considered a class effect of these agents [22]. Hypertension appears to be induced possibly by increasing vascular resistance (due to decreased NO and prostacyclin production), vascular rarefaction, and increased arterial stiffness [23, 24].

No patients experienced thromboembolic events or cholecystitis in this study.

Motesanib was rapidly absorbed following oral administration.

Values for t_{max} and $t_{1/2,z}$ were similar to those observed at the same doses in the US study [18]. However, exposure

Table 4 Pharmacokinetic parameters for motesanib following single-dose (day 1) and multiple-dose (day 21) oral administration

	Motesanib dose cohort		
	50 mg QD	100 mg QD	125 mg QD
Day 1			
n	3	3	9
t_{max}, h	0.25 (0.25–0.25)	0.5 (0.5–1.0)	1.0 (0.25–2.0)
C_{max}, ng/ml	462 (322–695)	521 (468–696)	792 (285–2410)
$t_{1/2,z}$, h	5.96 (5.07–6.12)	7.26 (6.35–9.24)	6.54 (4.04–8.11)
AUC_{0-inf}, μg h/ml	1.84 (1.17–1.88)	3.50 (1.56–5.85)	3.08 (2.35–6.61)
AUC_{0-24}, μg h/ml	1.77 (1.12–1.78)	3.16 (1.52–5.08)	2.84 (2.23–6.51)
CL/F, l/h	27.2 (26.6–42.7)	28.6 (17.1–64.1)	40.6 (18.9–53.1)
C_{24}, ng/ml	8.49 (6.83–12.5)	29.4 (3.53–50.2)	14.6 (4.68–37.0)
C_{48}, ng/ml	0.347 (0.310–0.715)	3.66 (0.547–11.3)	1.63 (BQL–4.94)
Day 21			
n	3	3	9
t_{max}, h	0.5 (0.5–2.0)	1.0 (1.0–2.0)	1.0 (0.25–2.0)
C_{max}, ng/ml	561 (267–669)	390 (351–636)	639 (272–1350)
$t_{1/2,z}$, h	3.81 (3.38–5.24)	4.83 (4.20–5.92)	4.12 (2.81–5.29)
AUC_{0-inf}, μg h/ml	NR	NR	NR
AUC_{0-24}, μg h/ml	1.31 (0.932–1.43)	2.36 (1.06–4.94)	1.99 (0.862–5.68)
CL/F, l/h	38.1 (35.0–53.6)	42.3 (20.2–94.6)	62.8 (22.0–145)
C_{24}, ng/ml	2.22 (1.24–4.64)	12.2 (2.81–45.3)	6.32 (0.979–33.8)
C_{48}, ng/ml	NR	NR	NR

AUC_{0-inf} = area under the plasma concentration versus time curve from time 0 to infinite time; AUC_{0-24} = area under the plasma concentration versus time curve from time 0–24 h postdose; CL/F = apparent clearance; C_{max} = maximum observed concentration after dosing; C_{24} = observed concentration at 24 h postdose; C_{48} = observed concentration at 48 h postdose; NR = not reported; QD = daily dose; t_{max} = time of maximum observed plasma concentration; $t_{1/2,z}$ = estimated terminal elimination half-life; BQL = below quantitation limit (0.5 ng/ml); All values are reported as the median (range)

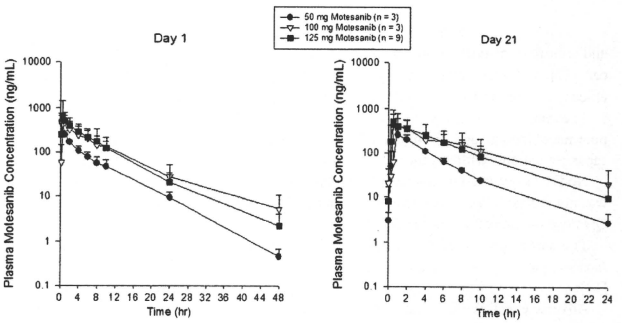

Fig. 1 Mean (+SD) plasma concentration–time profiles for motesanib after single-dose (day 1) and multiple-dose (day 21) administration in patients with advanced solid tumors

to motesanib was somewhat greater in this study than in previous motesanib studies. The reasons for this increased exposure are unclear.

Motesanib mean trough concentrations (C_{24}) were above the IC50 for human umbilical vein endothelial cell proliferation (4 ng/ml) [17] at dose ≥100 mg QD.

There was no evidence that motesanib accumulates in plasma following multiple-dose administration.

Motesanib exhibited encouraging antitumor activity in this study with all patients enrolled achieving a best response of stable disease. The duration of response in these patients seemed clinically meaningful. However,

Table 5 Tumor response per RECIST

	Motesanib dose cohort			All patients ($n = 15$)
	50 mg QD ($n = 3$)	100 mg QD ($n = 3$)	125 mg QD ($n = 9$)	
Patients with measurable/non-measurable disease at baseline, *n*	3	3	9	15
Response assessment, *n*				
Stable disease	3	3	9	15
Durable (>24 weeks) stable disease	0	0	5	5

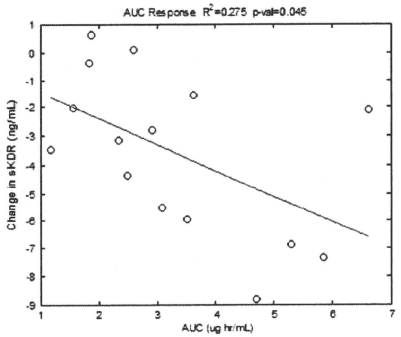

Fig. 2 Changes in serum sKDR compared to motesanib AUC

because of the small size of the study population, few firm conclusions can be drawn from this study regarding the clinical efficacy of motesanib. Bevacizumab, sunitinib malate, and sorafenib tosylate have demonstrated clinical efficacy, providing proof of concept that antiangiogenic agents can provide significant clinical benefit. In addition, multiple other small-molecule multikinase inhibitors including VEGFR as a target are currently under clinical development.

Adverse events generally reported to occur with increased incidence in patients receiving these investigational products include diarrhea, nausea, vomiting, hypertension, and fatigue [21, 25–27]. Some angiogenesis inhibitors have been associated with arterial and venous thrombosis [28, 29]. In this study, thrombosis was not observed.

The trends in the changes in the angiogenic cytokines follows patterns similar to those reported in the motesanib phase 1 study as reported in Rosen et al. [18]. These discrepancies could be associated with small sample size, and it should be taken the small sample size and paucity of decreased SLD measures into consideration at evaluation. The statistical analysis of the changes in the angiogenic factors in this study was limited by the small study size (15 patients). The results reported by Rosen et al. [18] were analyzed in larger study size of 69 patients.

Factors that may impact the comparison between the two studies are ethnicity, particular selection of tumor types, and the difference in when the tumor was assessed.

These results demonstrated that motesanib was tolerable in Japanese patients at doses up to 125 mg QD.

The safety profile of motesanib was similar to that observed in a US study.

These results, and the encouraging antitumor activity observed in this study, support the further development of motesanib for the treatment of patients with solid tumors.

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ORIGINAL REPORT

Association of DNA Repair Gene Polymorphisms With Response to Platinum-Based Doublet Chemotherapy in Patients With Non–Small-Cell Lung Cancer

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ABSTRACT

Purpose
To identify polymorphisms in DNA repair genes that affect responses to platinum-based doublet chemotherapy in patients with non–small-cell lung cancer (NSCLC).

Patients and Methods
In total, 640 patients with NSCLC who received platinum-based doublet chemotherapy in the National Cancer Center Hospital in Japan from 2000 to 2008 and whose responses were evaluated by Response Evaluation Criteria in Solid Tumors (RECIST) participated in a study of the association between response and genotypes for 30 single nucleotide polymorphisms (SNPs) in 27 DNA repair genes. Candidate SNPs were selected in a discovery set of 201 patients, and their associations were validated in an independent set of 439 patients by prespecified *P* value criteria.

Results
Homozygotes for the minor allele TP53-72Pro of the Arg72Pro SNP in the *TP53* gene showed a better response rate (54.3%) than those for the major allele TP53-72Arg (29.1%; $P = 4.4 \times 10^{-5}$) irrespective of therapeutic regimens, and minor allele homozygotes had significantly longer progression-free and overall survivals than major allele homozygotes (hazard ratio [HR], 0.85; 95% CI, 0.74 to 0.98; $P = .020$; and HR, 0.86; 95% CI, 0.74 to 0.99; $P = .039$). Minor allele carriers for SNP Lys940Arg in the poly (ADP-ribose) polymerase 1 (*PARP1*) gene showed a better response rate to the paclitaxel regimen (45.8%) than to the gemcitabine regimen (10.5%; *P* for interaction = .019).

Conclusion
Polymorphisms in the *TP53* and *PARP1* genes are involved in inter-individual differences in the response to platinum-based doublet chemotherapy in patients with NSCLC.

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INTRODUCTION

Non–small-cell lung cancer (NSCLC) is a major cause of cancer-related death with 5-year survival rates of < 20%.[1] Cytotoxic chemotherapy is the standard care for patients with advanced NSCLC. The standards of therapeutic regimens are platinum-based doublets (platinum plus another agent).[2] The drugs paired with platinum include microtubule-targeted agents (paclitaxel, docetaxel, or vinorelbine) and DNA-damaging agents (gemcitabine or irinotecan). The efficacy of each combination has been demonstrated to be similar by a series of trials in unselected patients with response rates of 30% to 40%.[3-5] Therefore, predictive factors for the efficacy of these chemotherapy regimens are being investigated for the development of customized therapies.

Considering that agents that damage DNA or disturb chromosomal integrity are used for chemotherapy, activities that repair DNA or chromosome damage possibly influence the outcome of patients with NSCLC after chemotherapy. In fact, expression of *ERCC1*, which is involved in the repair of DNA adducts generated by platinum, has been shown to be a possible predictive factor for the efficacy of the postoperative cisplatin-based adjuvant chemotherapy in resected tumors.[6,7] More recently, a single nucleotide polymorphism (SNP) in the *ERCC1* gene, rs11615, which affects *ERCC1* mRNA levels, was suggested to be associated with response (ie, tumor regression) of patients with advanced NSCLC to platinum-based chemotherapy.[8] Since SNPs can be examined by using blood cells, they will be promising biomarkers in the clinical

decision-making process for patients with advanced NSCLC. Reports on the association of SNPs in several other DNA repair genes with prognosis of patients with NSCLC who received chemotherapy also suggested their associations with the outcome of the patients.[7,9-14] However, sample sizes were small (50 to 250 patients), and only four to 15 genomic polymorphisms were investigated in those studies. In addition, the data in each trial were not confirmed by an independent validation set. Therefore, clinical importance of these SNPs still remains unclear.

We previously searched for nonsynonymous (ie, associated with amino acid changes) SNPs in 36 DNA repair genes involved in diverse intracellular processes that maintain genome integrity and identified 29 SNPs in 26 DNA repair genes, whose minor allele frequencies were more than 5% in Japanese patients[15] (Table 1). Thus, in this study, we conducted a single-hospital–based retrospective analysis of 640 patients with NSCLC to elucidate associations of these 29 SNPs and the ERCC1 SNP above[8] with the patients' outcome after platinum-based doublet chemotherapy. To minimize type I errors, the significance of candidate SNPs picked up by the first discovery set were validated by using the second independent validation set. We chose the response evaluated by the Response Evaluation Criteria in Solid Tumors (RECIST)[16] as the primary end point of outcome to search for predictive factors for the primary effect of chemotherapy.

Table 1. 30 SNPs in DNA Repair Genes

Pathway	Gene	SNP (rs number)	Amino Acid/ Nucleotide Change	Minor Allele Frequency				
				Japanese*	Japanese†	Chinese†	European†	African†
29 Nonsynonymous SNPs (associated with amino acid change)								
Base excision repair								
	PARP1	rs1805412	Val762Ala	0.40	0.46	0.48	0.17	0.01
		rs1136471	Lys940Arg	0.05	—	—	—	—
	APEX	rs1130409	Asp148Glu	0.38	0.32	0.46	0.51	0.28
	MBD4	rs140693	Glu346Lys	0.35	0.41	0.27	0.00	0.03
	MTH1	rs4866	Val83Met	0.09	—	—	—	—
	OGG1	rs1052133	Ser326Cys	0.48	0.52	0.50	0.22	0.14
	XRCC1	rs1799782	Arg194Trp	0.33	0.28	0.24	0.09	0.08
		rs25489	Arg280His	0.09	—	—	0.03	0.03
		rs25487	Arg399Gln	0.25	0.28	0.27	—	0.10
Nucleotide excision repair								
	XPG	rs17655	His1104Asp	0.42	0.48	0.56	0.73	0.46
	CSB	rs2228528	Gly399Asp	0.45	0.46	0.40	0.19	0.22
	XPC	rs2228001	Lys939Gln	0.40	0.34	0.38	0.41	0.26
	XPD	rs13181	Lys751Gln	0.05	0.08	0.06	0.33	0.18
Mismatch repair								
	MLH3	rs175080	Pro844Leu	0.18	0.14	0.13	0.43	0.41
	MSH3	rs26279	Thr1045Ala	0.24	0.22	0.37	0.22	0.40
	MSH6	rs1042821	Gly39Glu	0.32	—	—	—	—
DNA double-strand break repair								
	BRCA2	rs144848	Asn372His	0.22	0.31	0.21	0.29	0.13
	SNM1	rs3750898	His317Asp	0.26	0.26	0.10	0.27	0.74
	NBS1	rs1805794	Gln185Glu	0.50	0.46	0.49	0.28	0.16
	XRCC3	rs861539	Thr241Met	0.09	0.15	0.07	0.42	0.24
DNA damage response								
	TP53	rs1042522	Arg72Pro	0.33	0.23	0.49	0.41	0.67
DNA polymerase								
	POLD1	rs1726801	Arg119His	0.20	0.22	0.18	0.06	0.35
	POLI	rs8305	Thr731Ala	0.25	0.28	0.29	0.26	0.00
	REV1	rs3087386	Phe257Ser	0.33	0.30	0.37	0.50	0.30
	POLZ	rs462779	Thr1224Ile	0.35	0.43	0.49	0.82	0.38
Other pathways								
	BLM	rs28384991	Thr298Met	0.09	—	—	—	—
	FANCA	rs2239359	Ser501Gly	0.17	0.16	0.21	0.62	0.33
	FANCG	rs2237857	Thr297Ile	0.12	0.13	0.01	0.00	0.14
	WRN	rs1346044	Cys1367Arg	0.09	0.07	0.08	0.23	0.15
One synonymous SNP (not associated with amino acid change)								
Nucleotide excision repair								
	ERCC1	rs11615	C118T	—	0.29‡	0.22	0.65	0.02

Abbreviation: SNP, single nucleotide polymorphism.
*Frequency in Japanese determined by Sakiyama et al.[15]
†Frequency determined by the HapMap project.
‡Frequency in Japanese (T. Kohno, unpublished data).

PATIENTS AND METHODS

Selection of Study Population and Acquisition of Clinical Information

In total, 987 patients with NSCLC with clinical stages IIIA, IIIB, and IV tumors, who had not received prior platinum-based chemotherapy, were given platinum-based chemotherapy at the National Cancer Center Hospital in Tokyo, Japan, from 2000 to 2008 (Fig 1A). Clinical information was obtained by attending physicians and nurses. Of the 987 patients, 640 were eligible for the study according to the following criteria: they were not indicated for definitive chemoradiotherapy; they received a platinum-based doublet but not single or triplet chemotherapy; and their tumor response was evaluable according to RECIST[16] on the basis of data from computed tomography scans. However, those with clinical or radiologic evidence of early progression, such as emergence of new lesions, were included as patients with progressive disease (PD) in the analysis, even when unaccompanied by corresponding computed tomography scans, according to the definition in RECIST.[16] All patients were Japanese and were diagnosed with adenocarcinoma (ADC), squamous cell carcinoma (SQC), or other histologic types of NSCLC according to WHO classification[17,18] (Table 2).

Written informed consent was obtained from all patients for the use of blood cells for the analysis of genetic polymorphisms in association with clinical findings, including response to chemotherapy. Thus, 201 patients in the discovery set received therapy from 2000 to 2004, and 439 patients in the validation set received therapy from 2004 to 2008. Information on response in a subset of patients was obtained from the data in clinical trials conducted at the National Cancer Center Hospital.[3,19,20] This study was approved by the institutional review boards of the National Cancer Center. Smoking habit was recorded by pack-years. Patients with pack-years > 0 were defined as smokers, including both former and current smokers. Patients who report no smoking history (ie, pack-years = 0) were defined as never-smokers.

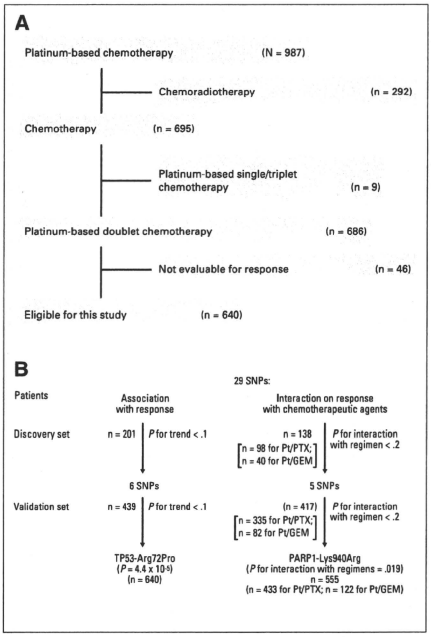

Fig 1. Patients and strategy. (A) Selection of eligible cases. (B) A two-phase screening of single nucleotide polymorphisms (SNPs) associated with responses to platinum-based doublet chemotherapy. Pt, platinum; PTX, paclitaxel; GEM, gemcitabine.

Chemotherapy

Patients were treated with one of the following regimens: (1) paclitaxel 200 mg/m^2 followed by cisplatin 80 mg/m^2, carboplatin at a dose calculated to produce an area under the serum concentration-time curve of 6.0 min · mg/mL, or nedaplatin 100 mg/m^2 on day 1, repeated every 3 weeks; (2) docetaxel 60 mg/m^2 followed by cisplatin 80 mg/m^2 on day 1, repeated every 3 weeks; (3) vinorelbine 25 mg/m^2 on days 1 and 8 and cisplatin 80 mg/m^2 on day 1, repeated every 3 weeks; (4) gemcitabine 1,000 mg/m^2 on days 1 and 8 and cisplatin 80 mg/m^2 or carboplatin to area under the serum concentration-time curve of 5.0 min · mg/mL on day 1, repeated every 3 weeks; or (5) irinotecan 60 mg/m^2 on days 1, 8, and 15 and cisplatin 80 mg/m^2 on day 1, repeated every 4 weeks. Each treatment was repeated for two or more cycles unless the patient met the criteria for PD or experienced unacceptable toxicity. Chemotherapy dosage was modified by toxicities in subsequent courses.

Genetic Analysis

A 20 mL whole-blood sample was obtained from each patient, and genomic DNA was extracted from whole-blood cells.[15] Genotyping for 30 SNPs in 27 genes was performed by pyrosequencing or TaqMan methods as previously described.[15,21]

Statistical Analysis

Patients were divided into two categories: responders were those with complete response and partial response, and nonresponders were those with stable disease and PD. Odds ratios (ORs) and 95% CIs for the response (ie, responder *v* nonresponder) according to genotypes were calculated as a measure of difference in the response rate against therapy. ORs were calculated by adjusting sex (male *v* female), age (increase by 10 years), performance status (0 *v* 1 to 2), smoking status (never-smoker *v* smoker), stage (III *v* IV), and chemotherapy (platinum plus a DNA-damaging agent *v* platinum plus a microtubule-targeting agent) by using an unconditional logistic regression analysis.[22] *P* value by the trend test was also calculated by using an unconditional logistic regression analysis under the same adjustments as above. Differences in the response between two chemotherapeutic regimens according to genotypes were examined by calculating *P* values for interaction with the regimens on the trend of OR for response.

A two-phase screening was used to search for SNPs associated with the response to chemotherapy (Fig 1B). In the first phase, 29 SNPs were examined for associations with the response and differences in the association according to regimens in 201 and 138 patients (for whom paclitaxel or gemcitabine therapy was used, respectively) in the discovery set. In the second phase, SNPs that showed *P* values < .1 by the trend test for association with the response and *P* values < .2 for interaction with the regimen were subjected to genotyping of 439 and 417 patients (for whom paclitaxel or gemcitabine was used, respectively) in the validation set. SNPs that showed *P* values < .1 for association with the response and *P* values < .2 for interaction with the regimen in patients in the validation set were further subjected to analysis in all 640 and 555 patients, respectively. Progression-free survival (PFS) was defined as the period from the first day of chemotherapy to the date of documentation of disease progression by RECIST and overall survival (OS) was defined as the period from the first day of chemotherapy to death. Hazard ratios (HRs) for PFS and OS and 95% CIs were calculated by using multivariate Cox proportional hazards models with adjustment for sex, age, histology, performance status, smoking status, clinical stage, and treatment as above. Statistical analyses were performed using JMP version 8.0 software (SAS Institute, Cary, NC). A level of $P < .05$ was considered significant, whereas a level of $P < .10$ was considered marginal.

Table 2. Patient Characteristics

Variant	All			Discovery Set*		Validation Set	
	No.	%	95% CI	No.	%	No.	%
Total patients	640			201		439	
Age, years							
Mean	57.9			57.2		58.2	
Range	22-78			22-78		26-74	
± Standard deviation	9.2			10.0		9.1	
Sex							
Male	402	62.8		136	67.7	266	60.6
Female	238	37.2		65	32.3	173	39.4
ECOG performance status							
0	218	34.1		46	22.9	172	39.2
1	402	62.8		153	76.1	249	56.7
2	20	3.1		2	1.0	18	4.1
Histologic cell type							
Adenocarcinoma	549	85.8		167	83.1	382	87.0
Squamous cell carcinoma	84	13.1		34	16.9	50	11.4
Others	7	1.1		0	0.0	7	1.6
Smoking habit							
Never-smoker	233	36.4		74	36.8	159	36.2
Smoker	407	63.6		127	63.2	280	63.8
Pack-years of smokers							
Mean	46.3			45.9		46.5	
± Standard deviation	29.6			29.4		29.7	
Stage							
III	172	26.9		60	29.9	112	25.5
IIIA	24	3.8		12	6.0	12	2.7
IIIB	148	23.1		48	23.9	100	22.8
IV	468	73.1		141	70.1	327	74.5
Tumor response							
Responder	231	36.1		74	36.8	157	35.8
CR	4	0.6		0	0.0	4	0.9
PR	227	35.5		74	36.8	153	34.9
Non-responder	409	63.9		127	63.2	282	64.2
SD	232	36.3		70	34.8	162	36.9
PD	177	27.7		57	28.4	120	27.3
Platinum-based regimens							
Platinum + a microtubule-targeted agent	476	74.4		129	64.2	347	79.0
Paclitaxel†	433	67.7		98	48.8	335	76.3
Docetaxel‡	8	1.3		2	1.0	6	1.4
Vinorelbine§	35	5.5		29	14.4	6	1.4
Platinum + a DNA-damaging agent	164	25.6		72	35.8	92	21.0
Gemcitabine¶	122	19.1		40	19.9	82	18.7
Irinotecan‖	42	6.6		32	15.9	10	2.3
PFS, median month							
Platinum + Paclitaxel	4.7		4.2 to 5.3				
Platinum + Gemcitabine	4.6		3.8 to 5.4				
Responder	6.1		5.7 to 6.4				
Nonresponder	3.0		2.7 to 3.3				

Abbreviations: ECOG, Eastern Cooperative Oncology Group; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; PFS, progression-free survival.

*Genotype for 29 nonsynonymous DNA repair gene single nucleotide polymorphisms were determined by Sakiyama et al.[15]

†Cisplatin or carboplatin or nedaplatin + paclitaxel.

‡Cisplatin + docetaxel.

§Cisplatin + vinorelbine.

¶Cisplatin or carboplatin + gemcitabine.

‖Cisplatin + irinotecan.

RESULTS

Association of a TP53-Arg72Pro SNP With Response to Platinum-Based Doublet Chemotherapy

Among 987 patients with NSCLC who were treated with platinum-based chemotherapy, 640 were eligible for this study (Fig 1A). Characteristics of these patients are summarized in Table 2. Genotypes for the 29 nonsynonymous SNPs in 26 DNA repair genes had been determined in 201 of the 640 patients in our previous study[15] (the discovery set in Table 2). Therefore, associations of these 29 SNPs with responses to chemotherapy were first investigated in these patients (Fig 1B). Six of the 29 SNPs fulfilled the criteria described above ($P < .1$ by the trend test; Appendix Table A1, online only); thus, they were further genotyped in the remaining 439 patients (the validation set in Table 2). Only one SNP, TP53-Arg72Pro, reproducibly showed an association that met the criteria ($P < .1$; Fig 1B and Appendix Table A1). In the analysis of all 640 patients, TP53-72Pro, the minor allele, was associated with a better response ($P = 9.5 \times 10^{-5}$ by the trend test; Table 3), and response rates increased according to the increase in the number of minor alleles (Fig 2A). Minor allele homozygotes showed a better response rate (54.3%) than major allele homozygotes (29.1%; $P = 4.4 \times 10^{-5}$). The association remained significant after Bonferroni correction (ie, $< 0.05/29 = 1.7 \times 10^{-3}$). Response rates of heterozygotes and homozygotes for the TP53-72Pro allele were higher in SQC than in ADC (Fig 2A and Table 3).

In the Cox proportional hazard model, minor allele homozygotes showed a significantly longer PFS than major allele homozygotes (HR, 0.85; 95% CI, 0.74 to 0.98; $P = .020$). The HR for progression of these homozygotes in SQC (HR, 0.67; 95% CI, 0.45 to 0.98; $P = .041$) was lower than that in ADC (HR, 0.89; 95% CI, 0.76 to 1.03; $P = .13$). Minor allele homozygotes showed a significantly longer OS than major allele homozygotes (HR, 0.86; 95% CI, 0.74 to 0.99; $P = .039$). The HR for death of these homozygotes in SQC (HR, 0.66; 95% CI, 0.43 to 0.98; $P = .037$) was lower than that in ADC (HR, 0.87; 95% CI, 0.74 to 1.02; $P = .13$).

SNP rs11615 (C118T) in the *ERCC1* gene was reported to be associated with response to platinum-based chemotherapy of NSCLC[8]; thus, it was also examined for association with response in all 640 patients. Minor allele homozygotes for the *ERCC1* SNP showed a higher response rate than others, consistent with a recent report[8]; however, the association was not statistically significant (Appendix Table A2, online only).

Differential Response According to Chemotherapeutic Regimens by PARP1 Genotypes

We next investigated whether or not SNPs in DNA repair genes affect responses differentially according to chemotherapeutic agents. Paclitaxel (433 patients; 68%) and gemcitabine (122 patients; 19%) were the most and second-most commonly used drugs in the platinum-based regimens (other drugs were also used but less frequently [< 10%; Table 2]). Therefore, differences in the response among the

Table 3. Association of *TP53* Genotypes With Response to Chemotherapy in 640 Patients With NSCLC

		Nonresponders		Responders						
NSCLC	Genotype	No.	%	No.	%	Response Rate (%)*	OR	95% CI	*P*	*P* by Trend Test
All	Arg/Arg	175	42.8	72	31.2	29.1	Reference			9.5×10^{-5}
	Arg/Pro	197	48.2	115	49.8	36.9	1.38	0.96 to 1.99	.082†	
	Pro/Pro	37	9.0	44	19.0	54.3	3.02	1.77 to 5.18	4.4×10^{-5}†	
	Dominant						1.63	1.15 to 2.30	.0053†	
	Recessive						2.48	1.54 to 4.04	2.1×10^{-4}†	
Adenocarcinoma	Arg/Arg	152	42.2	64	33.9	29.6	Reference			.0024
	Arg/Pro	176	48.9	90	47.6	33.8	1.19	0.81 to 1.77	.38‡	
	Pro/Pro	32	8.9	35	18.5	52.2	2.67	1.50 to 4.81	8.7×10^{-4}‡	
	Dominant						1.42	0.98 to 2.07	.062‡	
	Recessive						2.44	1.44 to 4.15	9.2×10^{-4}‡	
Squamous cell carcinoma	Arg/Arg	21	46.7	7	17.9	25.0	Reference			.0032
	Arg/Pro	19	42.2	23	59.0	54.8	3.63	1.10 to 13.5	.033‡	
	Pro/Pro	5	11.1	9	23.1	64.3	8.71	1.64 to 62.5	.010‡	
	Dominant						4.62	1.52 to 16.3	.0062‡	
	Recessive						3.85	1.02 to 17.6	.047‡	
Smoker	Arg/Arg	98	39.5	44	27.7	31.0	Reference			.0084
	Arg/Pro	124	50.0	88	55.3	41.5	1.52	0.97 to 2.41	.069§	
	Pro/Pro	26	10.5	27	17.0	50.9	2.31	1.19 to 4.50	.013§	
	Dominant						1.65	1.07 to 2.57	.023§	
	Recessive						1.78	0.99 to 3.23	.056§	
Never-smoker	Arg/Arg	77	47.0	28	38.9	26.7	Reference			.0052
	Arg/Pro	73	44.5	27	37.5	27.0	1.06	0.55 to 2.02	.87§	
	Pro/Pro	11	6.7	17	23.6	60.7	5.31	2.00 to 15.3	6.8×10^{-4}§	
	Dominant	161					1.56	0.86 to 2.86	.14§	
	Recessive						4.76	2.02 to 11.8	3.6×10^{-4}§	

Abbreviations: NSCLC, non–small-cell lung cancer; OR, odds ratio.
*Fraction of responder.
†OR for responder against nonresponder adjusted for sex, age, histology, smoking status, clinical stage, performance status, and treatment.
‡OR for responder against nonresponder adjusted for sex, age, smoking status, clinical stage, performance status, and treatment.
§OR for responder against nonresponder adjusted for sex, age, histology, clinical stage, performance status, and treatment.

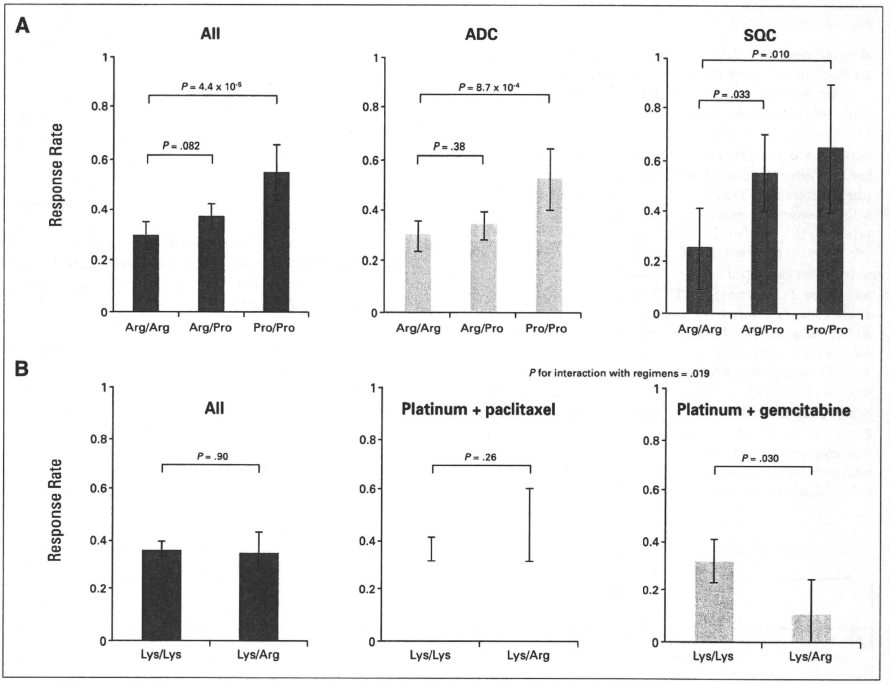

Fig 2. (A) Response rates according to *TP53* genotypes in (left) all patients and those with (middle) aenocarcinoma (ADC) and (right) squamous cell carcinoma (SQC). (B) Response rates according to *PARP1* genotypes in (left) all patients and those treated with (middle) platinum plus pacliteaxel or (right) platinum plus gemcitabine. Response rate is shown with its sampling variations estimated by 95% CI.

agents according to genotypes were investigated in 555 patients who received chemotherapy with either of these two regimens.

Among 201 patients in the discovery set, 138 received chemotherapy with regimens using paclitaxel (98 patients) or gemcitabine (40 patients; Fig 1B). Five of the 29 SNPs met the criteria in these 138 patients ($P < .2$ for interaction). Therefore, these five SNPs were further genotyped for 417 patients who received chemotherapy with regimens using paclitaxel (335 patients) or gemcitabine (82 patients) among 439 patients in the validation set. Only one SNP, poly (ADP-ribose) polymerase 1 (PARP1) -Lys940Arg, reproducibly showed $P < .2$ for interaction (Appendix Table A3, online only). This SNP showed a statistically significant interaction with the regimens on the response when analyzed in all 555 patients ($P = .019$ for interaction; Fig 1B, Appendix Table A4, online only), although the association did not remain significant after Bonferroni correction (ie, > 0.05 of 29 SNPs tested $= 1.7 \times 10^{-3}$). Heterozygotes for this SNP showed a better response rate to the paclitaxel regimen (45.8%) than to the gemcitabine regimen (10.5%; Fig 2B). There were no minor allele homozygotes for this SNP in this population.

PFS according to the PARP1-Lys940Arg genotype was compared between the two regimens. In the Cox proportional hazard model, the risk for progression of major allele homozygotes with the platinum/paclitaxel treatment was similar to that with the platinum/gemcitabin treatment (HR, 0.97; 95% CI, 0.86 to 1.09; $P = .60$). Conversely, the risk of heterozygotes with the platinum/paclitaxel treatment was smaller than that with the platinum/gemcitabine treatment, although it was not statistically significant (HR, 0.82; 95% CI, 0.59 to 1.17; $P = .27$). SNPs in *TP53* and *ERCC1* did not show differential associations according to regimens (Appendix Table A4).

DISCUSSION

An SNP in the *TP53* genes was shown to be associated with the response to platinum-based doublet chemotherapy. In this study, association results obtained by the discovery set were confirmed by using an independent validation set. The association of the p53-72Pro allele with a better response to platinum-based doublet chemotherapy

retained statistical significance after Bonferroni correction. Therefore, the results strongly indicate the importance of p53-Arg72Pro SNP as a determinant for the response to platinum-based chemotherapy.

TP53 is a tumor suppressor gene somatically mutated in 40% to 70% of NSCLCs.[23] p53-72Arg protein has a greater activity to induce apoptosis than p53-72Pro protein[24]; however, the relationship was reported as being the reverse in mutant p53 proteins.[25,26] p73, a p53-related protein, plays a role in apoptosis in anticancer agents for cancer cells carrying *TP53* mutations; however, its function is abrogated by mutant p53 proteins. The abrogating activity is greater in mutant p53 proteins with the Arg residue at codon 72 than in those with the Pro residue.[25,26] In an analysis of 25 patients with head and neck cancer, those with a *TP53* mutation on the 72Pro allele showed a better response than those with a mutation on the 72Arg allele with cisplatin-based chemoradiotherapy.[25] Similarly, in this study, the TP53-72Pro allele appeared to confer a better response to platinum-based doublet chemotherapy in patients with NSCLC (Fig 2A). In a previous study,[11] patients with NSCLC who carry the TP53-72Pro allele also showed a better OS after cisplatin-gemcitabine treatment, although the association did not reach statistical significance. These results indicate that p53 mutants with the Pro residue at codon 72 only weakly inhibit the function of p73 protein in NSCLC cells and therefore efficiently induce apoptosis of NSCLC cells treated with platinum and other anticancer agents. In fact, the effect of this SNP was more apparent in patients with SQC than in patients with ADC (Fig 1A), consistent with the fact that *TP53* mutations are more frequent in SQC than in ADC.[27] Since tumor specimens for examination of somatic *TP53* mutations were not available for these patients, *TP53* status in their tumor cells could not be determined. Therefore, we could not conclude whether this differential association was really due to differences in *TP53* mutations. An association study of patients with NSCLC informative for somatic *TP53* mutation will provide a more complete picture of the role of *TP53* SNP in chemotherapeutic responses.

The PARP1-Lys940Arg genotype was suggested to differentially affect the response according to chemotherapeutic agents (Fig 2B), although the association was not significant after Bonferroni correction and needs validation. The *PARP1* gene encodes poly (ADP-ribose) polymerase 1, which regulates multiple processes for DNA repair, such as DNA strand break repair.[28] It is noted that suppression of PARP activity has been recognized as a method of tumor suppression in breast and other cancers[29] and that a PARP inhibitor enhanced the cytotoxic activity of gemcitabine.[30] The biologic significance of the PARP1-Lys940Arg SNP is unknown at present; however, the lysine-arginine residue at codon 940 is located in the catalytic domain of the PARP1 protein.[31] Therefore, this polymorphism may cause differences in the activity of PARP1 protein that affect the response to some chemotherapeutic agents, in particular to DNA-damaging agents.

Interestingly, the frequencies of the TP53-72Pro allele are known to be different among ethnic populations, although those of the PARP1-940Arg allele in other ethnic populations are unknown at present (Table 1). Therefore, examination of these two SNPs in NSCLC patient populations other than Japanese will also help elucidate the mechanism of interethnic differences in the outcome of patients after chemotherapy, as recently discussed.[32]

Identification of polymorphisms associated with drug toxicities is also important to develop customized chemotherapies. For instance, the *UGT1A1* gene polymorphisms are known to be associated with the toxicity of irinotecan, such as neutropenia.[33] In this study, the *TP53* and *PARP1* SNPs were not associated with grade 4 hematologic toxicities, including neutropenia (data not shown). Therefore, genetic factors responsible for response are likely to be different from those for toxicity. In addition, associations of these two SNPs with responses were not significantly different according to smoking habit ($P > .05$ for interaction with smoking; for *TP53*, see Table 3); therefore, these SNPs are likely to contribute to the response irrespective of smoking.

Our study has several limitations. This is a single-institution retrospective study with various therapeutic regimens. Therefore, the effects of SNPs on differential responses according to chemotherapeutic agents were only preliminarily investigated. The results should be confirmed by a larger, preferably prospective, cohort using a defined set of agents. More extensive analyses of interaction between SNPs and responses to chemotherapeutic agents will also be worth performing. Another limitation of this study is that, although the *TP53* polymorphism was significantly associated with response to chemotherapy, differences in PFS and OS were only modest. We chose the response as the primary end point of efficacy to pick up subgroups for which chemotherapy does work. Although this information would be potentially valuable, clinical response alone would be inadequate to improve the outcome of patients with advanced NSCLC. Therefore, investigation of polymorphisms in other genes might provide more information for individually optimized chemotherapy. Indeed, a few other SNPs in DNA repair genes have been reported to be associated with prognosis of patients with NSCLC.[7,9-14] In addition to ERCC1-118T, the APEX-148Asp, XRCC1-399Arg, and XPD-751Gln alleles, which had been reported to be associated with favorable prognosis of patients,[9,13,14] were consistently more frequent in responders than in nonresponders in our study population (Appendix Table A2), although these SNPs did not fulfill the criteria as validated predictive factors in this study.

In conclusion, our extensive analysis of 30 SNPs in 27 DNA repair genes identified the *TP53* and *PARP1* SNPs as strong candidates for defining inter-individual differences in the response to platinum-based chemotherapy of NSCLC. Our results indicate the significance of SNPs in DNA repair genes in the outcome of patients with NSCLC and also imply the utility of these SNPs as predictive markers for responses to chemotherapy. Further investigation is warranted.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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The usefulness of mutation-specific antibodies in detecting epidermal growth factor receptor mutations and in predicting response to tyrosine kinase inhibitor therapy in lung adenocarcinoma

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ABSTRACT

Introduction: Among the mutations of epidermal growth factor receptor (*EGFR*), deletions in exon 19 (DEL), and point mutations in exon 21 (L858R) predict the response to EGFR-tyrosine kinase inhibitors (TKIs) in primary lung adenocarcinoma. The ability to detecting such mutations using immunohistochemistry (IHC) would be advantageous.
Methods: The molecular-based and IHC-based *EGFR* mutations were analyzed in 577 lung adenocarcinomas using high resolution melting analysis (HRMA) and 2 mutation-specific antibodies, respectively.
Results: In the molecular-based analyses, DEL was detected in 135 cases (23%), and L858R was detected in 172 cases (30%). In the IHC-based analyses, a positive reaction was detected in 59 cases (10%) for the DEL-specific antibody, and in 139 cases (24%) for the L858R-specific antibody. With the molecular-based results set as the gold standard, the sensitivity and specificity of the DEL-specific antibody were 42.2% and 99.5%, respectively, while the sensitivity and specificity of the L858R-specific antibody were 75.6% and 97.8%, respectively. The antibody specificities improved when the threshold for the mutation-positive reactions was set as >50% of immunopositive tumor cells. The significant predictors of the clinical response to EGFR-TKI were molecular-based *EGFR* mutations ($p < 0.001$) and IHC-based *EGFR* mutations ($p = 0.001$). However, a multivariate analysis revealed that only molecular-based *EGFR* mutations were significantly correlated with the clinical response ($p < 0.001$).
Conclusions: Mutation-specific antibodies demonstrated extremely high specificities, but their sensitivities were not higher than those of molecular-based analyses. However, IHC should be performed before a molecular-based analysis, because it is more cost-effective and can effectively select candidates for EGFR-TKI therapy.

1. Introduction

Many human receptor tyrosine kinases mediate signals that promote the proliferation and survival of cancer cells. Activation of tyrosine kinases appears to be the causal event in many human malignancies [1]. The importance of this finding is reflected in the development of new anticancer drugs that specifically target these activated proteins. The clinical success of tyrosine kinase inhibitors (TKIs), such as imatinib for the treatment of chronic myeloid leukemia and gastrointestinal stromal tumors, has prompted intensive efforts to identify and target additional oncogene kinases as a broad therapeutic strategy for selected patient populations [2,3].

A subset of non-small cell lung cancer (NSCLC), particularly adenocarcinomas, has activating mutations in the *epidermal growth factor receptor* (*EGFR*) gene [4,5]. The most prevalent *EGFR* mutations are deletions in exon 19 (DEL) and a point mutation at codon 858 in exon 21 (L858R); together, these account for more than 90% of all *EGFR* mutations. These 2 types of *EGFR* mutations cause sustained activation of *EGFR*, followed by the selective activation of Akt and signal transduction, and the activation of

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transcription signaling pathways: altogether, these promote cell survival [4,6].

EGFR-TKIs are competitive inhibitors of the adenosine triphosphate-binding clefts within the tyrosine kinase domain of *EGFR* [7]; they effectively inhibit the critical antiapoptotic signals transduced by the mutant receptors [6]. The clinicopathologic parameters of female gender, East Asian ethnicity, adenocarcinoma histology, and nonsmoking status are strong predictors of the response to EGFR-TKIs [4,5,8,9]. Moreover, the DEL and L858R mutations were also revealed to be strong predictors [10–14]. Therefore, the detection of such mutations provides both patients and physicians with important information regarding the optimal choice for therapy.

Direct sequencing is the gold standard method to detect *EGFR* mutations. However, to obtain precise data, high-quality DNA extracted from an adequate amount of pure tumor cells is required, and this is expensive and time-consuming. Recently, other indirect methods were developed to detect *EGFR* mutations, including Scorpion ARMS, the peptide nucleic acid-locked nucleic acid PCR clamp, mutant-enriched PCR, the smart amplification process, and high-resolution melting analysis (HRMA) [15,16]. These methods have high sensitivities, and can be applied to specimens in which cancer cell content is low. However, they invariably require technical labor and sophisticated instruments, and are therefore, not applied in most pathology laboratories.

Compared to molecular techniques, immunohistochemistry (IHC) is a fast and cost-effective method that can be performed in most pathology laboratories on not only fresh, but also archival, formalin-fixed tissue samples. Recently, some authors revealed the correlation between EGFR mutations and EGFR phosphorylation detected by IHC [17,18]. Additionally, EGFR phosphorylation antibodies exhibited a correlation with response to EGFR-TKIs [18]. However, these antibodies recognize EGFR phosphorylation regardless of mutational status. More recently, highly sensitive and specific rabbit monoclonal antibodies against the 2 most common mutations were developed for detecting *EGFR* mutations [19–24].

The main purpose of the present study was to explore the use of the 2 mutation-specific antibodies for DEL and L858R for detecting *EGFR* mutations. Additionally, we compared the molecular-based and the IHC-based *EGFR* mutational status to the response to EGFR-TKI.

2. Materials and methods

2.1. Case selection

After obtaining institutional review board approval, the specimens used in the present study were obtained from 577 Japanese patients who underwent a surgical resection for primary lung adenocarcinoma at the National Cancer Center Hospital, Tokyo, Japan, between 1993 and 2009. Histological diagnosis was based on the latest World Health Organization classification of lung tumors [25].

2.2. Analysis of EGFR mutational status by molecular technique

The materials analyzed for the molecular-based mutational status were as follows: fresh frozen (in liquid nitrogen), surgically resected tissue specimens from 505 patients (88%); methanol-fixed, paraffin-embedded, surgically resected tissue specimens from 36 patients (6%); and ethanol-fixed, imprint cytologic smears obtained from the fresh-cut surface of resected tumor specimens from 36 patients (6%). We used HRMA for detecting the DEL and L858R mutations, routinely performed at our institution. HRMA is well validated, and has been previously shown to accurately reflect *EGFR* mutational status [15].

2.3. Tissue microarray construction

The representative tumor regions to be sampled for the tissue microarray (TMA), were carefully selected and marked on a hematoxylin-eosin-stained slide. The TMAs were assembled using a manual tissue-arraying instrument (Azumaya, Tokyo, Japan). Considering tumor heterogeneity, 2 replicate 2-mm cores were routinely sampled from different regions of each tumor.

2.4. Immunohistochemistry

For the immunohistochemical staining, the 4-μm-thick TMA sections were deparaffinized. A heat-induced epitope retrieval with Target Retrieval Solution (Dako, Carpinteria, CA, USA) was performed. The primary antibody used were a rabbit monoclonal antibody against human *EGFR* with the DEL (E746-A750del) mutation (1:100, clone 6B6, Cell Signaling Technology, Danvers, MA, USA) and a rabbit monoclonal antibody against human *EGFR* with the L858R mutation (1:200, clone 43B2, Cell Signaling Technology). The antibodies were diluted in SignalStain (Cell Signaling Technology), and slides were incubated with each primary antibody for 1 h, at room temperature. The immunoreactions were detected using the EnVision Plus system (Dako) and 3,3′-diaminobenzidine, followed by counterstaining with hematoxylin. We used positive and negative controls for the IHC that previously confirmed the mutational status by using molecular analyses.

2.5. Immunohistochemical scoring system for mutation-specific antibodies

The immunoreactivity for each mutation-specific antibody was evaluated by using light microscopy at magnifications of 4 and 10× with objective lenses. Immunoreactivity was classified on the basis of cytoplasmic intensity. The following scoring system was used: negative intensity, 0 (defined as no immunoreactivity with any intensity); weak intensity, 1 (defined as the immunoreactivity only observed in 10× objective lenses); moderate intensity, 2 (defined as the immunoreactivity easily detected in 4× objective lenses, but less intense than the positive control); and strong intensity, 3 (defined as immunoreactivity equal to or stronger than the positive control; Fig. 1A and B). We also evaluated the extent of each intensity as a percentage (0–100%). Next, an expression score was obtained by multiplying the intensity by the percentage values (range, 0–300) for each core. Finally, the staining scores obtained in 2 cores were averaged, and the result was used as the representative score for each case. In the case of loss of tumor cells in 1 of the 2 cores during IHC, the staining score for the other core was used. We set the threshold at a staining score of 10; therefore, a staining score <10 was categorized as negative and a score ≥10 was categorized as positive. Additionally, we set another threshold for positive cases, defined as >50% of immunopositive tumor cells with any intensity.

2.6. Evaluation of the response to EGFR-TKI

Of the 577 patients, 116 received systemic therapy with EGFR-TKI gefitinib (250 mg daily) after tumor relapse. The therapeutic effect of gefitinib was complete response (CR) in 3, partial response (PR) in 61, stable disease (SD) in 13, and progressive disease (PD) in 37. Two patients were not evaluable for the clinical response due to the withdrawal of gefitinib caused by drug-induced liver dysfunction. The clinical response to gefitinib was determined using standard bidimensional measurements [26]. Responders were defined as patients with CR or PR, and non-responders were defined as patients with SD or PD.

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