ORIGINAL ARTICLE

Phase II trial of a non-platinum triplet for patients with advanced non-small cell lung carcinoma (NSCLC) overexpressing ERCC1 messenger RNA

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Abstract

Background: We prospectively evaluated the efficacy and toxicity of a non-platinum triplet regimen for patients with advanced non-small cell lung cancer (NSCLC) expected to be platinum-resistant.

Methods: Patients were diagnosed with NSCLC using endobronchial ultrasonography with a guide sheath as a core biopsy. RNA was immediately isolated from unfixed biopsy specimens, and quantitative real-time reverse transcription-PCR assays were performed to determine ERCC1 messenger RNA expression. Patients with advanced, untreated NSCLC showing high ERCC1 levels ($\Delta Ct \geq 6.5$) were assigned a non-platinum triplet regimen of irinotecan and paclitaxel plus bevacizumab. The primary end point was the objective response rate (ORR).

Results: A total of 141 untreated patients were evaluated and 30 patients were entered into this phase II trial. The ORR was 66.7% (95% confidence interval [CI] 47.2–82.7) and median progression-free survival (PFS) was 215 days. Grade 4 thrombosis occurred in one patient, but other toxicities were mild and controllable. Fifty-six patients were treated with platinum-containing regimens and 24 patients responded (ORR 42.8%, 95% CI 29.7–56.7). Twenty-nine of these patients had high ERCC1 levels, of which 6 patients responded; 27 patients had low ERCC1 levels, 18 patients responded ($P = 0.0053$ by Fisher's exact test).

Conclusion: The triplet combination might be effective for patients with advanced, untreated NSCLC overexpressing ERCC1. ERCC1 messenger RNA levels may be a predictive factor for response to platinum-containing regimens.

Introduction

Recently, tyrosine kinase inhibitors, such as gefitinib, erlotinib, afatinib, and crizotinib, have become first-line chemotherapy regimens for patients with advanced non-small cell lung cancer (NSCLC) harboring driver mutations,[1–7] although platinum-based chemotherapy is still considered first-line treatment for patients without driver mutations.[8] However, the toxicities of platinum-based regimens are still severe and clinical benefit is limited. Thus, predictive biomarkers for response to platinum-based regimens have been investigated. The ERCC1 protein has shown promise as a predictive biomarker for platinum-based chemotherapy, especially with cisplatin.[9–12]

ERCC1 protein levels in patients with NSCLC have mainly been evaluated by immunohistochemical analysis. However, Friboulet *et al.* reported that the use of currently available ERCC1 antibodies did not specifically detect the unique functional ERCC1 isoform.[13] Immunohistochemistry has limited usefulness to detect ERCC1 for use in guiding therapeutic decisions about cisplatin-containing regimens.

ERCC1 messenger RNA (mRNA) level has also been studied using reverse transcription (RT)-PCR assay.[14–17] However, mRNA is unstable, and extraction of mRNA from formalin-fixed paraffin-embedded (FFPE) tissue is difficult, suggesting limitations in the usefulness of mRNA to evaluate *ERCC1* expression. Fresh core biopsy samples without prior formalin fixation and paraffin embedding are often considered best for evaluating target mRNA. However, obtaining a sufficient unfixed core biopsy from patients with advanced NSCLC, especially non-squamous NSCLC, can be difficult because tumors are mainly located in the peripheral lung field. Computed tomography (CT)-guided percutaneous needle core biopsy is usually performed for such patients to obtain a core biopsy. This technique carries a high risk of pneumothorax and sample size is sometimes insufficient for additive biological analysis.[18,19]

Endobronchial ultrasonography with a guide sheath (EBUS-GS) is a new technique to diagnose lung cancer.[20,21] Ultrasonography allows for confirmation that the biopsy samples are actually obtained from within the tumor. We used biopsies obtained by EBUS-GS as core biopsies and evaluated the mRNA level of *ERCC1* in unfixed biopsy samples obtained from patients with suspected advanced non-squamous NSCLC.

We have previously reported the results of a randomized phase II trial comparing non-platinum doublets, irinotecan plus paclitaxel (IP) versus irinotecan plus gemcitabine (IG).[22] In that trial, the response rate achieved in the IP group was higher than in the IG group, while the toxicities of both regimens were controllable. On the other hand, bevacizumab, a recombinant monoclonal antibody blocking tumor angiogenesis that inhibits vascular endothelial growth factor (VEGF), is now commonly used in combination chemotherapy with irinotecan or paclitaxel for patients with advanced colorectal cancer or non-squamous NSCLC.[23,24] In the present phase II trial, we evaluated the efficacy and safety of non-platinum combination chemotherapy consisting of irinotecan plus paclitaxel plus bevacizumab for patients with advanced non-squamous NSCLC showing high mRNA levels of *ERCC1*. We also evaluated the relationship between mRNA levels of *ERCC1* and the efficacy of platinum-based chemotherapy.

Methods

Eligibility criteria

The eligibility criteria for this study were as follows: histologically-confirmed stage IIIB/IV non-squamous NSCLC (according to the 7th edition of the General Rule for Clinical and Pathological Record of Lung Cancer) with a core biopsy via EBUS-GS; delta Ct of *ERCC1* in biopsy sample ≥ 6.5[16] the absence of homozygous *UGT1A1*6* or *UGT1A1*28*; aged 20–75 years; no prior chemotherapy with EGFR-tyrosine kinase inhibitors (TKIs) or ALK inhibitors; no radiation therapy for the primary tumor; Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1; measurable lesions according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1; life expectancy of ≥ 12 weeks; and adequate bone marrow, renal, and hepatic function. The major exclusion criteria were: history or presence of hemoptysis or bloody sputum, tumor invading or abutting major blood vessels, a history of radiation therapy to the lung field, or coexistence or history of interstitial lung disease.

This study was performed in accordance with the Declaration of Helsinki. The study protocol was reviewed and approved by the institutional review boards of the participating institutions and written informed consent was obtained from all patients.

Real time-PCR

Total RNA was extracted from unfixed frozen tissue using an RNeasy Plus Mini Kit (Qiagen GmbH, Dusseldorf, Germany). Template RNA (approximately 100 ng) was used for complementary DNA synthesis using a High Capacity cDNA Reverse Transcription Kit (Life Technologies, Waltham, MA, USA). Complementary DNA (equivalent to 10 ng total RNA) and Platinum qPCR Super Mix (Life Technologies) was used for TaqMan real time-PCR for *ERCC1* and Actin, Beta (ACTB). RT-PCR was carried out using a Sequence Detection System 9700HT (Life

Technologies). Relative expression was calculated as follows: delta-Ct = Average Ct (*ERCC1*) − Average Ct (ACTB), Relative Expression = $2^{-\text{deltaCt}}$. This analysis was independently performed by FALCO Biosystems, Ltd. (Kyoto, Japan).

Treatment

All patients received paclitaxel (150 mg/m^2), irinotecan (50 mg/m^2), and bevacizumab 15 mg/kg by intravenous infusion on day 1, and irinotecan (50 mg/m^2) by intravenous infusion on day 8, repeated every four weeks. Each patient received a minimum of three cycles until the onset of progressive disease or unacceptable toxicity. The maximal number of chemotherapy cycles was six, and bevacizumab maintenance was administered until disease progression, unacceptable toxicity, or patient refusal.

Assessment of treatment

Before treatment, all patients underwent a complete medical history and physical examination, chest radiography, chest and abdominal CT, a radionuclide bone scan or positron emission tomography CT, brain CT or magnetic resonance imaging, and electrocardiography. Complete blood cell counts and blood chemistry studies were also performed and repeated at least twice a week until treatment discontinuation. Scans or radiographs used to assess response were obtained every four to six weeks.

The response was investigator-determined according to RECIST version 1.1. All adverse events were recorded and classified by grade according to the Common Terminology Criteria for Adverse Events version 3.0.

Additional cohort

To evaluate the efficacy of a platinum-containing regimen for each *ERCC1* of high and low expression, patients that did not show *ERCC1* expression (delta-CT ≥ 6.5) were added to the analysis set as an additional cohort.

Statistical analysis

The primary end point was overall response rate (ORR). A Simon optimal two-stage design was chosen to determine the total number of patients required for the study.[24] Assuming an ORR of 30% for standard therapy, a target response rate of 60% was established. With alpha = 0.05 and beta = 0.10, the estimated number of patients required was 28.

Overall survival (OS) was defined as the interval from the start of treatment to death from any cause. Progression-free survival (PFS) was defined as the interval from the start of treatment to either progressive disease or death, whichever came first. Survival curves were plotted using the Kaplan–Meier method.

This trial was registered with University Hospital Medical Information Network (UMIN000006514).

Results

Patient characteristics

Between September 2012 and March 2015, the *ERCC1* mRNA expression level was evaluated in 141 patients (range of delta-Ct: 3.9–8.5); 92 patients showed delta-CT ≥ 6.5. Of those, 30 patients with advanced non-squamous NSCLC were enrolled in the trial (Fig 1). The patient characteristics are presented in Table 1. Twenty-seven patients were diagnosed by EBUS-GS, while three patients were diagnosed by other core biopsy methods, such as thoracoscopic or transbronchial lymph node biopsy. All patients were treated and able to be assessed for toxicities, but two patients refused chemotherapy during the first cycle and asked to receive only supportive care, thus we were unable to evaluate the response in these patients.

Safety

We delivered a total of 106 treatment cycles, with a median of 3 (range 1–6) treatment cycles per patient. The range of bevacizumab maintenance was 0–9 cycles. Table 2 lists the
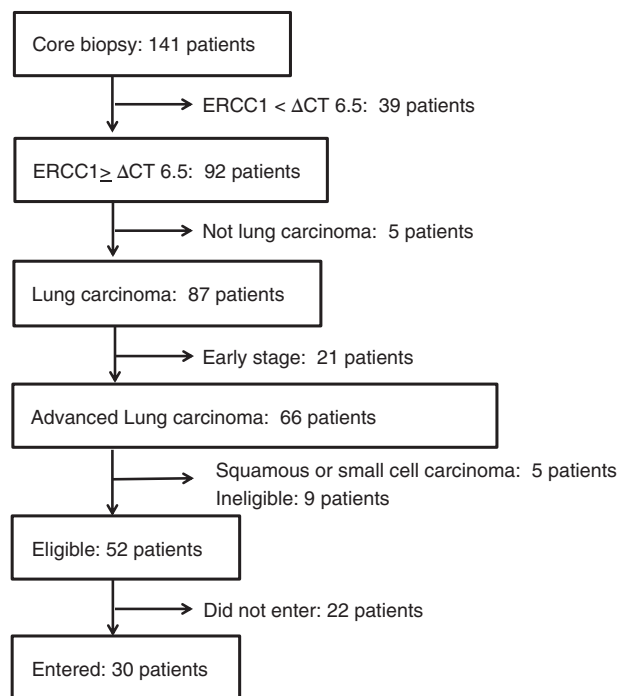
Figure 1 Study scheme.

Table 1 Patient characteristics

Characteristics	Number of patients	%
Age		
Median	64	
Range	37–75	
Gender		
Men	13	43
Women	17	57
Stage		
IIIB	1	3
IV	29	97
Histology		
Adenocarcinoma	30	100
Other	0	0
ECOG PS		
0	14	47
1	16	53
Mutation status		
EGFR deletion 19	4	13
EGFR L858R	5	17
EGFR others	1	3
EML4-ALK	0	0
Others	0	0
Diagnosis		
EBUS-GS	27	90
Others	3	10

EBUS-GS, endobronchial ultrasonography with a guide sheath; ECOG PS, Eastern Cooperative Oncology Group performance status.

incidence of hematological and non-hematological toxicities. Neutropenia was the most common grade 3/4 adverse event and occurred in 47% (14/30) of patients. Other grade 3/4 hematological toxicities included leukopenia (27%, 8/30) and anemia (3%, 1/30). Grade 3/4 non-hematological toxicities included febrile neutropenia (23%, 7/30), hypertension (7%, 2/30), duodenal ulcer, ileus, bleeding, thrombosis, pneumonitis, increased alanine transaminase and aspartate transaminase levels (3%, 1/30 each). No treatment-related death occurred. In addition, there were no severe complications requiring hospitalization as a result of EBUS-GS during the study.

Efficacy

Twenty patients achieved a partial response, 7 achieved stable disease, and one exhibited progressive disease. Figure 2 shows the best tumor changes from baseline in this study. All evaluable patients achieved tumor reduction. As mentioned previously, two patients were not evaluable because of treatment cessation. On the basis of intent-to-treat analysis, ORR was 66.7% (95% confidence interval [CI] 47.2–82.7). The median PFS was 229 (95% CI 169–294) days and the median survival time (MST) 857 (95% CI 331–NA) days (Fig 3). The one-year PFS rate was 15.2%. The one and two-year OS rates were 69.3% and

Table 2 Toxicities occurring in all treatment courses

	No. of patients (*n* = 30)		
Toxicity	Grade 1–2 (%)	Grade 3 (%)	Grade 4 (%)
Hematologic			
Leukopenia	10 (33%)	7 (23%)	1 (3%)
Neutropenia	8 (27%)	4 (13%)	10 (33%)
Anemia	7 (23%)	1 (3%)	0
Thrombocytopenia	1 (3%)	0	0
Non-hematologic			
Febrile neutropenia	6 (20%)	6 (20%)	1 (3%)
Anorexia	10 (33%)	0	0
Nausea	3 (10%)	1 (3%)	0
Vomiting	1 (3%)	0	0
Diarrhea	13 (43%)	0	0
Constipation	5 (17%)	0	0
Hiccups	2 (7%)	0	0
Duodenal ulcer	1 (3%)	1 (3%)	0
Ileus	0	1 (3%)	0
Hypertension	5 (17%)	2 (7%)	0
Bleeding	1 (3%)	1 (3%)	0
Thrombosis	2 (7%)	0	1 (%)
Neuropathy	9 (30%)	0	0
Pneumonitis	1 (3%)	1 (3%)	0
Thrombosis	2 (7%)	1 (3%)	0
Rash	5 (17%)	1 (3%)	0
Pruritus	1 (3%)	0	0
Fatigue	5 (17%)	0	0
Arthritis	1 (3%)	0	0
Myositis	1 (3%)	0	0
Alopecia	9 (30%)	0	0
AST	4 (13%)	1 (3%)	0
ALT	4 (13%)	1 (3%)	0
Hypercholesterolemia	1 (3%)	0	0
Hypoalbuminemia	3 (10%)	0	0
Hyponatremia	1 (3%)	0	0

ALT, alanine aminotransferase; AST, aspartate aminotransferase.
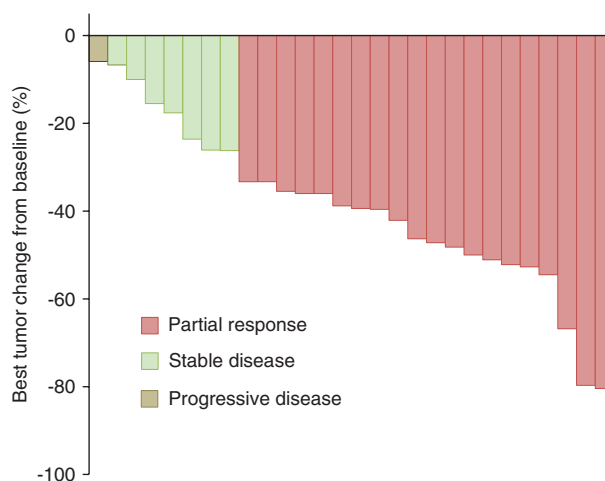
Figure 2 Best tumor change from baseline.

54.9%, respectively. OS data were not sufficiently mature at the time of analysis to present results here.

At the time of this analysis, 56 patients with advanced NSCLC had received platinum-based chemotherapy. These regimens are summarized in Table 3. Carboplatin plus pemetrexed plus bevacizumab and cisplatin plus pemetrexed were the most commonly used regimens. Platinum-based chemotherapy was used after triplet therapy immediately in all patients. Responses for the treatment and delta Ct of *ERCC1* in these patients are shown in Table 3. Six of 29 patients with high *ERCC1* responded to platinum regimens, with an ORR of 20.7%. By comparison, 18 of 27 patients with low *ERCC1* responded, with an ORR of 66.7%. The response to the platinum regimens in patients with high *ERCC1* was statistically significantly high compared to patients with low *ERCC1* (*P* = 0.0053 by Fisher's exact test).

Discussion

We evaluated the efficacy and safety of a non-platinum combination regimen – irinotecan and paclitaxel plus bevacizumab – for patients with advanced non-squamous NSCLC expressing *ERCC1* mRNA at a high level. The ORR was 66.7% and met the primary end point of this study. We observed a median PFS of 229 days and an MST of 32.0 months. However, the OS data were insufficient at the time of this analysis, as only two events had occurred. In this trial, grade 3 or 4 neutropenia and leukocytopenia were dominant toxicities (47% and 27%,

respectively) in accordance with our previous report about an IP regimen.[19] Febrile neutropenia also occurred in 43% of patients. Gastrointestinal toxicities such as nausea, vomiting, and diarrhea were mild and controllable compared to our previous report. Bleeding or thrombosis occurred in patients in this trial but not in our previous study. These adverse events are closely related to the VEGF antibody, thus we believe that bevacizumab might responsible for these toxicities. However, these VEGF antibody-specific toxicities were also controllable and we have shown that this treatment modality was well tolerated in the eligible patients. In additional analysis, a total of 56 patients with advanced NSCLC were evaluated for *ERCC1* mRNA expression and were treated with platinum-based chemotherapy. Eighteen of 27 patients with low *ERCC1* expression responded to platinum regimens (ORR 66.7%), while six of 29 patients with high *ERCC1* expression responded (ORR 20.7%). Compared to the high *ERCC1* population, the response to platinum-based chemotherapy was statistically significant in patients with a low *ERCC1* level.

The ERCC1 enzyme is thought to be an important enzyme in mediating resistance to platinum by removing platinum-induced DNA adducts.[12] Olaussen *et al.* were the first to report that patients with ERCC1-negative NSCLC based on immunohistochemical staining appear to benefit from adjuvant cisplatin-based chemotherapy,[9] and ERCC1 has come to be recognized as a promising predictive factor in platinum-based chemotherapy. Since then, ERCC1 has been evaluated in many clinical trials and analyses by several immunohistochemical methods.[10,11] Friboulet *et al.*
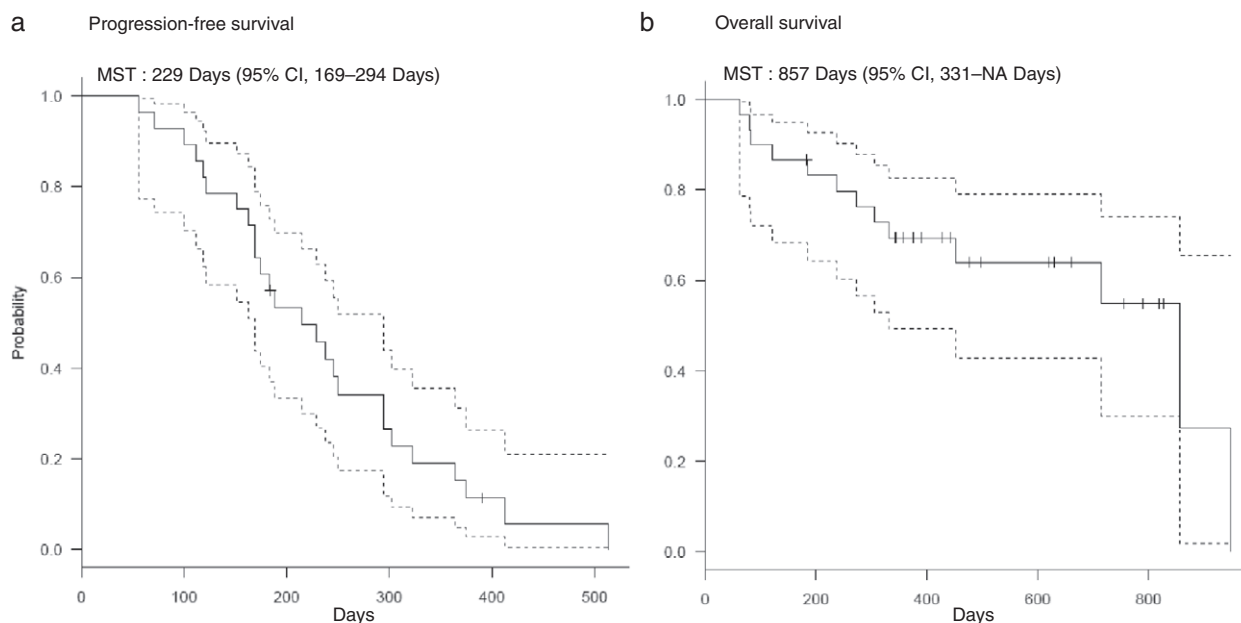
Figure 3 Progresion-free survival and Overall survival. CI, confidence interval; MST, median survival time.

Table 3 Responses to platinum regimens

ERCC1 high: ORR 20.7% (6/29)		ERCC1 low: ORR 66.7% (18/27)	
ERCC1 delta Ct	Response	ERCC1 delta Ct	Response
8.5	PD	6.4	PR
8.0	PD	6.4	PR
7.9	PD	6.4	SD
7.8	PR	6.4	PR
7.6	PR	6.3	SD
7.4	SD	6.3	NE
7.4	SD	6.2	PR
7.4	SD	6.2	PR
7.4	NE	6.2	SD
7.3	PD	6.2	PR
7.3	PD	6.1	PR
7.3	SD	6.1	PR
7.2	SD	6.1	PR
7.1	SD	6.1	PR
7.0	SD	5.9	SD
7.0	PD	5.9	SD
7.0	SD	5.9	PR
6.9	PD	5.8	PR
6.8	PD	5.8	PR
6.7	SD	5.8	PR
6.7	SD	5.5	PR
6.7	PD	5.4	PR
6.6	PD	5.2	PD
6.6	PR	5.2	SD
6.6	PR	5.0	PR
6.6	PR	5.0	PD
6.5	SD	3.9	PR
6.5	PD		
6.5	PR		

NE, not evaluable; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease.

reported that the currently available ERCC1 antibodies did not specifically detect the unique functional isoform of ERCC1 and concluded that immunohistochemistry may be of limited usefulness to guide therapeutic decision-making.[13]

The relationship between *ERCC1* mRNA expression and resistance to platinum regimens has been corroborated in patients with advanced NSCLC in previous studies. However, these analyses were almost all conducted using FFPE tissue. The instability of RNA compared to DNA is well recognized, and mRNA is destroyed or damaged in FFPE tissue. Thus, unfixed tissue obtained by core biopsy must be used to evaluate mRNA expression. In advanced lung cancer, percutaneous CT-guided core biopsy is considered a standard method of obtaining core biopsy samples from primary tumors. However, potential complications, such as pneumothorax or air embolization, are associated with this method.[18] EBUS-GS is a recently developed bronchoscopic method that makes it possible to confirm that samples are obtained from within a tumor based on ultrasonography

imaging.[20,21] We adopted this method in our trial and designated the biopsy obtained by this method as a core biopsy. As a result, in this trial we examined the relationship between *ERCC1* mRNA levels and the efficacy of platinum chemotherapy. EBUS-GS is probably one of the safest methods to obtain core biopsy samples from primary tumors in advanced NSCLC.

We did not compare the efficacy of non-platinum combination chemotherapy to the platinum combination in patients with high levels of ERCC1 because platinum combination therapy is now strongly recommended as first-line treatment for patients with advanced NSCLC.[8] However, our data indicated the possibility that *ERCC1* mRNA overexpression extracted from an unfixed core biopsy tissue is a predictive factor for response to platinum-containing regimens. In addition, as mentioned previously, EBUS-GS may be a useful method for obtaining a core biopsy. We recommend a randomized, prospective trial to confirm our results using unfixed biopsy specimens collected by EBUS-GS.

In conclusion, the triplet combination of irinotecan and paclitaxel plus bevacizumab might be effective for patients with advanced, untreated NSCLC overexpressing *ERCC1*. In addition, *ERCC1* mRNA levels extracted from unfixed lung biopsy specimens obtained by EBUS-GS may also be predictive for a response to platinum-containing regimens.

Disclosure

No authors report any conflict of interest.

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