Lung Cancer 93 (2016) 43–46

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Sorafenib treatment for patients with *RET* fusion-positive non-small cell lung cancer

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ARTICLE INFO

Article history:
Received 24 September 2015
Received in revised form 3 December 2015
Accepted 25 December 2015

Keywords:
RET
Sorafenib
Non-small cell lung cancer

ABSTRACT

Background: RET fusions were recently identified in non-small cell lung cancer (NSCLC) and are considered as a potential therapeutic target of NSCLC. Sorafenib, a multi-kinase inhibitor, has potent anti-*RET* activity. We conducted a study to evaluate the efficacy of sorafenib in a small number of patients with *RET* fusion-positive NSCLC.
Materials and methods: Eligible patients had advanced or recurrent NSCLC, were more than 20 years old, had undergone treatment with one or more previous chemotherapy regimens, had an Eastern Cooperative Oncology Group performance status 0–2, had adequate organ function, and provided informed consent. The presence of the *RET* fusion gene was confirmed by a split FISH assay. The patients were treated twice daily with 400 mg of sorafenib taken orally. The treatment was continued until either disease progression or unacceptable toxicity.
Results: From March 2012 to April 2013, three patients were enrolled. The responses to sorafenib included one patient with stable disease (SD) and two patients with progressive disease (PD). One patient took sorafenib for twelve months. The most common toxicities were palmar–plantar erythrodysesthesia syndrome, hypertension, and diarrhea.
Conclusion: Since sorafenib did not show dramatic responses, we suggest testing other RET inhibitors for the treatment of *RET* fusion-positive NSCLC. This study was registered at UMIN as trial number 000007515.

1. Introduction

Recently, a number of oncogenic gene alterations have been identified in non-small cell lung cancer (NSCLC). Several classes of targeted therapies have been developed in molecularly-defined subsets of NSCLCs. Among them, activating somatic mutations in epidermal growth factor receptor tyrosine kinase (*EGFR*) and rearrangement of the anaplastic lymphoma kinase gene (*ALK*) are associated with better outcomes when targeted by selective tyrosine kinase inhibitors [1–3].

RET (rearranged during transfection) is a transmembrane tyrosine kinase that functions as the receptor for growth factors from the glial-derived neurotrophic factor family [4]. *RET* is a well-known oncogenic driver in thyroid cancers. Activating somatic mutations in *RET* are common in sporadic medullary thyroid cancer (MTC), and *RET* fusions are identified in a subset of papillary thyroid cancers [5]. Recently, we identified *RET* fusions in a subset of NSCLC through an integrated molecular- and histopathology-based screening system. *RET* fusions are present in about 1% of NSCLC patients, and occur in younger patients with lighter smoking exposure [6]. Three other groups found *RET* fusions using different screening strategies simultaneously [7–9].

There are several small molecules, including sorafenib, sunitinib, vandetanib, and cabozantinib which have been shown pre-clinically to inhibit *RET* kinase activity, and have clinical activity for advanced MTC. Sorafenib, a multi-kinase inhibitor, is already approved for the treatment of advanced renal cell carcinoma, advanced primary hepatocellular carcinoma, and advanced thyroid cancer. Sorafenib targets multiple intracellular (c-CRAF, BRAF, and mutant BRAF) and cell surface kinases (KIT, FLT-3, RET,

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http://dx.doi.org/10.1016/j.lungcan.2015.12.011
0169-5002/

Table 1
Patients characteristics.

Patient number	1	2	3
Gender	Female	Male	Female
Age (years)	62	38	75
PS (ECOG)	1	1	1
Smoking	Never	Never	Never
Histology	Unclassified	Adenocarcinoma	Adenocarcinoma
Subtype	–	Papillary	Solid with mucin production
Grade	–	Moderately	Poorly
Stage	IV	Recurrence after surgery	Recurrence after surgery
EGFR mutation	None	None	None
ALK fusion	Negative	Negative	Negative
RET fusion	Positive	Positive	Positive
RET partner gene	KIF5B	Unknown	CCDC6
Prior number of chemotherapy	3	2	1
Efficacy of sorafenib			
Response	PD	PD	SD
Time to progression	18 days	43 days	371 days
Duration of treatment	18 days	43 days	373 days

Abbreviations: PS, performance status; ECOG, Eastern Cooperative Oncology Group; PD, progressive disease; SD, stable disease.

RET/PTC, VEGFR-1, VEGFR-2, VEGFR-3, and PDGFR-ß). Precinical studies reported that sorafenib has potent anti-*RET* activity (the IC_{50} against *RET* is 5.9–47 nM), and clinical studies suggested that sorafenib has activity against thyroid cancers that have sustained oncogenic *RET* activation [10]. Furthermore, in a preclinical study it was found that Ba/F3 cells with the *KIF5B-RET* fusion, which is common in *RET* fusion-positive NSCLC, are sensitive to sorafenib [9]. Therefore, it was worth evaluating the anti-tumor activity of sorafenib for *RET* fusion-positive NSCLC. We conducted a study to evaluate the efficacy of sorafenib in patients with *RET* fusion-positive NSCLC.

2. Materials and methods

2.1. Study population

Patients were required to have histologically confirmed *RET* fusion-positive advanced or recurrent NSCLC, and were refractory to treatment with one or more previous chemotherapy regimens. Other inclusion criteria included age of 20 years or over, Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0–2, life expectancy of at least 3 months, and adequate organ function. Patients were excluded from the trial for any of the following reasons: uncontrolled malignant pleural or pericardial effusion, a concomitant serious illness contraindicating chemotherapy, pregnancy, or breast-feeding. All patients provided written informed consent. The study protocol was approved by our institutional ethics committee and was registered with the UMIN Clinical Trials Registry as UMIN 000007515 (http://www.umin.ac.jp/ctr/).

2.2. Identification of RET fusion

We identified *RET* fusion using our screening system of kinase fusions in NSCLC of our institution. In our screening system, *RET* rearrangements were identified using the spirit fluorescent in situ hybridization (FISH) assay, fusion FISH assay, RT-PCR assay, or a genomic PCR assay [11,12]. When these analyses were not in agreement, we further examined the tissue by more than one method, including either rapid amplification of cDNA ends (RACE) or inverse RT-PCR assays, and determined a definitive diagnosis.

2.3. Treatment

All patients were treated twice daily with 400 mg of sorafenib taken orally. The treatment was continued until either disease progression, unacceptable toxicity, discontinuation of sorafenib for any reason for ≥21 days, or patient withdrawal. If treatment-related toxicity, such as grade 3 or recurrent grade 2 non-hematologic toxicities, grade 2 skin toxicity, hypertension, and grade 4 hematologic toxicities, was observed, the sorafenib dose was reduced to 400 mg once daily in one case and then to 400 mg every other day.

2.4. Assessment

Adverse reactions were monitored, graded, and recorded according to the National Cancer Institute Common Toxicity Criteria version 4.0. Efficacy was assessed by a physician on the basis of the antitumor effect according to the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. The response was confirmed for at least 4 weeks (for a complete response or partial response: PR) or for 6 weeks (for stable disease: SD) after it was first documented.

2.5. Statistical consideration

Since *RET* fusion-positive NSCLC comprise only 1–2% of NSCLC cases, it is difficult to recruit a large number of the patients. Therefore, we performed an exploratory study evaluating the efficacy of sorafenib in 3 patients without statistical consideration.

3. Results

From March 2012 to April 2013, three patients were enrolled in this study. The patient characteristics are summarized in Table 1.

All patients were non-smokers. Based on histology, two of the cancer types were adenocarcinoma and one was an unclassified NSCLC. *RET* fusions were identified in all three patients by a split FISH assay. The fusion partner was identified in two patients as *KIF5B-RET* and *CCDC6-RET* using the fusion FISH assay. No *EGFR* mutations and no *ALK*, or *ROS-1* fusion genes were identified in any of the patients.

The first patient was a 62-year-old female who had stage IV unclassified NSCLC with the *KIF5B-RET* fusion gene. She had received three prior chemotherapy regimens, but the disease did not respond to these treatments. After palliative radiation for the thorax and whole brain, she participated in this study. On day 14 of the treatment, she felt pain in the inguinal region and a left ilium fracture was observed. The fracture was due to a bone metastasis. Rapidly progressing multiple liver metastases were observed in CT-scan on day 18 (Fig. 1A). Regarding toxicities, the patient

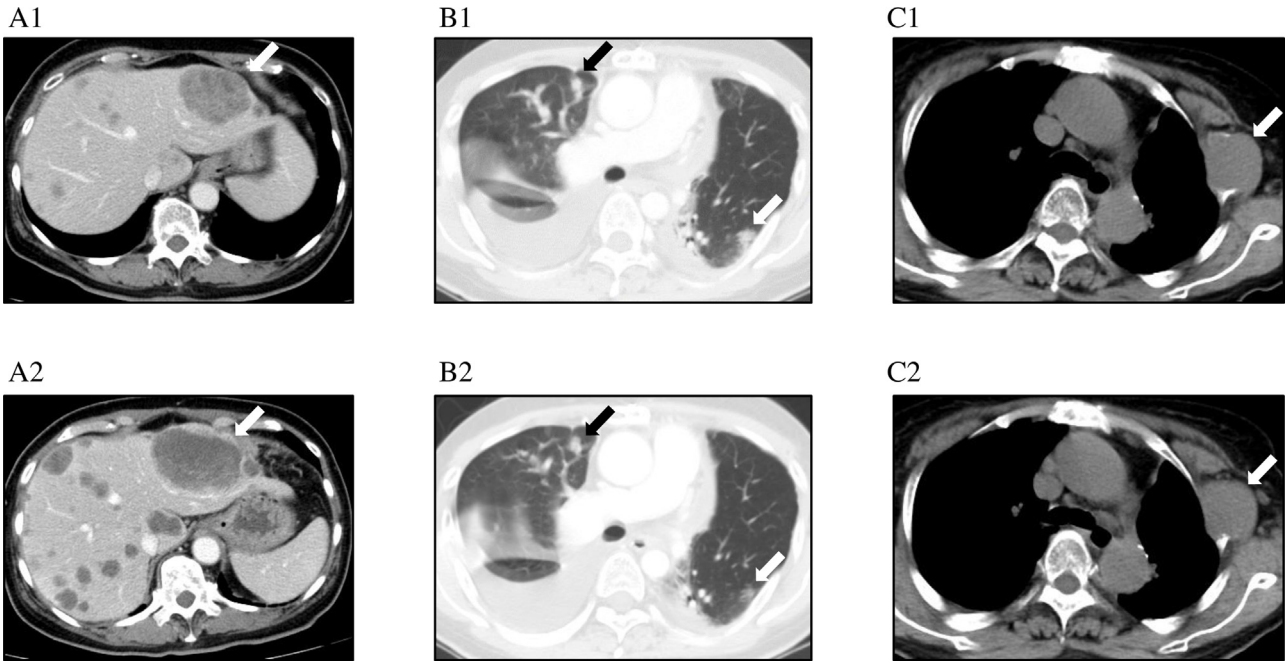

Fig. 1. Radiographically changes during sorafenib treatment.
(A1) Baseline chest CT of the first patient with KIF5B-RET showing liver metastasis. (A2) Repeat imaging on day 18 of treatment showing that the liver metastasis were increased in number and size. (B1) Chest CT of the second patient with RET fusion-positive NSCLC showing 2 lung metastases. (B2) Repeat imaging on the fourth week of treatment showing lung metastases were minimally decreased. (C1) Baseline imaging of the third patient with CCDC6-RET showing chest wall metastasis. (C2) Stable disease at the fourth week of treatment. Responses have been maintained 12 months.

experienced grade 2 hypertension, grade 1 aspartate aminotransferase increased, grade 1 serum amylase increased, and grade 1 lipase increased.

The second patient was a 38-year-old male who was diagnosed with a moderately differentiated, papillary adenocarcinoma, and *RET* fusion by the split FISH assay. *KIF5B-RET* and *CCDC6-RET* fusions were negative by the fusion FISH assay and the RT-PCR assay, and his *RET* partner gene was not identified in this study. He had a recurrence of the cancer three years after thoracic surgery. He had received two prior chemotherapy regimens, and the responses of these chemotherapies were SD. After palliative radiation for the whole brain, he participated in this study. In the fourth week, his target lesions were minimally decreased (−20.4%) (Fig. 1B). However, his pleural effusion continued increasing in the sixth week. Regarding toxicities, the patient experienced grade 1 palmar–plantar erythrodysesthesia syndrome, grade 1 diarrhea, grade 1 white blood cell decreased, and grade 1 blood bilirubin increased.

The third patient was a 75-year-old female who was diagnosed with a poorly differentiated, solid adenocarcinoma with mucin production and *CCDC6-RET* fusion gene. She had a recurrence of the cancer four years after thoracic surgery. She had received decetaxel monotherapy with SD before enrolling this study. After starting sorafenib treatment, her tumors were slightly decreased (−4.0%), and her tumor-related pain improved during sorafenib treatment. Her response to sorafenib was maintained clinically and radiographically for twelve months (Fig. 1C). Regarding toxicities, the patient experience grade 3 palmar–plantar erythrodysesthesia syndrome, grade 3 infection, grade 2 hypertension, grade 2 nausea, grade 1 diarrhea, grade 1 white blood cell decreased, grade 1 neutrophil counts decreased, and grade 1 aspartate aminotransferase increased. She needed two separate dose reductions due to her grade 3 palmar–plantar erythrodysesthesia syndrome and a grade 3 infection.

4. Discussion

This is the first report to evaluate the efficacy of sorafenib for *RET* fusion-positive NSCLC. Although dramatic responses were not observed in the three patients, sorafenib may have some anti-tumor activity for *RET* fusion-positive NSCLC. Tumor shrinkage of target lesions and symptom improvements were observed in the patients and a stable disease for one year was seen in a patient who had rapidly progressing disease after prior chemotherapy.

As compared with other potential *RET* kinase inhibitors, such as cabozantinib and vandetanib, the anti-tumor activity of sorafenib does not seem to be impressive. Drilon et al. reported preliminary data for the first three patients treated with the cabozantinib on a prospective phase II trial for patients with *RET* fusion-positive NSCLCs, and they observed two PR and one SD among the 3 patients [13]. The efficacy of vandetanib were also reported in two cases with *RET* fusion-positive NSCLC [14,15]. Recently, Platt et al. reported a retrospective analysis of four phase III randomized NSCLC trials of vandetanib, and none of the four patients with *RET* fusion-positive NSCLC had an objective response to vandetanib [16]. To date, only a few cases with *RET* fusion-positive NSCLCs treated with *RET* kinase inhibitors have been reported. Based on similar preclinical findings, sorafenib, vandetanib, and cabozantinib were examined in MTC, and improved PFS as compared with placebo in phase III studies [17–19]. In these studies, the objective response rates of sorafenib, vandetanib, and cabozantinib were 12.2%, 45%, and 28%, respectively. These reports suggested that currently available muti-kinase inhibitors, which have potent anti-*RET* activity in preclinical models, have some activity for *RET* fusion-positive NSCLC patients. But the responses were not dramatic as compared to that with either EGFR-TKI for *EGFR* mutated NSCLC or ALK-TKI for NSCLC with the *ALK* fusion gene. One of the reasons for the lack of a dramatic response is the weak anti-*RET* activity of these drugs. Indeed, preclinical studies showed the IC_{50} against

RET of these drugs were relatively higher than the IC_{50} against other targets, such as VEGFRs [20].

Currently, several clinical trials to evaluate the efficacies of *RET* inhibitors (vandetanib, cabozantinib, ponatinib, sunitinib, and lenvatinib) for *RET* fusion-positive NSCLC patients are underway (UMIN000010095, NCT01639508, NCT01813734, NCT01823068, NCT01829217, NCT01877083). The results of these trials will clarify the usefulness of *RET* inhibitors for *RET* fusion-positive NSCLC.

In conclusion, sorafenib seems to have some clinical activity for *RET* fusion-positive NSCLC, however, it does not show a dramatic response with *RET* fusion-positive NSCLC patients. We suggest testing other *RET* inhibitors rather than sorafenib for the treatment of *RET* fusion-positive NSCLC.

Conflict of interests

This study was supported by the Japanese Foundation for Cancer Research. Kengo Takeuchi reports consultancies from Nichirei and Chugai, grants from LSI medience and Chugai, and honoraria from Chugai, Kyowa Kirin, Pfizer, Nichirei, Meiji, Otsuka, Dainihonsumitomo, Nihonshinyaku, and Eisai outside the submitted work. Atsushi Horiike, Takeshi Uenami, Yuko Kawano, Azusa Tanimoto, Kyohei Kaburaki, Keita Kudo, Noriko Yanagitani, Fumiyoshi Ohyanagi, Noriko Motoi, Yuichi Ishikawa, Takeshi Horai, and Makoto Nishio have nothing to disclose.

Acknowledgments

We would like to express our deepest gratitude to Dr. Tetsuo Noda (Director of the Cancer Institute of Japanese Foundation for Cancer Research) who provided excellent feedback and valuable comments. We gratefully acknowledge the patients, their families, their caregivers, and the investigators who participated in this study. Finally, we would like to thank the Japanese Foundation for Cancer Research for a grant that made it possible for us to complete this study.

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