De Novo MET Amplification in Chinese Patients With Non–Small-Cell Lung Cancer and Treatment Efficacy With Crizotinib: A Multicenter Retrospective Study

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Abstract

We investigated the clinicopathologic characteristics, treatment, and prognosis of non–small-cell lung cancer patients with de novo mesenchymal–epithelial transition (MET) amplification. The results demonstrated that patients with MET amplification had a trend toward a high prevalence of the solid predominant subtype of adenocarcinoma and of brain metastases. Patients with de novo MET amplification benefit from crizotinib treatment, especially those with high-level amplification.

Background: De novo mesenchymal–epithelial transition (MET) amplification represents an uncommon oncogenic event in patients with non–small-cell lung cancer, and little is known about the clinicopathologic characteristics, treatment, and prognosis of these patients. **Patients and Methods:** Patient data were retrospectively collected in 5 hospitals in China from 2014 to 2016. All MET amplification was identified with fluorescence in-situ hybridization. A MET/centromere ratio (MET/CEN) of ≥ 1.8 was defined as positive for MET amplification. **Results:** Amplification of the de novo *MET* gene was identified in 47 patients with lung cancer. Thirty-two patients had a MET/CEN > 5, while 12 patients had intermediate-level amplification and only 3 had low-level amplification. Nine of 40 patients with advanced stage disease had brain metastases, and 15 had a solid predominant subtype of adenocarcinoma. Fifteen patients were treated with crizotinib. Of these, 11 patients (73.3%) had a partial response, 3 (20%) stable disease, and 1 (6.7%) progressive disease. The median progression-free survival of the 15 patients treated with crizotinib was 6.5 months (95% confidence interval, 2.7–10.3). Notably, treatment efficacy was more pronounced in patients with high-level MET amplification than in those with intermediate-level amplification (8.6 vs. 4.4 months, $P = .008$). The overall survival of patients with and without crizotinib treatment was 31.0 and 13.7 months, respectively ($P = .001$). **Conclusion:** We observed a trend toward a high prevalence of the solid predominant subtype of adenocarcinoma and of brain metastases in this group of patients. Patients with de novo MET amplification benefit from crizotinib treatment, especially those with high-level amplification.

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Introduction

Non–small-cell lung cancer (NSCLC) is the leading cause of cancer-related deaths in the world.[1] A growing number of genetic alterations and related inhibitors have been identified in NSCLC, most notably associated with epidermal growth factor receptor (EGFR), chromosomal rearrangements involving the anaplastic lymphomakinase gene (*ALK*), and the c-ros oncogene 1 (*ROS1*).[2-6]

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The mesenchymal–epithelial transition (MET) receptor is a potential therapeutic target in NSCLC.[7-10] Although MET amplification has been identified as a common gene alteration in patients resistant to EGFR tyrosine kinase inhibitors (TKIs), with a frequency of 5% to 20%, it has been described as a rare oncogenic event in EGFR-TKI treatment-naive patients with NSCLC.[11-15]

Although preliminary data from case reports have demonstrated promising efficacy of crizotinib treatment in NSCLC patients with de novo MET amplification,[16,17] studies that have included a large number patients with NSCLC and de novo MET amplification are lacking. In fact, to our knowledge, no prospective study has been published evaluating the efficacy of crizotinib treatment in patients with de novo MET amplification. In addition, there are limited data on the clinicopathologic characteristics, treatment, and prognosis of patients with de novo MET amplifications. In this multicenter retrospective study, we describe the characteristics, treatment, and survival of 47 patients with NSCLC and de novo MET amplification.

Patients and Methods

Patients

We conducted this multicenter retrospective study involving 5 hospitals in China between January 2014 and December 2016. The pathologic type of NSCLC was determined according to the 2015 World Health Organization histologic classification, and the subtype of adenocarcinoma was confirmed according to International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society criteria. The following patients were excluded: (1) patients who had been treated with EGFR-TKIs or other inhibitors before MET detection, (2) patients with evidence of pulmonary metastases of small-cell lung cancer or other carcinoma, and (3) patients who died of other diseases unrelated to NSCLC. The study was approved by the institutional ethics committee at all the study sites.

MET Fluorescence In-Situ Hybridization Assay

MET amplification was conducted using a MET/centromere ratio (MET/CEN) 7q dual-color fluorescence in-situ hybridization probe (Vysis, Abbott Molecular, Des Plaines, IL). Formalin-fixed, paraffin-embedded slides 4 to 6 μm thick were used for detection. The MET amplification status was defined according to the 80081001 study,[18] as follows: MET/CEN7 ratio < 1.8, negative; low: ≥ 1.8 to ≤ 2.2; intermediate: > 2.2 to < 5.0; high: > 5.0. Details of the procedure are provided elsewhere.[15]

Tumor Evaluation

Tumor responses were measured according to the Response Evaluation Criteria in Solid Tumors 1.1. Objective tumor responses included complete response, partial response (PR), stable disease, and progressive disease. The disease control rate was defined as the addition of the objective responses and stabilization rates (complete response + PR + stable disease).

Statistical Analysis

Survival analysis, including progression-free survival (PFS) and overall survival (OS), was conducted by the Kaplan-Meier method. PFS was defined as the length of time from treatment with crizotinib to disease progression or last follow-up. OS was determined as the length of time from date of diagnosis to date of death or last follow-up. $P < .05$ was considered to indicate statistical significance. The median follow-up time of the 47 patients was 32 months (range, 12–72 months). The last follow-up date was August 30, 2017.

Table 1 Clinicopathologic Feature Comparison Among Patients for De Novo Mesenchymal–Epithelial Transition Amplification

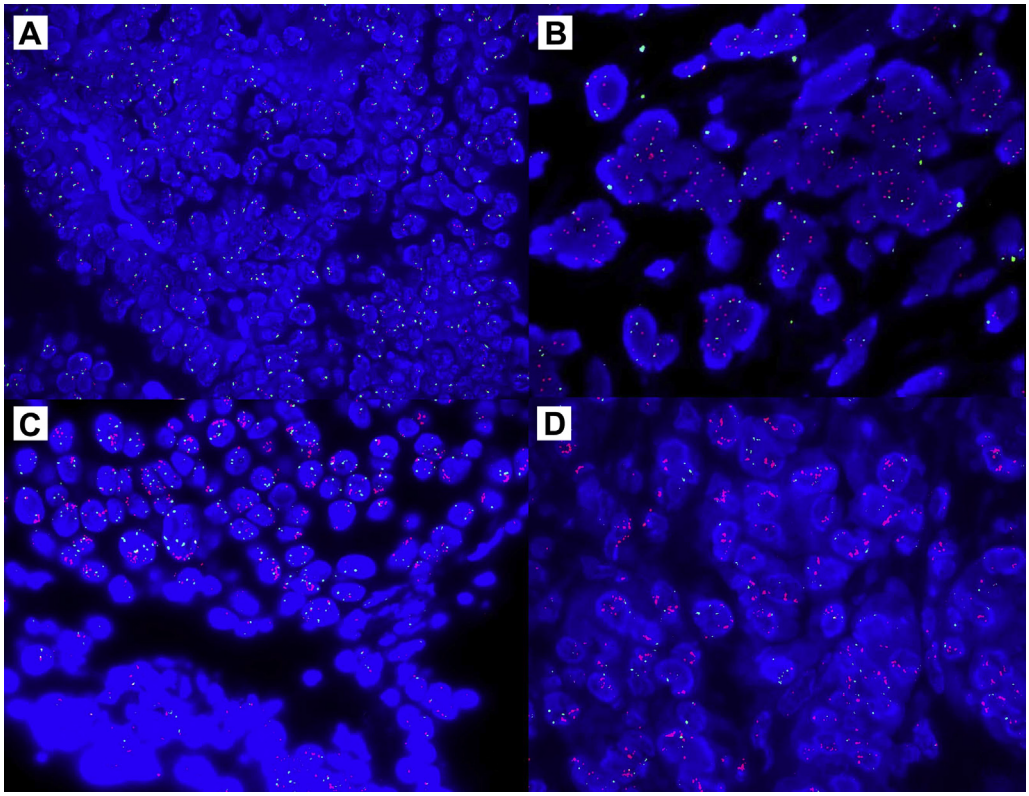
Characteristic	N	%
Gender		
Male	29	61.7
Female	18	38.3
Age at Diagnosis		
< 60 years	18	38.3
≥ 60 years	29	61.7
Smoking History		
Yes	22	46.8
No	25	53.2
Histology		
Adenocarcinoma	41	87.2
Nonadenocarcinoma	6	12.8
Stage at Diagnosis		
I-IIIA	7	14.9
IIIB/IV	40	85.1
Metastasis Site at Advanced Stage		
Lung	16	40.0
Brain	9	22.5
Bone	7	17.5
Others	8	20.0
Met Amplification		
>5	32	68.1
2.2–5	12	25.5
1.8–2.2	3	6.4
Crizotinib Treatment		
Yes	15	31.9
No	32	68.1

Results

Clinicopathologic Characteristics

Forty-seven patients were identified to have de novo MET amplification. The median age was 63 years old (range, 29–78 years); 29 (61.7%) were male and 18 (38.3%) female. Twenty-two subjects (46.8%) were ever-smokers or smokers, whereas 25 (53.2%) were nonsmokers. Forty-one (87.2%) were diagnosed with adenocarcinoma, while a diagnosis of nonadenocarcinoma was made in 6 (12.8%). Of the 41 patients with adenocarcinoma, 37 were further classified as subtypes, with the most frequent subtype being solid predominant adenocarcinoma (15 patients). According to the 7th edition of the tumor, node, metastasis classification system staging system, 7 patients (14.9%) were classified as having stage I-IIIA disease, and 40 (85.1%) had stage IV disease. Among the 40 patients with advanced stage disease, the most frequent site of disease

Figure 1 Different MET Amplification Levels. (A) Negative. (B) Low Level. (C) Intermediate Level. (D) High Level



Abbreviation: MET = mesenchymal–epithelial transition.

involvement was the lung (n = 16), followed by brain (n = 9), bone (n = 7), and other (n = 8). The clinical characteristics of all patients are presented in Table 1.

Molecular Characteristics

Among the 47 patients, 32 had high-level MET amplification, 12 had intermediate-level amplification, and 3 had low-level amplification (Figure 1). *EGFR* and *ALK* genes were detected by reverse transcriptase PCR in all 47 patients. EGFR mutations were observed in 3 cases, one of whom displayed low-level amplification (MET/CEN7 = 1.8), one intermediate-level amplification (MET/CEN7 = 2.75), and one high-level amplification (MET/CEN7 > 5). *ROS1* was detected in 17 patients. Of interest, one *ROS1*-positive patient had high-level amplification (MET/CEN7 > 5), and one had intermediate-level amplification (MET/CEN7 = 2.26).

A trend of more patients with intermediate- and low-level amplification tended to have a concurrent gene (*EGFR/ALK/ROS1*) compared to patients with high-level amplification (20% vs. 6.3%, $P = .36$).

There was no significant difference in gender ($P = .63$), age ($P = .63$), smoking status ($P = .54$), histologic type ($P = .69$), or brain metastases ($P = .77$) between patients with high-level and intermediate/low-level MET amplification. We found that there were more patients with high-level MET amplification in the advanced stage of disease ($P = .004$). Correlations between different MET amplification status and clinicopathologic characteristics are listed in Table 2.

Treatment Efficacy

Fifteen of the 47 patients with MET amplification were treated with crizotinib as a second or further-line treatment. Among the 15 patients, 11 had high-level amplification and 4 had intermediate-level amplification. All of the 15 patients were assessed for tumor response. Eleven (73.3%) of the 15 patients had a PR, 4 had stable disease (26.7%), and one had progressive disease. For the 11 patients with PR, 10 had high-level amplification and 1 had intermediate-level amplification. The median PFS for all patients was 6.5 months (95% CI, 2.7−10.3). The median PFS for patients with high and intermediate-level amplification was 8.6 and 4.4 months, respectively ($P = .008$). The efficacy data is presented in Figure 2 and Table 3.

Survival Analysis

Among the 47 patients, 40 had advanced stage disease, 7 had undergone surgery at the time of diagnosis, 4 were lost to follow-up and could not be included in the survival data (one had advanced stage disease, and 3 were postsurgery patients). We analyzed the survival data of 39 patients with advanced stage disease. The median OS with high, intermediate and low-level amplification was 16.2,

Table 2 Clinicopathologic Feature Comparison Between Patients With High and Intermediate/Low-Level Amplification

Characteristic	High Level (N)	Intermediate/Low Level (N)	*P*
Gender			.63
Male	19	10	
Female	13	5	
Age at Diagnosis			.63
<60 years	13	5	
≥ 60 years	19	10	
Smoking History			.54
Yes	14	8	
No	18	7	
Histology			.69
Adenocarcinoma	28	13	
Nonadenocarcinoma	4	2	
Stage at Diagnosis			.004
I-IIIA	1	6	
IIIB/IV	31	9	
Metastasis site at advanced stage			
Brain Metastasis			.77
Yes	7	2	
No	25	13	

13.5, 17.2 months, respectively ($P = .277$). No difference was observed between those patients who were not treated with crizotinib according to their amplification status of high, intermediate and low-level amplification (13.7 vs. 13.5 vs. 17.2 months, $P = .929$). However, there was a significant survival difference for patients with and without crizotinib treatment (31.0 vs. 13.7 months, $P = .001$), respectively (Figure 3).

The median OS for the 15 patients with crizotinib was 31.0 months. A trend of OS difference was observed between patients with high and intermediate-level MET amplification (31.5 vs.18.0 months, $P = .06$).

Discussion

In this study of NSCLC patients with de novo MET amplification, there was a high prevalence of brain metastases and predominant solid subtype of adenocarcinoma. High-level amplification was the most frequent finding, and treatment with crizotinib was associated with the most promising efficacy in this

Figure 2 Duration of Response With Crizotinib Treatment in 15 Patients. Arrow Indicates Continuation to Crizotinib Treatment

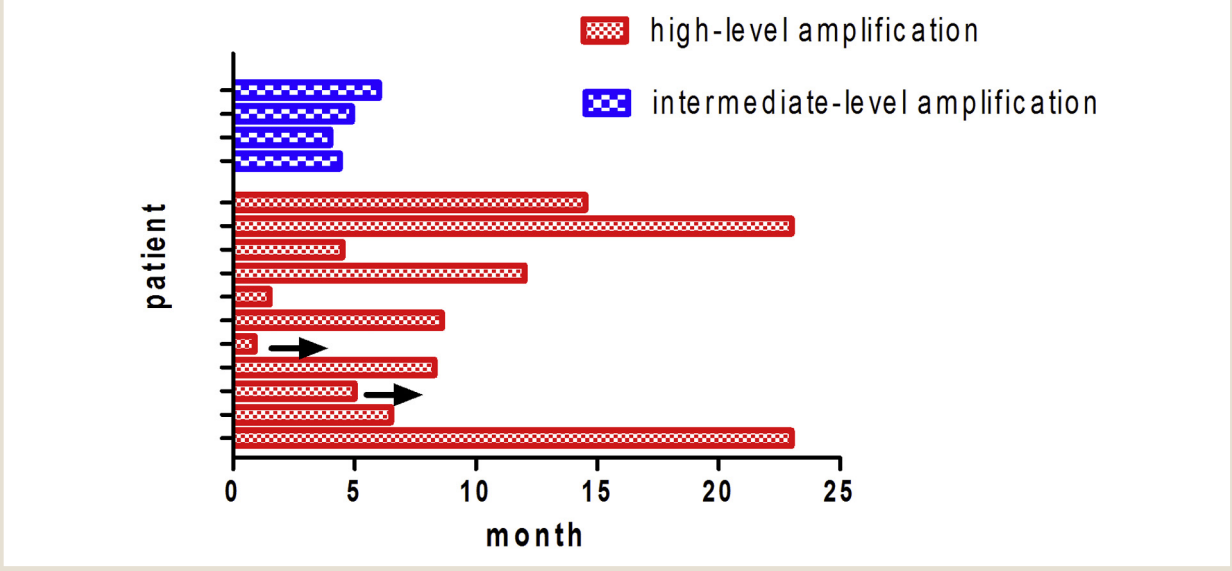


Table 3 Efficacy of Crizotinib in 15 Patients With MET Amplification

Response	Total (N = 15)	High Level (N = 11)	Intermediate Level (N = 4)
Complete response	0	0	0
Partial response	11	10	1
Stable disease	3	1	2
Progressive disease	1	0	1
Response rate	73.3%	90.9%	25.0%
Disease control rate	93.3%	100%	75%
Median progression-free survival	6.5 months	8.6 months	4.4 months
Median overall survival	31.0 months	31.5 months	18.0 months

Abbreviation: MET = mesenchymal–epithelial transition.

group of patients. To our knowledge, this is the first report to date of a case series evaluating de novo MET amplification in Asian patients with lung cancer.

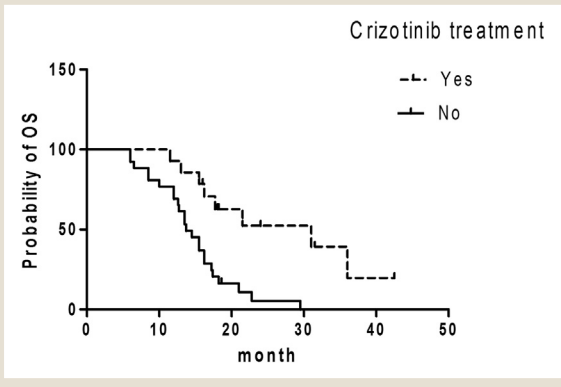
It is well known that molecular profiles of Chinese patients with lung cancer are different from those of western populations. The frequency of EGFR-mutated lung adenocarcinoma ranges from 30% to 60% in the Chinese population, but is less than 20% among westerners.[19,20] The incidence of MET exon 14 mutation in lung adenocarcinoma in Chinese individuals has been reported to be lower than that described in data based on western populations. However, data on the prevalence of de novo MET amplification has been controversial due to the application of several alternative definitions in the published literature.[10,14,21] The reported prevalence of de novo MET amplification in NSCLC ranges from 1% to 10%.[13] Currently, the primary definition for MET amplification most widely used in clinics to select patients for treatment with MET inhibitors is MET/CEN $\geq$ 1.8. In our previous study of 791 individuals, the frequency of de novo MET amplification was less than 1%.[15] Similarly, only 0.84% of patients had high-level MET amplification in the 80081001 study.[18] In the present study, becuse the data were not based on epidemiologic data, the frequency of MET amplification could not be determined.

In our previous study, the most common clinical characteristics of de novo MET amplification observed were advanced stage disease and the predominant solid subtype of adenocarcinoma.[15] This present study, which includes a larger number of patients with de novo MET amplification, supports this observation. Interestingly, patients with de novo MET amplification were also more likely to have brain metastases, though we need a larger study to confirm these clinicopathologic characteristics.

The *MET* gene is increasingly emerging as a clinically relevant biomarker for predicting the response to treatment with MET inhibitors. However, currently efficacy data have been mostly evaluated for MET exon skipping and have mostly been based on case reports.[22,23] Patients with high-level amplification are usually the most selective for response to treatment. The first case report of MET-amplified lung cancer was from the 80081001 study (NCT00585195). The patient was a 77-year-old woman with advanced adenocarcinoma of the lung, in whom a high-level MET amplification (MET/CEP7 ratio > 5) was detected. She received crizotinib and achieved a durable PR. At the 2014 American Society of Clinical Oncology meeting, preliminary results including 14 patients with de novo MET amplification were reported. One of 6 patients with intermediate-level MET amplification had a PR, and 3 of 6 patients with high-level MET amplification had a PR. Responses were not seen in patients with low-level MET amplification.[18] Of note, the details of PFS and OS were not reported for the 14 patients. Our results showed a similar response rate in our patients compared to the data from the 80081001 study. In addition, we found that patients with high-level amplification were the most responsive to crizotinib treatment, while those with intermediate amplification were less responsive. A longer PFS was also observed in patients with high-level versus intermediate/low-level amplification (8.6 vs. 4.4 months, P = .008), respectively. To our knowledge, this is the first study with detailed information of crizotinib efficacy in patients with MET amplification.

For the rare frequency of de novo MET amplification, the survival data for this gene are lacking. The survival analysis in the present study revealed a shorter OS in patients who did not receive a MET inhibitor compared to those who did. No difference of OS was observed in patients without crizotinib treatment according to the MET amplification level. We could indicate from this results that the MET amplifications may not be a prognostic factor for survival but rather a predictor of responsiveness to treatment with a MET inhibitor. To our knowledge, our study is the first to compare survival for patients treated with MET-directed therapy compared to those who did not receive targeted therapy.

Figure 3 Overall Survival Comparison for Patients With and Without Crizotinib Treatment (31.0 vs. 13.7 Months, P = .001)



Our study has certain limitations that should be considered. Although the number of patients with de novo MET amplification was the largest to date, only 15 patients were treated with crizotinib. Hence, efficacy results must be viewed with caution, and additional efficacy data regarding MET inhibitors must be collected in future studies. In addition, more details of concurrent gene alterations, which may be meaningful for clinical treatment, was not investigated in the present study. Besides, scholars raised several new proposals to define a clear-cut high-level amplification category (ie, MET/CEN7 ratio 2.0 or average gene copy number 6.0 per tumor cell).[13] We will explore the feasibility for these new proposals in patients who received MET inhibitor.

In summary, our data showed that patients with MET amplification may benefit from MET inhibitor treatment, especially patients with high-level amplification. Due to the small number of patients treated with MET inhibitor in the current study, further research into treatment efficacy targeting MET amplification in lung cancers is greatly needed in this molecular subset of patients.

Clinical Practice Points

- De novo MET amplification represents an uncommon oncogenic event in patients with NSCLC.
- A trend toward a high prevalence of the solid predominant subtype of adenocarcinoma and of brain metastases in NSCLC patients with de novo MET amplification.
- Patients with de novo MET amplification benefit from crizotinib treatment, especially those with high-level amplification.

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Disclosure

The authors have stated that they have no conflict of interest.

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