ORIGINAL ARTICLE

The Unique Characteristics of *MET* Exon 14 Mutation in Chinese Patients with NSCLC

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ABSTRACT

Introduction: Predictive biomarkers of mesenchymal-to-epithelial transition factor (MET)-targeted therapy remain elusive. Since the discovery of the MNNG HOS Transforming gene (*MET*) exon 14 mutation, it has been found to have the best potential to become one precise biomarker for MET-targeted therapy. Here, we present the unique characteristics of *MET* exon 14 mutations in Chinese patients with NSCLC.

Methods: A total of 1296 patients with NSCLC were screened for *MET* exon 14 mutations. Next-generation sequencing was performed on the DNA of 968 patients and Sanger sequencing was conducted on complementary DNA of the other 328 patients. Immunohistochemical analysis and fluorescence in situ hybridization were also performed on all specimens.

Results: Twelve patients had *MET* exon 14 mutations. These accounted for only 0.9% of adenocarcinoma. Thus, the mutations were present at less than half the frequency of their occurrence in Western patients (0.9% versus 3% in Chinese and white patients, respectively, $\chi^2 = 15.1$, $p < 0.001$). Samples from six patients with *MET* exon 14 mutations were analyzed using immunohistochemical analysis and fluorescence in situ hybridization. We found no significant relationships among the mutation, *MET* amplification, and MET overexpression. In two patients who received crizotinib, only one patient (who exhibited *MET* amplification) experienced a partial response; the progression-free survival was 9 months. However, it remains unclear whether the sensitivity of this patient to crizotinib was conferred by the *MET* exon 14 mutation per se or by *MET* amplification. In the other patient with concomitant *MET* exon 14 skipping and *KRAS G12D* mutation, the disease progressed in only 1 month.

Conclusions: *MET* exon 14 mutation per se may not be sufficiently robust for use in defining a subset of NSCLCs. Further research on *MET* exon 14 mutations, *MET* amplification, and MET overexpression is required. Maybe a panel of biomarkers will be necessary in the future.

Keywords: NSCLC; *MET* exon 14; Clinical characteristics; Chinese; Biomarker

Introduction

The discovery of targetable genomic alterations has brought the treatment of NSCLC into the era of precision medicine. Since then a new term, *driver gene*, has entered the sights of clinical oncologists, and the novel associated concept has encouraged the development of more effective targeted therapies for certain subsets of patients with lung cancer. The median overall survival has been prolonged to 3.5 years in patients whose tumors harbor driver genes,[1] including *EGFR,*[2–4] anaplastic lymphoma receptor tyrosine kinase gene (*ALK*),[5–7] and *ROS1.*[8] Moreover, MNNG HOS Transforming gene (*MET*) has been identified as a novel promising target. It has been

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suggested that either a *MET*-activating mutation or genomic amplification of *MET* plays a role in lung cancer.[9,10]

However, unlike work with *EGFR* or *ALK*, clinical studies on *MET*-targeting cancer therapeutics have yielded mixed outcomes. The utility of *MET* as a lung cancer target once seemed in doubt. The newly defined cutoff point for *MET* amplification (*MET*-to–centromere 7 [CEP7] ratio $\geq$5) has begun to dispel this pessimism.[11] Last year the discovery of *MET* exon 14 mutations further focused attention on *MET*-targeting therapies.[12] In fact, *MET* exon 14 skipping was first recognized in patients with NSCLC more than 10 years ago.[13,14] Loss of exon 14 leads directly to deletion of the intracellular mesenchymal-to-epithelial transition factor (MET) juxtamembrane domain, which contains critical regulatory elements that promote MET protein degradation. These include tyrosine 1003, the binding site for Cbl (an E3 ubiquitin ligase).[15,16] As a result, *MET* exon 14 mutation prolongs MET signaling and increases the oncogenic potential.

In 2015, two important papers reported gratifyingly that almost 3% of patients with lung adenocarcinomas harbored an *MET* exon 14 mutation and responded effectively to MET inhibitors such as crizotinib.[17,18] Very recently, Awad et al. reported results consistent with the earlier findings. Furthermore, the clinical characteristics of patients harboring *MET* exon 14 mutations were described in detail.[19] Thus, *MET* exon 14 mutations have become a novel target for precision therapy for NSCLC.

However, East Asian patients with lung cancer have their own molecular characteristics that differ from those of whites, in particular in terms of *EGFR* and *KRAS* status.[20] Thus, to explore the clinical features of Chinese patients with the novel *MET* target, we sought *MET* mutations in patients at our institution and attempted to treat those with these mutations with crizotinib. We hoped that *MET* exon 14 mutation status would define a subset of patients with lung cancer for whom we could optimize genotype-directed therapies.

However just like the expression "life is just a rugged mountain road and you would never know what will be next around the corner," when we reviewed our screening data and attempted to summarize the clinical features of Chinese patients, we came to conclusions different from those of Western studies.

Materials and Methods

Study Population

Our study population was composed of patients treated between January 1, 2013, and November 1, 2015. Tumor biopsy specimens were obtained using a protocol approved by our institutional review board. All patients provided written informed consent.

Next-Generation Sequencing

We profiled DNA from all tissue samples using a capture-based targeted sequencing panel. Human genomic regions totaling 2.02 megabases in size, including selected exons and introns of 295 genes, were captured using 120–base pair (bp) probes. DNA was fragmented into segments 200 to 250 bp in length, captured by the 120-bp probes, and sequenced by obtaining paired 2$\times$150-bp reads. After DNA extraction (QIAamp DNA FFPE Tissue Kit [QIAgen, Hilden, Germany]), DNA concentrations were measured using the Qubit dsDNA assay (Invitrogen, Carlsbad, CA). The DNA quality was confirmed by checking that the A260/A280 ratio was 1.8:2.0. DNA was hybridized with the capture probes (the baits), selected using magnetic beads, and polymerase chain reaction (PCR)-amplified. Then a bioanalyzer (Qubit and Agilent 2100 [Agilent Technologies, Santa Clara, CA]) was used to perform high-sensitivity assays assessing DNA quality and size range. All samples were sequenced on a NextSeq 500 platform (Illumina, Inc., San Diego, CA); we obtained pair-end reads. For tissue samples, we aimed to achieve an average sequencing depth of 1000$\times$ for all targeted regions.

Sanger Sequencing

RNAs used in the Sanger sequence protocol were extracted from samples stored at −80°C using AllPrep DNA/RNA mini-kits (Qiagen). RNA was reverse-transcribed into complementary DNA using the PrimeScript reverse transcriptase PCR kit from Takara Bio (Kusatsu, Japan). The reverse transcription conditions were as follows: 37°C for 60 minutes and 75°C for 15 minutes. Next, the PCR products were screened for *MET* exon 14 mutations using an ABI 7500 platform (Applied Biosystems, Foster City, CA).

IHC Analysis for MET

In line with WHO and International Association for the Study of Lung Cancer guidelines, tumor histological features were classified by a board-certified pathologist with expertise in thoracic malignancies.

Immunohistochemical (IHC) analysis and fluorescence in situ hybridization (FISH) were performed on specimens from patients with *MET* exon 14 mutations. IHC was performed on formalin-fixed paraffin-embedded tissue sections 4 mm in thickness. An anti-MET primary antibody (rabbit monoclonal anti-human; catalog no. 8198 [Cell Signaling Technology, Danvers, MA]) was applied at a dilution of 1:300. MET was scored using the criteria defined in the MetMab Phase III trial in patients with advanced NSCLC (positive: $\geq$50% of tumor cells positive for membranous and/or cytoplasmic cellular MET immunostaining of moderate-to-strong intensity [i.e., staining grade $\geq$2+]).[21]

FISH to Detect MET

Deparaffinized sections 4 μm thick were subjected to dual-color FISH using a MET/CEN7q Dual Color FISH Probe (Vysis, Abbott Molecular, Des Plaines, IL). Tissue sections were immersed in tris(hydroxymethyl) aminomethane–ethylenediaminetetraacetic acid (pH = 8.0) and then washed in phosphate-buffered saline, digested with a protease solution at 37°C for 7 to 8 minutes, and washed in phosphate-buffered saline once more. After codenaturation of the sections and probe at 80°C for 5 minutes, the sections were hybridized at 37°C for 14 to 18 hours and subsequently counterstained with 4,6-diamino-2-phenylindole. The results were evaluated by reference to two criteria: a *MET*/CEP7 ratio of 2.0 or higher[22] and the criterion of Cappuzzi (positivity: a mean of ≥5 copies per cell or clustered gene amplification evident in all nuclei).[23]

Results

Patient Characteristics

MET exon 14 mutations were identified in 12 of 1296 patients with NSCLC (1.0%), including 1101 adenocarcinomas, 136 adenosquamous carcinomas, 20 squamous carcinomas, four lymphoma carcinomas, three sarcomatoid carcinomas, three metastatic adenocarcinomas, and 29 poorly differentiated NSCLCs. The clinical and pathological characteristics of all patients are listed in Table 1. Furthermore, Figure 1 shows the genetic profile of patients receiving next-generation sequencing (NGS). Of the 12 mutations detected, five were detected by Sanger sequencing.

Genomic deletions were evident in seven of the 12 patients (58.3%), and point mutations were found in the other five patients. Of the seven deletions, five were precise *MET* exon 14 deletions, and two occurred partly within exon 14. All five point mutations disrupted the splice donor site of intron 14.

The median age of the 10 patients with stage IV adenocarcinoma was 59 years (range, 45–77 years); five (41.7%) were women and six (50%) were never-smokers. At the time of cancer diagnosis, two patients (16.7%) with *MET* exon 14 mutations had stage I NSCLC and the other 10 (83.3%) had stage IV disease. In terms of histological features, 10 patients (83.3%) had adenocarcinomas, one (8.3%) had an adenosquamous carcinoma, and one (8.3%) had a squamous carcinoma.

IHC Analysis and FISH

Of the 12 patients with *MET* exon 14 mutations, six yielded sufficient tissue for performance of IHC and FISH. The results are shown in Figure 2. Three patients with stage IV adenocarcinomas exhibited strong MET expression (≥50% of tumor cells were positive in terms of membranous or cytoplasmic staining of moderate to strong intensity [grade ≥ 2+]). Genomic copy number analysis showed that only one patient exhibited a concurrent high-level *MET* copy gain (*MET* gene copy number per tumor nucleus >5). It is a pity to say that our small sample size rendered it difficult to ascertain relationships among the three variants.

Table 1. Clinical Characteristics of Patients Screened for *MET* Exon 14 Mutation (N = 1296)

Variable	Value
Median age (range), y	60 (19-98)
Sex	
Male	770 (59.4%)
Female	526 (40.6%)
Smoking status	
Smoker	325 (25.1%)
Nonsmoker	368 (28.4%)
Unknown	603 (46.5%)
Histological diagnosis	
Adenocarcinoma	1101 (85.0%)
Squamous carcinoma	136 (10.5%)
Adenosquamous carcinoma	20 (1.5%)
Sarcomatoid carcinoma	3 (0.2%)
Lymphoma carcinoma	4 (0.3%)
Metastatic adenocarcinoma	3 (0.2%)
NSCLC, poorly differentiated	29 (2.2)
TNM stage	
I	65 (5.0%)
II	30 (2.3%)
III	124 (9.6%)
IV	953 (73.5%)
Unknown	124 (9.6%)
Previous treatment	
No treatment	463 (35.7%)
Targeting treatment	163 (12.6%)
Chemotherapy	308 (23.8%)
Targeting and chemotherapy	305 (23.5%)
Surgery	57 (4.4%)

Note: Values are presented as n(%) unless otherwise indicated.
MET, MNNG HOS Transforming gene.

Variation in the Responses to Crizotinib

Two patients received targeted crizotinib treatment. One experienced a partial response; the PFS was 9 months. In the other, the disease progressed in only 1 month.

Case 1. Stage IV NSCLC (a moderately differentiated adenocarcinoma) was diagnosed in 45-year-old male heavy smoker. Initial molecular testing revealed no *EGFR, BRAF,* or phosphatase and tensin homolog gene (*PTEN*) mutation or any rearrangement in *ALK* or *ROS1*. But *KRAS G12D* mutation was confirmed. After two-line chemotherapy, the disease eventually progressed. An *MET* exon 14 splice site mutation (c.2888_3028del) was identified by Sanger sequencing (Fig. 3*A*); it was associated with

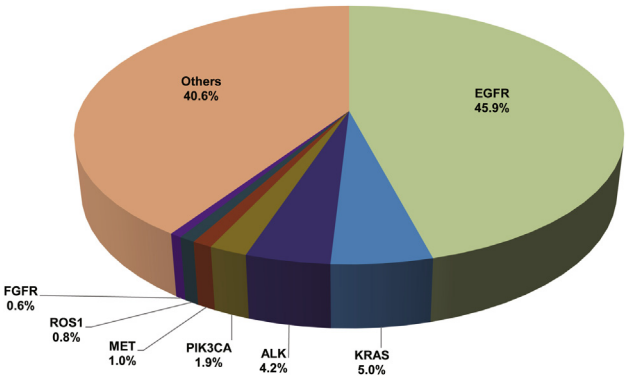

Figure 1. Distribution of genotypes among 968 patients receiving next-generation sequencing. *FGFR*, fibroblast growth factor receptor gene; *MET*, MNNG HOS Transforming gene; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha gene; *ALK*, anaplastic lymphoma receptor tyrosine kinase gene.

moderate MET overexpression (100%++) (Fig. 3*B*) but no *MET* amplification (*MET*/CEP7 ratio = 1.0) (Fig. 3*C*). We prescribed crizotinib 250, mg orally twice daily. Unfortunately, progressive disease developed and the PFS was only 1 month (Figs. 3*D* and *E*).

Case 2. The patient was a 76-year-old male never-smoker in whom stage IV lung adenocarcinoma was diagnosed. Imaging revealed widespread metastatic disease in the lung and the T2 thoracic vertebra. NGS revealed no activating mutation in *KRAS*, *EGFR*, or *BRAF*, and no genomic rearrangement in *ALK* or *ROS1*.

However a c.2888_2919 deletion, TCTTTCTCTCTG TTTTAA, was identified in *MET* exon 14 in 38.4% of 333 reads (Fig. 4*A*) in association with moderate MET overexpression (80%++) (Fig. 4*B*) and *MET* amplification (mean copy number >5.0 per tumor cell [Fig. 4*C*]). The patient received crizotinib, 250 mg orally twice daily; repeat imaging 4 weeks later showed dramatic improvements in multiple lesions throughout the body (Figs. 4*D* and *E*). The response persisted for 9 months.

Discussion

Detection of variation in *MET* exon 14 has become a milestone on the road of searching for MET-relevant predictive biomarkers. Such variation may serve as a precise biomarker of MET-targeted therapy such as *MET* amplification. Most previous studies have been performed on white patients; data on changes in *MET* exon 14 in Asians are rare. Here, we describe the largest, to the best of our knowledge, single-institution study to figure out the incidence of *MET* exon 14 mutations in and the clinical features of Chinese patients with lung cancer with such mutations. When we retrospectively analyzed our data, several interesting findings deserving further consideration and exploration emerged.

First, we found that *MET* exon 14 skipping occurred in only 0.9% of lung adenocarcinomas, which is less than half the frequency previously observed (0.9% versus 3% in Chinese and white patients, respectively, $\chi^2 = 15.1$, $p < 0.001$).[17] For this situation, we consider

ID	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
Mutation	Exon 14 del	c.2888_2919del	Exon 14 del	Exon 14 del	Exon 14 del	Exon 14 del	c.G3082A	c.G3082C	c.G3082A	c.2888_2888-13del	c.T3082+2C	c.G3082+1C
IHC	100%++	80%++	70%+++ 30%++	Negative	Negative	Negative	NA	NA	NA	NA	NA	NA
FISH	Negative (ratio1:1)	copies>5	Negative (ratio1:1)	Negative (ratio1:1)	Negative (ratio1:1)	Negative (ratio1:1)	NA	NA	NA	NA	NA	NA
Crizotinib ORR/PFS	PD 1m	PR 9m	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Gender	M	M	F	M	F	M	M	F	F	F	M	M
Age	45	76	50	58	41	49	77	65	45	60	66	68
Histology	ADC	ADC	ADC	ADC	ADC	ADC	ADC	ADC	ADC	ADC	SCC	ASC
Stage	IV	IV	IV	IV	I	I	IV	IV	IV	IV	IV	IV
Previous treatment	Chemo therapy	Chemo therapy	Chemo therapy	No treatment	Surgery	Chemo therapy	Chemo therapy +erlotinib	No treatment	Radio therapy	Chemo therapy	Chemo therapy	Surgery +Chemo therapy

Figure 2. The immunohistochemical (IHC) and fluorescence in situ hybridization (FISH) results for patients with MNNG HOS Transforming gene (*MET*) exon 14 mutation. del, deletion; NA, not applicable; ORR, objective response rate; PFS, progression-free survival; ADC, adenocarcinoma; SCC, squamous cell carcinoma; ASC, adenosquamous carcinoma.

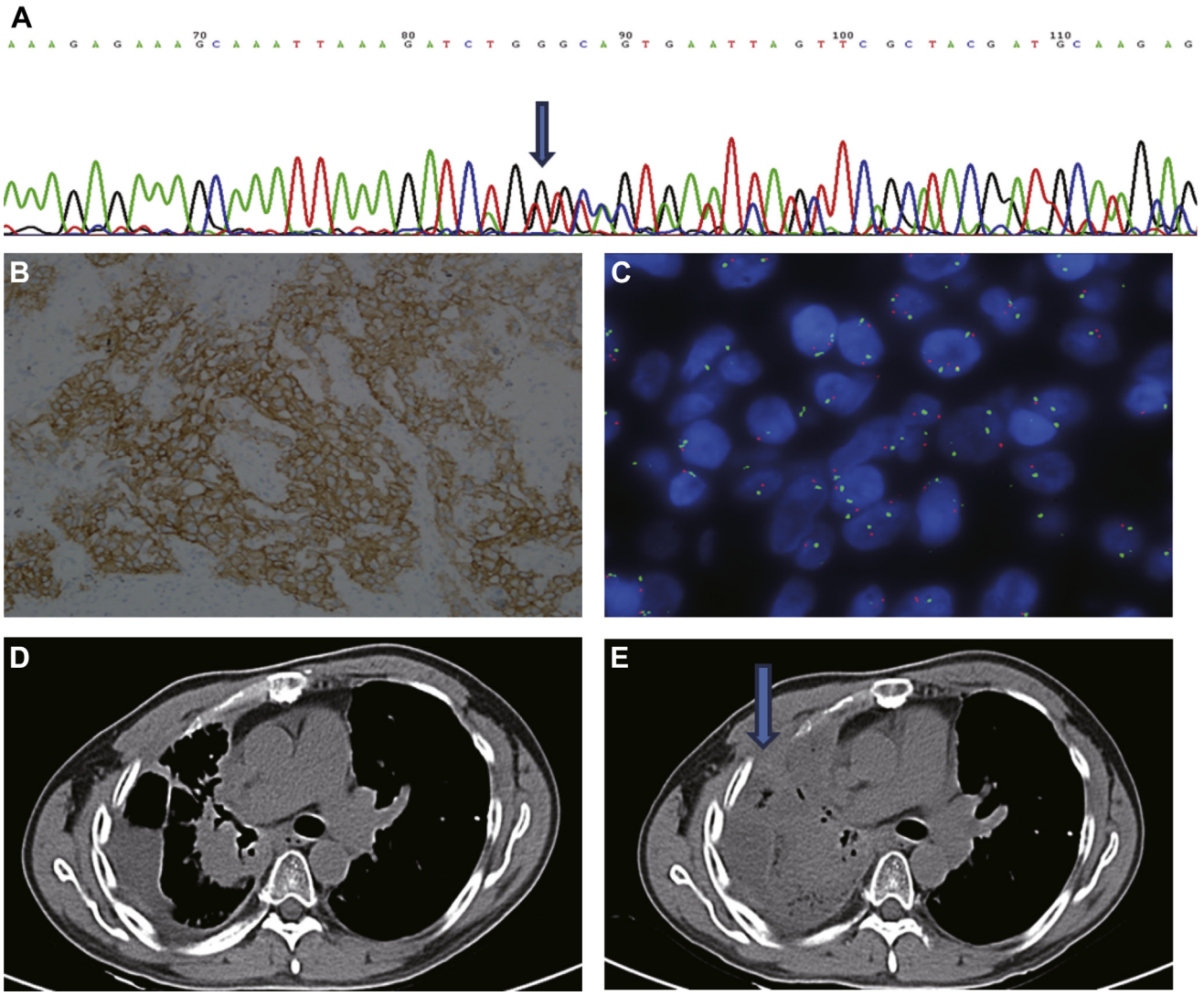

Figure 3. Response to crizotinib in a patient with MNNG HOS Transforming gene (*MET*) exon 14 mutation and concurrent *KRAS G12D* mutation. A 45-year-old female heavy smoker with a moderately differentiated stage IV adenocarcinoma was found by Sanger sequencing to have (*A*) a *MET* exon 14 c.2888_3028 deletion (*arrow*), (*B*) moderate mesenchymal-to-transition factor (MET) overexpression (100%++), but no *MET* amplification (the *MET*-to-centromere 7 ratio was 1.0) (*C*). A chest computed tomography scan (*axial view*) is shown before (*D*) and after (*E*) treatment with the cellular mesenchymal-to-epithelial transition factor inhibitor crizotinib (*arrow* indicates increased tumor in one month).

that ethnic differences could explain this variation. The molecular profiles of Chinese patients with lung cancer are different from those of white patients. For example, the frequency of *EGFR*-mutated NSCLCs is 28.2% in Chinese populations but less than 10% among whites.[24] Hence, we believe that the incidence of *MET* exon 14 mutation in lung adenocarcinomas in Chinese individuals may be not as high as that in the West. In terms of clinical features, *MET* exon 14 mutations occur at a young median age, 59 years in Chinese patients with stage IV adenocarcinoma, which is similar to the median age of patients with *ALK* and *ROS1* rearrangements.[25,26] In terms of detection method, the previous *MET* exon 14 mutations have been detected by NGS. We detected mutations in five patients using Sanger sequencing. Thus, we believe that Sanger sequencing could also effectively detect *MET* exon 14 mutations.

Moreover, it seems that patients with *MET* exon 14 mutations did not respond to targeted treatment as effectively as previously reported in the literature.[9,18,27–29] In one patient with concomitant *MET* exon 14 skipping and *KRAS G12D* mutation, disease progression was evident after only 1 month. In most cases, mutations are assumed to mutually exclude each other.[30] Here in our study, we described *MET* exon 14 skipping combined with *KRAS G12D* mutation for the first time. Maybe for this patient *KRAS G12D* mutation was the oncogenic driver, not *MET* exon 14 skipping. Although the

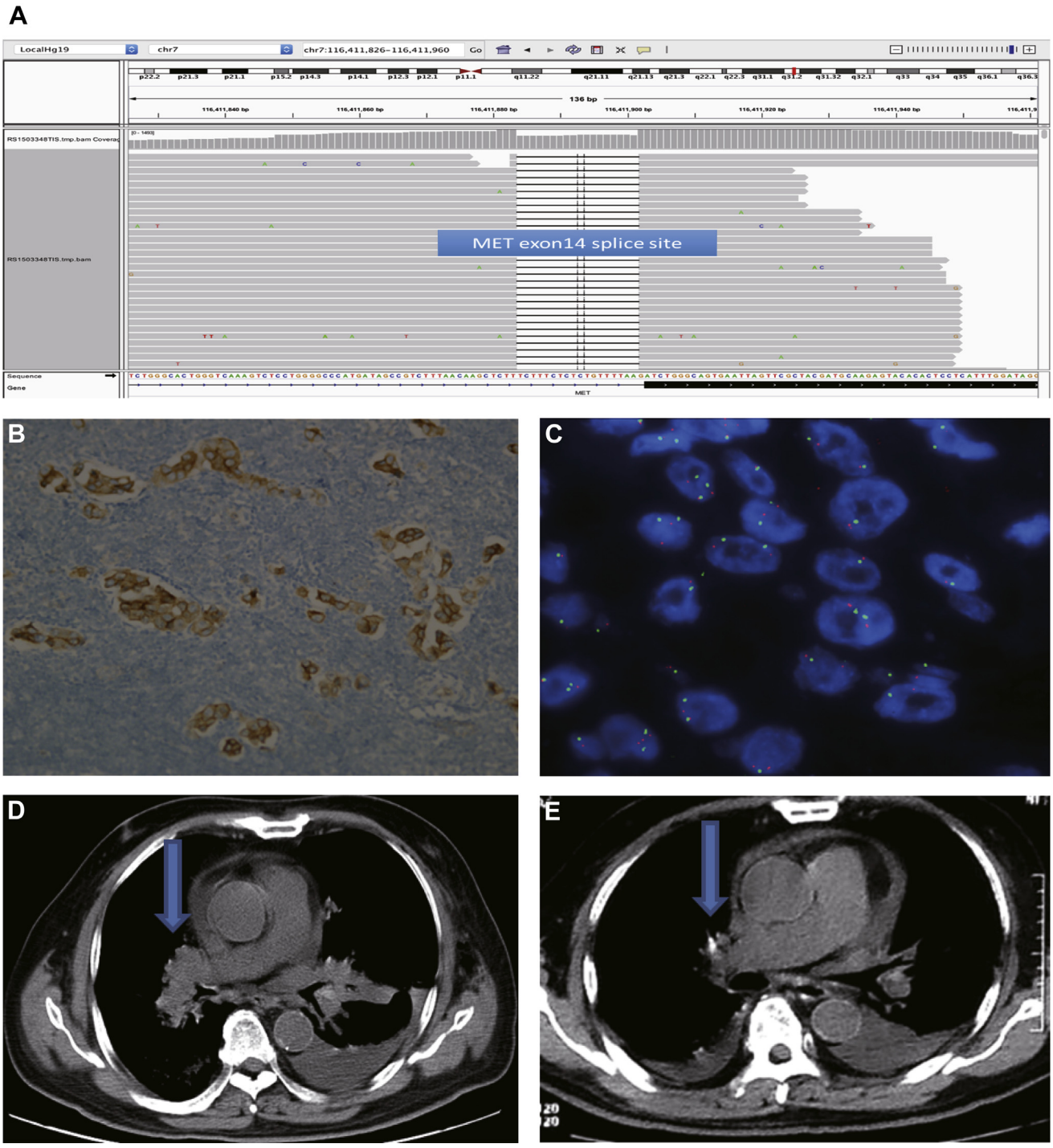

Figure 4. Response to crizotinib in a patient with concomitant MNNG HOS Transforming gene (*MET*) exon 14 mutation and *MET* amplification. Stage IV lung adenocarcinoma was diagnosed in a 76-year-old male never-smoker. A c.2888_2919 deletion, TCTTTCTCTCTGTTTTAA, was identified by next-generation sequencing in *MET* exon 14 in 38.4% of 333 reads (*A*) in association with moderate mesenchymal-to-epithelial transition factor overexpression (80%++) (*B*) and *MET* amplification (mean copy number >5.0 per tumor cell *(C)*. A chest computed tomography scan (*axial view*) is shown before (*arrow* indicates primary tumor before treatment) (*D*) and 4 weeks after (*E*) treatment with the mesenchymal-to-epithelial transition factor inhibitor crizotinib (*arrow* indicates decreased tumor after treatment).

other patient enjoyed a partial response, he exhibited concurrent de novo *MET* amplification. Camidge et al. found that patients with de novo *MET* amplifications exhibited significantly improved responses to crizotinib.[10] Thus, it remains unclear whether the crizotinib sensitivity of our second patient was conferred by an *MET* exon 14 mutation or *MET* amplification. Further studies are necessary to determine whether *MET* exon 14

mutation per se induces responsiveness to cellular MET inhibitors.

Although targeted therapy has increased the median overall survival of patients with lung cancer with driver genes to 3.5 years, only 47.9% of Chinese patients can benefit from driver gene–focused treatment. The remaining patients with advanced lung cancer continue to be treated with chemotherapy or radiotherapy. Thus, further novel targets based on definitive biomarkers are urgently required.[24] It was most encouraging when *MET* was identified as a promising target. However, more definitive biomarkers are required to optimize targeted therapy.

In the way of biomarker exploration, detection of *MET* exon 14 mutations by sequencing, screening for *MET* amplification by FISH, and quantification of MET overexpression by IHC analysis have all been used in work on MET-targeted therapy. In addition, the *MET* exon 14 mutation has been shown to be associated with both *MET* amplification and MET overexpression.[19,31] Unfortunately, we found no significant correlation among these three parameters. As our sample size was small, this conclusion must be cautiously interpreted. Apart from the low incidence of mutation and the inconsistent responses to crizotinib treatment, we suggest that the *MET* exon 14 mutation may not be precise enough biomarker to define a subset for Chinese patients with NSCLC. In terms of *MET* amplification and overexpression, 33% and 15% of patients have been reported to exhibit partial responses to crizotinib[10] and INC280, respectively.[32] We noted that both *MET* amplification and overexpression partly predicted patient responses, but the most precise biomarker remains elusive. However, we suggest that there is still a long way to go before the *MET* exon 14 mutation per se becomes a precise enough biomarker for Chinese patients. The three potential biomarkers need to be further researched in the future.

There were certain limitations to our study. Although the number of patients initially enrolled was large, regrettably, only six patients exhibited one of the three *MET* variants and only a few patients received crizotinib. Hence, our results must be assessed with caution. We continue to seek *MET* exon 14 mutations in our patients and we plan to present more data in the future.

In conclusion, we suggest that *MET* exon 14 mutation per se may not be sufficiently robust for use in defining a subset of NSCLCs in Chinese patients. Further research on *MET* exon 14 mutations, *MET* amplification, and MET overexpression is required. In addition, the effects of racial differences on experimental data cannot be overlooked. Ethnic variation must be considered when future clinical studies are planned.

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