ORIGINAL ARTICLE

MET Amplification and Efficacy of Nivolumab in Patients With NSCLC

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ABSTRACT

Introduction: *MET* amplification is an important genetic alteration in NSCLC. Unlike in patients with *EGFR* and *ALK* alterations, the efficacy of immune checkpoint inhibitors in patients with *MET*-amplified NSCLC remains unknown.

Methods: An exploratory analysis of a prospective, multi-institutional cohort comprising 200 patients with advanced or recurrent NSCLC treated with nivolumab monotherapy was performed, and *MET* amplification was defined as a *MET*-to-CEP7 ratio of greater than or equal to 2 using fluorescent in situ hybridization. High-level and low-level *MET* gains were also defined as *MET* signals $\geq$10/nuclei and 10> *MET* signals $\geq$5/nuclei, respectively. Overall response rates (ORRs) and survival outcomes were evaluated on the basis of the *MET* gene copy number status.

Results: Among 175 patients eligible for analysis, *MET* amplification was detected in 13 tumors (7.4%). Four (2.3%) high-level and 14 (8.0%) low-level *MET* gains were also detected. There were no considerable differences in ORRs in accordance with the *MET* gene copy number status. Similarly, no significant differences in both progression-free survival (PFS) and overall survival (OS) were observed between patients with and without *MET*-amplified NSCLC (log-rank, $p = 0.813$ for PFS, and $p = 0.855$ for OS). Among 101 adenocarcinomas, ORRs in patients with high-level and low-level *MET* gains (50.0% for both, $p = 0.049$) were significantly higher than those without *MET* gains (17.6%), yet survival outcomes for both PFS and OS did not improve.

Conclusions: *MET* amplification was not associated with greater benefit of nivolumab treatment in patients with NSCLC. Further studies are warranted to prioritize immune checkpoint inhibitors in the treatment regimen for patients with *MET* amplification.

Keywords: Lung cancer; MET; Nivolumab; Amplification; Immune checkpoint inhibitor

Introduction

MET amplification, in particular high-level amplification, is considered an important oncogenic alteration found in approximately 1% to 5% of treatment-naive patients with NSCLC.[1–3] Another *MET* gene alteration, a

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MET exon 14 (*MET*ex14) skipping mutation, is established as a driver and definitive druggable target. As a result of pivotal clinical trials revealing considerable efficacy of MET inhibitors in patients with *MET*ex14, targeted drugs for this type of lung tumors have been approved.[3,4] In contrast, it remains a matter of debate whether *MET* amplification is a true actionable driver of NSCLC and whether *MET* amplification is a definitive target of *MET*-directed therapies,[1,5] because *MET* amplification is more genetically heterogeneous than *MET*ex14, with a wide range of *MET* gene copy numbers and frequently observed comutations.[6,7] Accordingly, clinical trials have exhibited the inconsistent efficacy of MET inhibitors against *MET*-amplified tumors.[2,8]

Along with targeted therapies, programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) inhibitors have transformed the treatment landscape of cancer and have substantially improved outcomes of a subset of patients with NSCLC. Nevertheless, the efficacy of immunotherapy using these agents is not universally high, and robust predictive biomarkers are still needed to select patients who are likely to benefit from this line of treatment. The relationship between the efficacy of immunotherapy and tumor-specific genotypes represented by driver oncogenic alterations has been a major research focus. For example, patients with *EGFR* mutations or *ALK* fusions have benefited less from PD-1/PD-L1 inhibitors compared with their wild-type (WT) counterparts,[9–11] whereas *KRAS*-mutant tumors are more frequently responsive to immunotherapy, particularly when *TP53* is comutated.[12,13] Nevertheless, the efficacy of PD-1/PD-L1 inhibitors is yet to be determined for tumors with other less common drivers, such as *BRAF*, *ROS1*, *RET*, and *HER2* gene alterations. For *MET*-amplified NSCLC, there is very little evidence regarding the efficacy of PD-1/PD-L1 inhibitors compared with that of MET-directed therapies. Currently available data on the basis of registry analysis reveal that the response in patients with *MET* gene alterations (N = 36) including both *MET* amplification (n = 13) and *MET*ex14 (n = 23) is comparable with that of patients with *KRAS* mutation, albeit this study does not provide *MET* amplification-specific data.[14] Another recent cohort study suggested that patients with *MET* amplification potentially derive greater survival benefit from immune checkpoint inhibitors (ICIs) than standard chemotherapy,7 the rationale of which is supported by our previous study revealing *MET* gene copy numbers correlate with PD-L1 expression and the amount of tumor-infiltrating lymphocytes (TILs).[15] Although these findings seem promising for the use of PD-1/PD-L1 inhibitors in patients with *MET*-amplified NSCLC, the relationship between ICI efficacy and *MET* amplification is yet to be evaluated in a prospectively designed cohort. Further studies are therefore urgently required to address this unmet clinical need.

In addition to *MET* amplification and *MET*ex14, MET protein expression has been analyzed in several clinical trials for MET inhibitors. Nevertheless, MET immunohistochemical assessment has not been regarded as a predictive marker to date.[16,17] Furthermore, a recent prospective study revealed that MET expression is associated with response to ICIs.18 Thus, the significance of MET expression in patients receiving ICI therapy should be externally evaluated with the knowledge of *MET* gene alterations.

Here, we evaluated the association of *MET* amplification, gene copy number gains, and MET expression with efficacy of nivolumab monotherapy in patients with advanced NSCLC, using specimens and data collected in our previous prospective cohort study.[19]

Materials and Methods

Study Population

This study is an exploratory post hoc analysis of our previous multicenter cohort study that prospectively enrolled 200 patients with advanced NSCLC treated with a PD-1 inhibitor, nivolumab, between July 2016 and December 2018.[19] Our previous study evaluated the efficacy of nivolumab monotherapy according to *PD-L1* gene copy number status, as determined by fluorescence in situ hybridization (FISH). The present study evaluated the *MET* gene copy number status using the same specimens used in our previous study. Data analysis was conducted from February 2020 to April 2020. The study protocol was approved by the institutional ethical committee of Hamamatsu University School of Medicine (#18-280) in addition to each participating institution. All participants provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki and followed the Strengthening the Reporting of Observational Studies in Epidemiology reporting guidelines. The study was registered in the University Hospital Medical Information Network in Japan (UMIN000022505).

Eligibility criteria of patients included those who had clinical stage III, IV, or recurrent NSCLC after surgical resection; the details of these criteria are described in our previous study.[19] They should be more than or equal to 18 years of age, have an Eastern Cooperative Oncology Group performance status of 0 to 2, and have both the appropriate quality and quantity of formalin-fixed, paraffin-embedded specimens for biomarker analysis. The main exclusion criteria included patients with interstitial lung diseases, uncontrolled brain metastases, autoimmune diseases, or other severe complications. The enrolled

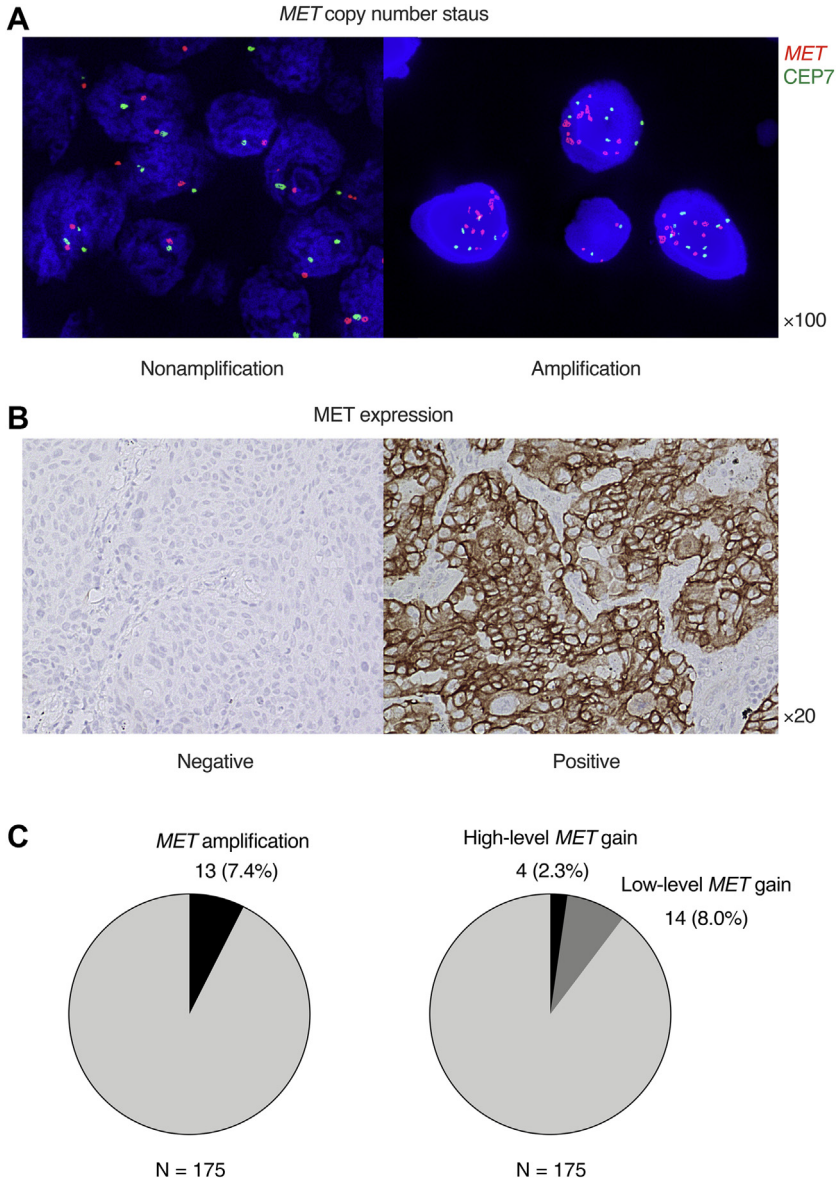

Figure 1. Representative images of MET alteration status. (*A*) Representative images of *MET* gene status, such as non-amplification and amplification with fluorescent in situ hybridization (magnification, ×100), revealing *MET* probes (red), chromosome 7 centromere probes (green), and nuclei (blue). (*B*) Representative images of negative and positive MET expression (magnification, ×20). (*C*) Proportion of *MET* amplification and aberrant *MET* gain categories in the entire cohort.

patients were treated with nivolumab with a dosage of 3 mg/kg of body weight biweekly until disease progression, occurrence of unacceptable toxicity, or withdrawal of consent. The clinical evaluation for treatment response was performed every four cycles. The efficacy of nivolumab was evaluated using Response Evaluation Criteria Solid Tumors, version 1.1. Overall response rate (ORR) was calculated as the percentage of patients who achieved either complete or partial response. Disease control rate was defined as the total percentage of patients achieving complete response, partial response, and stable disease.

Biomarker Evaluation

MET gene copy numbers were evaluated by FISH using previously described protocols.[15,20] Fluorescent probes for the *MET* gene locus on chr7q31.2 (bacterial artificial chromosome clone RP11-153D24; Advanced GenoTechs, Tsukuba, Japan) and for the centromere, a reference locus of *MET* (alpha satellite) region of chromosome 7 (chr7q31.2; bacterial artificial chromosome clone RP11-435D24; Advanced GenoTechs), were used. Probe signals were detected and calculated in at least 50 nuclei in more than three areas, at a magnification of ×100 (Fig. 1*A*). Then, the ratio of the mean targeted

signal to the mean centromere enumeration probe (CEP) 7 signal (*MET*/CEP7) was determined. *MET* amplification was primarily defined as having an average *MET*-to-CEP7 ratio of greater than or equal to 2.0 per nucleus.[15,21,22] In accordance with previous publications, we have also applied the secondary definition for *MET* copy number status, which categorizes *MET* gene status into the following three groups by average *MET* copy numbers/nucleus[23,24]: high-level *MET* gain (*MET* signals $\geq$10), low-level *MET* gain (10> *MET* signals $\geq$5), and no *MET* gain (5> *MET* signals). MET and PD-L1 protein expression were immunohistochemically evaluated as per the staining protocol previously described.[15] Antibodies that target MET (1:200 dilution, SP44, Abcam, Cambridge, MA), PD-L1 (1:100 dilution, E1L3N, Cell Signaling Technology, Danvers, MA), and CD8 (1:200 dilution, C8/144B, Nichirei, Tokyo, Japan) were applied. The degree of MET protein expression in tumor cells was evaluated using an H-score method. The H-score was calculated by multiplying the percentage of stained tumor area (0%–100%) by the staining intensity scored from 0 to 3, with a range from 0 to 300 (Fig. 1*B*). PD-L1 expression was evaluated using the tumor proportion score[25] (TPS) (Supplementary Fig. 1*A*). MET overexpression was defined as greater than 50% of cells stained with an intensity of 2+ or more.[15,16,26] PD-L1 positivity was defined as TPS greater than or equal to 1% as indicated in the previous reports.[25,27,28] CD8-positive TILs (CD8+TILs) were counted as the average numbers in the both epithelial and surrounding stroma components under high-power fields ($\times$20)[15,29] (Supplementary Fig. 1*B*). *EGFR* mutations and *ALK* rearrangements were confirmed using clinically approved tests.

Statistical Analysis

Progression-free survival (PFS) was defined as the time from nivolumab injection to the date of progression or death from any cause. Overall survival (OS) was defined as the time from nivolumab administration to the date of death from any cause. Fisher's exact test was used to analyze categorical variables, and the Mann-Whitney *U* test was used for continuous variables. Survival time was estimated using the Kaplan-Meier method, and the estimates of all the study subjects were compared using log-rank tests. Univariate models with Cox proportional hazards regression models were used to calculate hazard ratios (HRs). Statistical analyses were conducted using the R software (version 3.2.0, The R Foundation for Statistical Computing, Vienna, Austria) and GraphPad version 8.01 (GraphPad Software Inc, San Diego, CA). Results were considered statistically significant at p values less than 0.05.

Results

Patient Characteristics

Among 200 patients enrolled, 25 were not eligible owing to insufficient materials (18 cases [9.0%]) or poor quality of tissue specimens for FISH or immunohistochemistry (IHC) analyses (seven cases [3.5%]). The resulting 175 eligible patients had 101 (57.7%) adenocarcinomas, 62 (35.4%) squamous cell carcinomas, and 12 (6.9%) tumors with other histologic features. The details of the study participants are found in Table 1. The median age was 69 years (range: 43–83 y), with most of the patients being male (n = 141, 80.6%). In addition, 100 patients (57.1%) had an Eastern Cooperative Oncology Group performance status of 0 and 30 patients (17.1%) had no smoking history. There were 33 (18.9%) stage III, 123 (70.3%) stage IV, and eight (10.8%) postoperative recurrent cases. Nivolumab was administered as the second-line (n = 81, 46.3%) and third-line (n = 52, 29.7%) treatments in most patients. *EGFR* mutations were found in 15 patients (10.5%). No patient had received previous immunotherapy or MET inhibitors. MET inhibitors were not administered as a poststudy treatment during the observational period of this study. EGFR tyrosine kinase inhibitors were administered previously in 20 patients. The median (interquartile range) observation period was 12.7 (5.3–20.4) months. At data lock in February 2020, a total of 112 patients (64.0%) had died and 15 patients (8.6%) were still continuing nivolumab treatment. Nivolumab was discontinued owing to progression of the disease in 118 patients (67.4%) and adverse effects in 35 patients (20.0%).

MET Gene Copy Number Status

The median (range) *MET* gene copy number was 2.84 (1.83–12.67), and the median (range) *MET*-to-CEP7 ratio was 1.14 (0.38–4.22; Supplementary Fig. 2*A*–*B*). There was a positive correlation between *MET* copy numbers and *MET*-to-CEP7 ratios ($\rho = 0.793$, $p < 0.001$; Supplementary Fig. 2*C*). Furthermore, 13 tumors (7.4%) harbored *MET* amplification (Fig. 1*C* and Supplementary Table 1). Regarding the secondary definition for *MET* copy number status, high-level and low-level *MET* gains were identified in four (2.3%) and 14 patients (8.0%), respectively (Fig. 1*C*). There were no significant differences in patient and tumor characteristics according to *MET* gene copy number status (Table 1). High MET expression by IHC analysis was observed in 45 (25.7%) of the tumors. There was a significant association between *MET* gene copy number and MET expression (Supplementary Fig. 3*A*–*B*). The TPS of PD-L1 expression ranged from 0% to 92% with

Table 1. Patient Characteristics in Patients With NSCLC

		MET Copy Number Status			*MET* Gene Signals (/Nucleus)			
	Total	Non-Amp	Amp		No Gain (*MET* <5)	Low Gain (5≤ *MET* <10)	High Gain (10≤ *MET*)	
Characteristics	N = 175	n =162 (92.6)	n = 13 (7.4)	*p* Value	n = 157 (89.7)	n = 14 (8.0)	n = 4 (2.3)	*p* Value
Age, y	69 (43–83)	69 (43–83)	68 (45–74)	0.131	69 (43–83)	68.5 (51–82)	70.5 (45–73)	0.949
Sex								
Male	141 (80.6)	130 (80.2)	11 (84.6)	1.000	127 (80.9)	10 (71.4)	4 (100.0)	0.568
Female	34 (19.4)	32 (19.8)	2 (15.4)		30 (19.1)	4 (28.6)	0 (0)	
Smoking status								
Ever	145 (82.9)	134 (82.7)	11 (84.6)	1.000	130 (82.8)	11 (78.6)	4 (100.0)	0.866
Never	30 (17.1)	28 (17.3)	2 (15.4)		27 (17.2)	3 (21.4)	0 (0)	
ECOG PS								
0	100 (57.1)	94 (58.0)	6 (46.2)	0.437	93 (59.2)	6 (42.9)	1 (25.0)	0.337
1	67 (38.3)	61 (37.7)	6 (46.2)		57 (36.3)	7 (50.0)	3 (75.0)	
2	8 (4.6)	7 (4.3)	1 (7.7)		7 (4.5)	1 (7.1)	0 (0)	
Stage								
III	33 (18.9)	31 (19.1)	2 (15.4)	0.479	30 (19.1)	2 (14.3)	1 (25.0)	0.674
IV	123 (70.3)	112 (69.1)	11 (84.6)		108 (68.8)	12 (85.7)	3 (75.0)	
Postoperative recurrent	19 (10.8)	19 (11.7)	0 (0)		19 (12.1)	0 (0)	0 (0)	
Histologic type								
Adenocarcinoma	101 (57.7)	92 (56.8)	9 (69.2)	0.410	91 (58.0)	8 (57.1)	2 (50.0)	0.453
Squamous cell carcinoma	62 (35.4)	59 (36.4)	3 (23.1)		55 (35.0)	6 (42.9)	1 (25.0)	
Others[a]	7 (4.0)	7 (4.3)	0 (0)		7 (4.5)	0 (0)	0 (0)	
NOS	5 (2.9)	4 (2.5)	1 (7.7)		4 (2.5)	0 (0)	1 25.0)	
Number of previous systemic regimens								
1	81 (46.3)	74 (45.7)	7 (53.8)	0.565	71 (45.2)	8 (57.1)	2 (50.0)	0.203
2	52 (29.7)	50 (30.9)	2 (15.4)		49 (31.2)	1 (7.1)	2 (50.0)	
≤3	42 (24.0)	38 (23.5)	4 (30.8)		37 (23.6)	5 (35.7)	0 (0)	
Previous platinum-based chemotherapy	165 (94.3)	152 (93.8)	13 (100.0)	1.000	148 (94.3)	13 (92.9)	4 (100.0)	0.672
Palliative radiotherapy[b]	14 (8.0)	12 (7.4)	2 (15.4)	0.279	11 (7.0)	3 (21.4)	0 (0)	0.172
Tissue collection method								
Surgical resection	30 (17.1)	29 (17.9)	1 (7.7)	0.878	29 (18.5)	0 (0)	1 (25.0)	0.395
TBB	100 (57.1)	90 (55.6)	10 (76.9)		88 (56.1)	10 (71.4)	2 (50.0)	
EBUS-TBNA	25 (14.3)	23 (14.2)	2 (15.4)		22 (14.0)	3 (21.4)	0 (0)	
Thoracoscopic pleural biopsy	9 (5.1)	9 (5.6)	0 (0)		7 (4.4)	1 (7.2)	1 (25.0)	
CT-guided lung biopsy	6 (3.4)	6 (3.6)	0 (0)		6 (3.8)	0 (0)	0 (0)	
Others[c]	5 (2.8)	5 (3.1)	0 (0)		5 (3.2)	0 (0)	0 (0)	
EGFR mutation status								
Mutant	15 (8.6)	15 (9.3)	0 (0)	0.378	13 (8.3)	2 (14.3)	0 (0)	0.491
Wild	128 (73.1)	116 (71.6)	12 (92.3)		116 (73.9)	8 (57.1)	4 (100.0)	
Unknown	32 (18.3)	31 (19.1)	1 (7.7)		28 (17.8)	4 (28.6)	0 (0)	
ALK translocation status								

(continued)

Table 1. Continued

Characteristics	Total	*MET* Copy Number Status			*MET* Gene Signals (/Nucleus)				
		Non-Amp	Amp		No Gain (*MET* <5)	Low Gain (5≤ *MET* <10)	High Gain (10≤ *MET*)		
	N = 175	n =162 (92.6)	n = 13 (7.4)	*p* Value	n = 157 (89.7)	n = 14 (8.0)	n = 4 (2.3)		*p* Value
Positive	1 (0.6)	1 (0.6)	0 (0)	0.248	1 (0.6)	0 (0)	0 (0)		0.472
Negative	129 (73.7)	117 (72.2)	12 (92.3)		116 (73.9)	9 (64.3)	4 (100.0)		
Unknown	45 (25.7)	44 (27.2)	1 (7.7)		40 (25.5)	5 (35.7)	0 (0)		
The number of nivolumab cycles	4 (1–92)	4 (1–92)	4 (1–50)	0.462	4 (1–92)	5 (1–52)	2 (1–5)		0.126

Note: Variables are presented as n (%) or variable (range).
[a]Other histologic types include one adenosquamous cell carcinoma, one large cell carcinoma, and four large cell neuroendocrine carcinomas.
[b]They were concurrently performed during nivolumab therapy.
[c]Other methods include two ultrasonography-guided lymph node biopsies, two liver biopsies, and one muscle biopsy.
Amp, amplification; ECOG PS, Eastern Cooperative Oncology Group performance status; NOS, not otherwise specified; TBB, transbronchial biopsy; EBUS-TBNA, endobronchial ultrasonography-guided transbronchial needle aspiration; CT, computed tomography.

a positive rate of 33.1% (58 of 175), which was not significantly associated with *MET* gene signals (Supplementary Fig. 3*C*). To characterize the tumor immune microenvironment on the basis of MET status, we evaluated the number of CD8+TILs and found no significant difference in their abundance according to *MET* copy numbers or expression (Supplementary Fig. 4*A–C*).

Response to Nivolumab in Relation to MET Gene Copy Number Status

The ORR and disease control rate were 19.4% (95% confidence interval [CI]: 24.2%–26.0%) and 50.3% (95% CI: 43.0%–57.6%), respectively. No significant difference in ORRs was observed between patients with *MET* amplification (23.1%, 95% CI: 7.5%–50.9%) and those without amplification (19.1%, 95% CI: 13.8%–25.9%) ($p = 0.719$; Fig. 2*A*). Among three *MET* gain categories, there were also no significant differences in ORRs: 25.0% (95% CI: 3.4%–71.1%) of patients with high-level *MET* gain, 28.6% (95% CI: 11.3%–55.0%) of low-level *MET* gain, and 18.5% (95% CI: 13.1%–25.3%) of non–*MET* gain responded ($p = 0.495$; Supplementary Fig. 5*A*). Similarly, no significant difference according to MET expression was observed. The ORRs were 24.4% (95% CI: 14.1%–38.8%) for MET positive and 17.7% (95% CI: 12.0%–25.2%) for MET negative, respectively ($p = 0.382$; Supplementary Fig. 6*A*).

Survival Outcomes According to MET Gene Copy Number Status

For the entire cohort, the median PFS was 2.8 months (95% CI: 1.8–3.9 mo) and the median OS was 14.1 months (95% CI: 11.8–17.9 mo). No significant difference in PFS was observed between patients with *MET* amplification and those without *MET* amplification (log-rank, $p = 0.813$; Fig. 2*B*), with a median PFS of 3.5 (95% CI: 1.1–13.4) months for the former and 2.6 (95% CI: 1.8–3.9) months for the latter. The HR for PFS was 0.9 (95% CI: 0.5–1.7) for patients with *MET* amplification. In addition, there was no significant difference in PFS according to the *MET* gain categories (log-rank, $p = 0.629$; Supplementary Fig. 5*B*), with the HRs for low- and high-level *MET* gains in reference to non–*MET* gain being 0.6 (95% CI: 0.3–1.2) and 1.0 (95% CI: 0.5 –3.4), respectively. Similarly, there were no significant differences in OS according to the *MET* amplification status (log-rank, $p = 0.855$; Fig. 2*C*) and the *MET* gain categories (log-rank, $p = 0.337$; Supplementary Fig. 5*C*). In addition, there were no significant differences in PFS and OS when stratified by MET positivity (log-rank, $p = 0.494$ for PFS, and $p = 0.703$ for OS; Supplementary Fig. 6*B–C*).

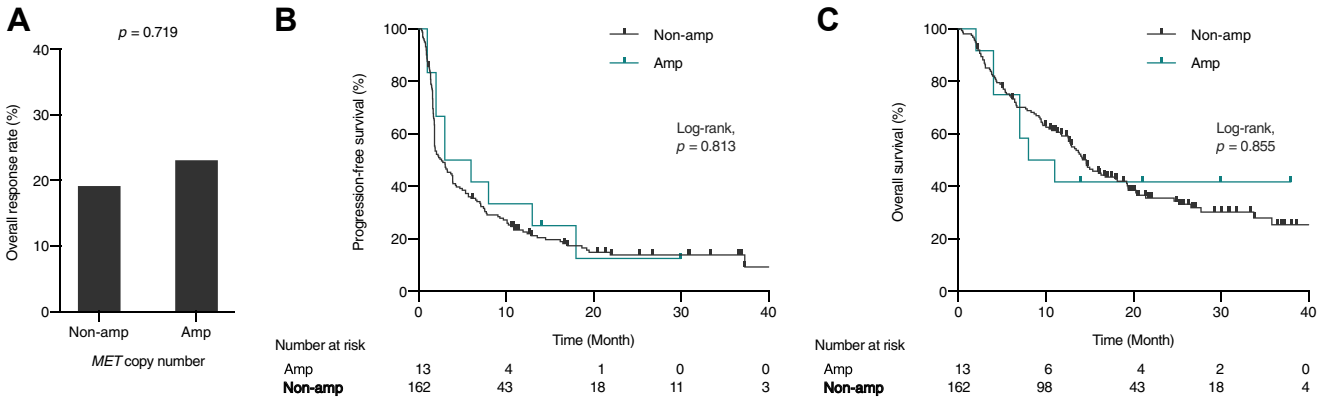

Figure 2. Response to nivolumab therapy and survival of patients with respect to *MET* amplification (*MET* amp). (*A*) Overall response rates pertaining to *MET* amp ($p = 0.719$). (*B*) Progression-free survival pertaining to *MET* amp (log-rank, $p = 0.813$). (*C*) Overall survival pertaining to *MET* amp (log-rank, $p = 0.855$).

Response and Survival Outcomes According to MET Gene Copy Number Status in Patients With Adenocarcinoma

Considering nine of the 13 (69.2%) *MET*-amplified tumors were adenocarcinomas, we conducted subgroup analyses focusing on the 101 patients with adenocarcinoma evaluated to exclude potential histologic bias in efficacy of the therapy. In this cohort, there were two (2.0%) high-level and eight (7.9%) low-level *MET* gains. Although no significant difference in ORRs was observed on the basis of the presence of *MET* amplification (33.3% versus 19.6%, $p = 0.389$; Fig. 3*A*), ORRs were significantly differed on the basis of the *MET* gain categories ($p = 0.049$; Fig. 3*B*) with a better response in patients with *MET* gains compared with those without. Notably, one patient with high-level *MET* gain responded well to nivolumab with 69% shrinkage in tumor size by the Response Evaluation Criteria Solid Tumors (Fig. 3*C*). In the other patient with high-level *MET* gain, nivolumab was terminated after as early as two cycles of treatment because of immune-related interstitial lung disease, and thus, the efficacy could not be evaluated. Nevertheless, this improvement of ORRs in patients with *MET* gains was not translated into better survival outcomes for both PFS (Fig. 3*D*) and OS (Fig. 3*E*).

The mutual exclusion of *MET* amplification and *EGFR* mutations in our adenocarcinoma cohort, in addition to the already known poor response to ICIs in patients with *EGFR* mutation,[9–11] motivated us to compare the efficacy of nivolumab by stratifying patients with adenocarcinoma into the following three categories (excluding one patient with an *ALK* translocation): *MET*-amplified/*EGFR* WT (n = 9), non–*MET*-amplified/*EGFR*-mutant (n = 12), and non–*MET*-amplified/*EGFR* WT (n = 79) groups. Despite not being statistically significant, it was notable that no response was documented in patients with *EGFR* mutation (Fig. 4*A*), who evidently had a significantly worse PFS (log-rank, $p = 0.001$ for overall; Fig. 4*B*). There was a similar trend in OS among the three groups (log-rank, $p = 0.077$ for overall; Fig. 4*C*). Importantly, no significant differences were observed in response to nivolumab treatment and patient survival between patients with *MET* amplification/*EGFR* WT and those without *MET* amplification/*EGFR* WT (Fig. 4*A–C*).

Discussion

To our knowledge, this study is the first to evaluate the efficacy of PD-1/PD-L1 inhibitors specifically focusing on *MET* amplification in a prospective cohort. We revealed that the increase of the *MET* gene copy number was not associated with greater efficacy of nivolumab in patients with NSCLC. This lack of influence of *MET* amplification on nivolumab efficacy is supported in part by the lack of association between *MET* copy numbers and the tumor immune microenvironment represented by the density of CD8+TILs in this study. Even in the era of widespread combined chemotherapy and immunotherapy as the first-line treatment of patients with NSCLC, this study provides insightful information on the effect of PD-1/PD-L1 blockade in patients with *MET* amplification. Although our study had an exploratory nature, it could help clinicians prioritize agents, such as ICIs, in the treatment regimen for patients with *MET* amplification.

There are several accepted biomarkers for predicting efficacy of ICIs in patients with NSCLC.[30,31] At present, PD-L1 expression evaluated by IHC is widely used to guide the selection of patients who should receive ICI therapy.[31,32] We previously reported the positive association of *MET* gene copy numbers evaluated by FISH with PD-L1 expression and a density of TILs in resected NSCLC specimens,[15] which are inconsistent with the present study probably owing to different disease stages between the two studies and limited sample size in the

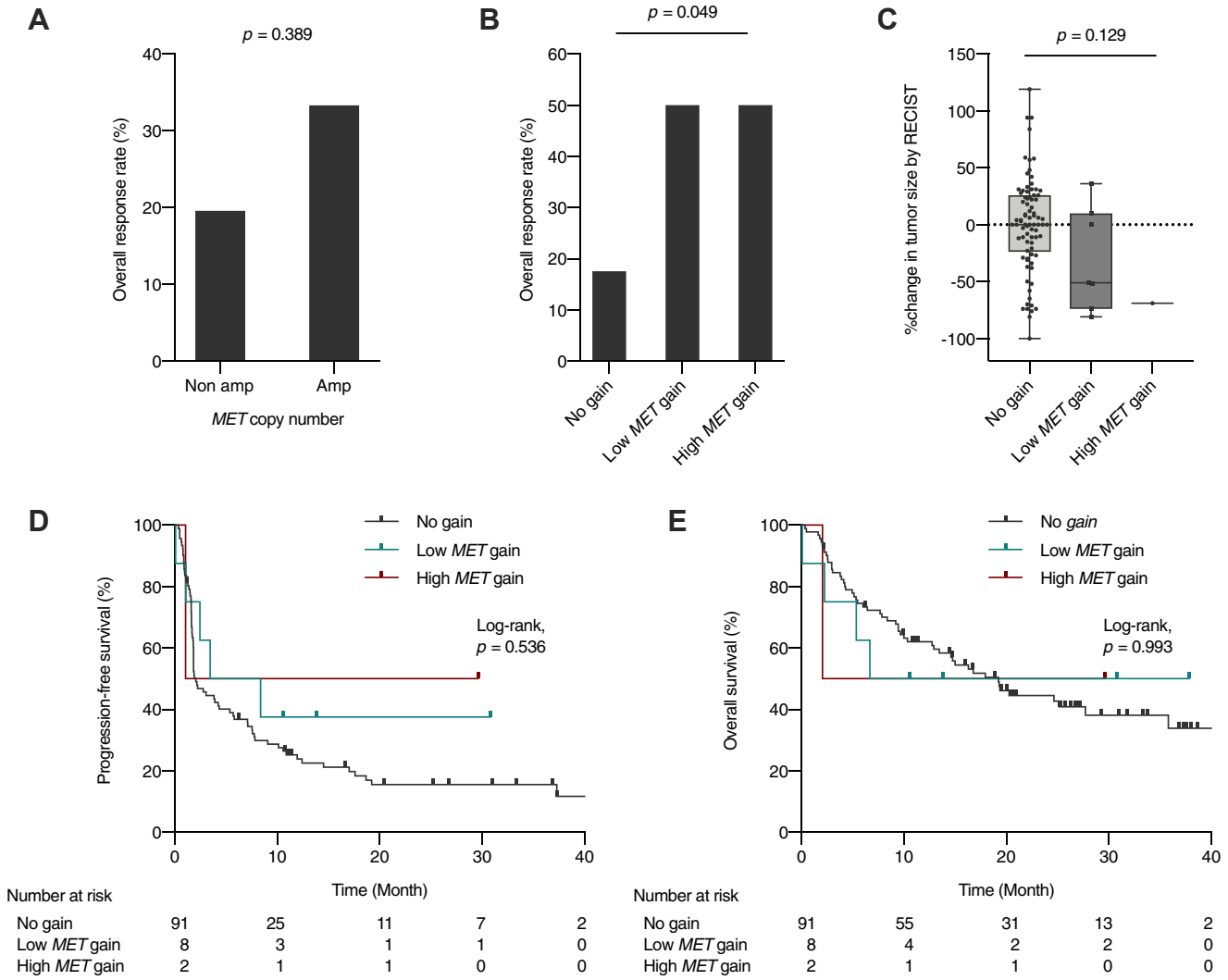

Figure 3. Response to nivolumab therapy and survival of patients with respect to *MET* gene copy numbers among adenocarcinomas. (*A*) Overall response rates with regard to *MET* amplification (*MET* amp) ($p = 0.389$). (*B*) Overall response rates with regard to *MET* gain categories ($p = 0.049$ for overall). (*C*) Percentage changes in tumor size on the basis of RECIST criteria ($p = 0.129$ for overall). (*D*) Progression-free survival pertaining to *MET* gain categories (log-rank, $p = 0.536$ for overall). (*E*) Overall survival pertaining to *MET* gain categories (log-rank, $p = 0.993$ for overall). RECIST, Response Evaluation Criteria Solid Tumors.

present study. Nonetheless, the association of *MET* amplification with the reported inflamed tumor microenvironment could be explained, in part, by the fact that the MET signaling pathway is mediated by JAK/STAT3, which is downstream of the interferon-γ pathway, resulting in expression of PD-L1 in *MET*-amplified tumors.[33] In addition, *MET* amplification was found to be associated with a higher tumor mutation burden, which is another predictive biomarker for ICI therapy.[34] These findings suggest that *MET*-amplified tumors may generate an inflamed tumor microenvironment and, thus, these tumors are likely to respond to a PD-1/PD-L1 axis blockade. Nevertheless, there are very limited studies exploring the association of ICI efficacy with *MET* amplification. Studies have focused more on *MET*ex14 than on *MET* amplification in this context,[35] although the relevance of both *MET* gene alterations is considered similar.[34,35] This may result from a nonbivariate nature of gene copy number status in contrast to gene mutation status, which makes its establishment as a solid biomarker for targeted therapy (e.g., against *MET*[1,2,5] and *HER2*[36] amplification) difficult. Although our results reveal that there are no clear associations between the efficacy of nivolumab and *MET* amplification, contrary to our expectations, this should be interpreted with caution considering *MET* amplification is regarded as a negative prognostic factor for NSCLC.[24,37,38] The present study also did not have a control arm. Therefore, the possibility that a shorter survival period, particularly for OS in patients with high-level *MET* gains, might have been

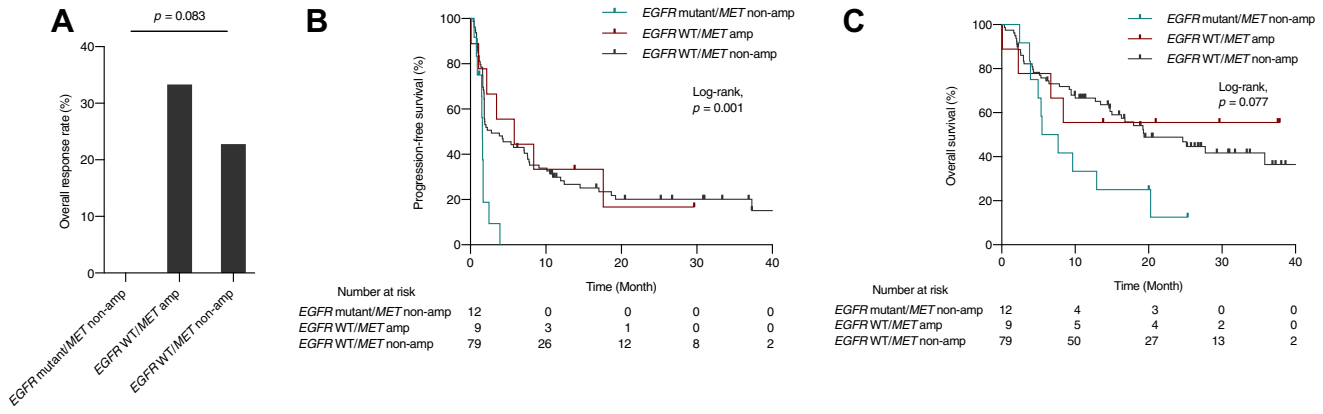

Figure 4. Response to nivolumab and survival of patients with respect to *MET* amplification (*MET* amp) and *EGFR* mutation status in adenocarcinomas. (*A*) Overall response rates pertaining to *MET* copy number and *EGFR* mutation status ($p = 0.083$ for overall). (*B*) Progression-free survival pertaining to *MET* amp and *EGFR* mutation status (log-rank, $p = 0.001$). (*C*) Overall survival pertaining to *MET* amp and *EGFR* mutation status (log-rank, $p = 0.077$). WT, wild-type.

interfered by the prognostic effect cannot be excluded. To more clearly determine the effect of *MET* amplification on nivolumab efficacy, we took patients with *EGFR* mutation as the refractory group control to nivolumab in the adenocarcinoma cohort. Importantly, although we observed no response and dismal survival outcomes in patients with *EGFR* mutation, response and survival outcomes in patients with *MET* amplification were distinct from those in patients with *EGFR* mutation but similar to that of those with EGFR-wild-type/non–*MET* amplification. These results suggest that *MET* amplification provides a distinct rationale for indication of immunotherapy from other established canonical driver genes, such as *EGFR* mutations. Therefore, our results do not preclude the use of PD-1/PD-L1 blockers for patients with *MET*-amplified NSCLC.

In addition to our study, one registry-based study revealed that exposure to ICI therapy is associated with superior survival outcomes as opposed to chemotherapy among patients with *MET*-amplified NSCLC.[7] Another study similarly revealed that patients with *MET*-amplified NSCLCs treated with immunotherapy have longer PFS than the non–*MET*-amplified counterparts.[39] This study further revealed that *MET* amplification is related to immune response-related pathways, DNA damage response, and repair pathways. In clear contrast, a recent study with multiple Memorial Sloan Kettering Cancer Center cohorts (N = 229) of lung adenocarcinoma treated with ICI revealed that patients with increased *MET* copy numbers (>5) evaluated by a polymerase chain reaction-based technique and FISH have worse survival outcomes than those without.[40] This refractory nature to ICI was exemplified by the dampened STING pathway by MET signaling and was rescued by MET inhibitors. These contrasting conclusions from multiple studies including ours should be addressed in future studies.

MET overexpression is observed in 25% to 75% of NSCLC,[41] and it was reported to potentially predict worse survival outcomes.[42] MET overexpression was also reported to be associated with PD-L1 expression and TILs[15,43] and to tend to be associated with more favorable outcomes with ICI therapy, irrespective of smoking history or PD-L1 positivity.[18] Nevertheless, the present study revealed no considerable association between MET expression and the efficacy of ICI therapy. MET expression has been found to be unreliable for predicting not only *MET*ex14 and *MET* amplification but also MET inhibitors.[5,44] Our findings further indicate that MET expression is not useful as a predictive biomarker for ICIs.

This study has several limitations. First, it is of an exploratory nature, as it was originally planned and designed to evaluate the significance of *PD-L1* amplification in patients with NSCLC treated with nivolumab. Second, there is no consensus on the definition of *MET* amplification by FISH. Therefore, we applied not only the main definition of *MET*/CEP7 greater than or equal to 2.0[15,21,22] but also the secondary definition of copy number gain[23,24] according to previous relevant studies. In addition, the modality to define the *MET* gene copy number status is not firmly established. Although next-generation sequencing–based techniques are convenient and widely accepted, the FISH technique is regarded as the gold standard[5,45] and has been used in numerous previous trials.[1,8,46,47] Third, patients in this study had a treatment history before nivolumab, and *MET* amplification could be acquired as a resistance mechanism to the previous therapy.[48–50] Nevertheless, a great most biopsy specimens were obtained at the time

of diagnosis. Furthermore, we could not exclude the possibility that some tumors that were *MET* amplification negative might possess *MET* amplification during nivolumab treatment. Finally, *MET* amplification is known to frequently possess other co-occurring genomic alterations, such as *MET*ex14, *TP53*, *EGFR*, *KRAS*, and *KEAP1* mutations.[1,7,51] Unfortunately, we could not evaluate comutations mainly because of a lack of biopsy specimens for NGS analyses. Further studies should characterize the impact of comutations on the biology and microenvironmental interactions in *MET*-amplified NSCLC.

In conclusion, our exploratory study revealed that *MET* gene copy number and MET expression may not be associated with the efficacy of nivolumab. Our findings provide important information for precision medicine of patients with *MET* amplification. Future larger prospective studies are required to confirm our findings in a more comprehensive manner.

CRediT Authorship Contribution Statement

Katsuhiro Yoshimura: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing - original draft.

Yusuke Inoue: Investigation, Methodology, Writing - review & editing.

Naoki Inui: Writing – review & editing, Supervision.

Masato Karayama: Data curation, Formal analysis.

Hideki Yasui, Hironao Hozumi, Yuzo Suzuki, Kazuki Furuhashi, Tomoyuki Fujisawa, Noriyuki Enomoto, Yutaro Nakamura: Data curation.

Haruhiko Sugimura, Takafumi Suda: Supervision.

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Data Availability

All data and supplementary information within the article were provided from the corresponding author upon reasonable request.

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2021.100239.

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