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ORIGINAL REPORT

MET Exon 14 Mutations in Non–Small-Cell Lung Cancer Are Associated With Advanced Age and Stage-Dependent *MET* Genomic Amplification and c-Met Overexpression

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ABSTRACT

Purpose

Non–small-cell lung cancers (NSCLCs) harboring mutations in *MET* exon 14 and its flanking introns may respond to c-Met inhibitors. We sought to describe the clinical, pathologic, and genomic characteristics of patients with cancer with *MET* exon 14 mutations.

Patients and Methods

We interrogated next-generation sequencing results from 6,376 cancers to identify those harboring *MET* exon 14 mutations. Clinical characteristics of *MET* exon 14 mutated NSCLCs were compared with those of NSCLCs with activating mutations in *KRAS* and *EGFR*. Co-occurring genomic mutations and copy number alterations were identified. c-Met immunohistochemistry and real-time polymerase chain reaction to detect exon 14 skipping were performed where sufficient tissue was available.

Results

MET exon 14 mutations were identified in 28 of 933 nonsquamous NSCLCs (3.0%) and were not seen in other cancer types in this study. Patients with *MET* exon 14–mutated NSCLC were significantly older (median age, 72.5 years) than patients with *EGFR*-mutant (median age, 61 years; $P < .001$) or *KRAS*-mutant NSCLC (median age, 65 years; $P < .001$). Among patients with *MET* exon 14 mutations, 68% were women, and 36% were never-smokers. Stage IV *MET* exon 14–mutated NSCLCs were significantly more likely to have concurrent *MET* genomic amplification (mean ratio of *MET* to chromosome 7, 4.3) and strong c-Met immunohistochemical expression (mean H score, 253) than stage IA to IIIB *MET* exon 14–mutated NSCLCs (mean ratio of *MET* to chromosome 7, 1.4; $P = .007$; mean H score, 155; $P = .002$) and stage IV *MET* exon 14–wild-type NSCLCs (mean ratio of *MET* to chromosome 7, 1.2; $P < .001$; mean H score, 142; $P < .001$). A patient whose lung cancer harbored a *MET* exon 14 mutation with concurrent genomic amplification of the mutated *MET* allele experienced a major partial response to the c-Met inhibitor crizotinib.

Conclusion

MET exon 14 mutations represent a clinically unique molecular subtype of NSCLC. Prospective clinical trials with c-Met inhibitors will be necessary to validate *MET* exon 14 mutations as an important therapeutic target in NSCLC.

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INTRODUCTION

In the past decade, the discovery of targetable genomic alterations in non–small cell-lung cancer (NSCLC) has revolutionized treatment of patients whose tumors harbor mutations in genes such as *EGFR*,[1-3] *ALK*,[4-6] and *ROS1*.[7,8] More recently, activating mutations and genomic amplification in the mesenchymal-to-epithelial transition (*MET*) gene have been recognized as a potentially important therapeutic target in NSCLC.[9-14] With a number of c-Met inhibitors already in clinical use,[15] prospective identification of *MET* genomic alterations may help guide treatment of a subset of patients with lung cancer toward more effective genotype-directed therapies.

c-Met is the tyrosine kinase receptor for hepatocyte growth factor. The intracellular c-Met juxtamembrane domain is encoded in part by

MET exon 14 and contains critical regulatory elements, including tyrosine 1003, the direct binding site for Cbl, an E3 ubiquitin ligase that promotes c-Met protein degradation.[16] First recognized in NSCLC more than 10 years ago, somatic mutations in the *MET* gene can cause exon 14 skipping, and the resulting mutant receptor demonstrates increased c-Met signaling and oncogenic potential.[17,18]

With the advent of improved sequencing technologies, routine detection of *MET* exon 14 mutations has become more feasible. Several recent reports have shown that patients with *MET* exon 14–mutant NSCLC may respond to treatment with c-Met inhibitors such as crizotinib and cabozantinib.[9-13] In our study, we screened a large cohort of diverse tumor types at our institution and found *MET* exon 14 mutations in 28 patients with NSCLC. Here, we describe the unique clinical, molecular, and pathologic characteristics of this cohort and describe the case of a patient who achieved a major partial response to crizotinib.

PATIENTS AND METHODS

Study Population

The study population was composed of patients at the Dana-Farber Cancer Institute who consented to an institutional review board–approved prospective cohort study for cancer sequencing between August 1, 2013, and May 1, 2015.[19,20] Demographic and clinical characteristics were collected from participants who had provided written informed consent for a separate institutional review board–approved clinical research protocol.

Next-Generation Sequencing

Next-generation sequencing (NGS) performed on 282 cancer-related genes (Appendix Table A1, online only) is described in the supplemental methods section. Because criteria for copy-number cutoffs by NGS have not been well established, a modified approach to the clinically validated fluorescent in situ hybridization–based criteria for amplification used in our laboratory (≥ 3:1 ratio of MET to CEP7) was applied. High-level gene copy gain (amplification) required evidence for focal *MET* gain at a ratio of 3:1 or greater of *MET* to chromosome 7, defined as the mean copy number of the *MET* gene (averaged across all the sequenced exons and targeted introns of the *MET* gene) normalized against the mean copy number of chromosome 7 (excluding regions of focal high amplification or deep deletions affecting other genes). Low copy gain was defined as a *MET* to chromosome 7 ratio of greater than 1 and less than 3. These criteria were similarly applied to other analyzed genes in this study.

Immunohistochemistry

A board-certified pathologist with expertise in thoracic malignancies (L.M.S.) classified each tumor histology according to WHO and International Association for the Study of Lung Cancer guidelines.[21,22] Immunohistochemical (IHC) staining for c-Met, ALK, and ROS1 was performed on formalin-fixed paraffin-embedded tissue sections 4 μm in thickness and scored using published criteria.[23-26] Clone names and staining conditions are listed in Appendix Table A2 (online only). Staining for c-Met was scored semiquantitatively on a four-tier scale from 0 (absent) to 3 (strong membranous and cytoplasmic staining). Intensity scores were multiplied by percentage of tumor cells staining to generate an H score (maximum score, 300).

Qualitative Real-Time Polymerase Chain Reaction for Detection of MET Exon 14 Skipping

RNA was extracted from formalin-fixed paraffin-embedded samples per protocol (AllPrep DNA/RNA mini kit; Qiagen, Hilden, Germany) and quantitated using Molecular Probes Quant-iT RiboGreen RNA assay kit (Life Technologies, Carlsbad, CA). FAM-labeled primer–probe sets specific for *MET* exon 14 deletion products and *MET*–wild-type products were combined with 10 ng of RNA in individual reactions. Qualitative real-time polymerase chain reaction (qRT-PCR) of test samples and positive and negative controls with 40 amplification cycles was performed in duplicate on an ABI 7900 RT-PCR system (Life Technologies) according to manufacturer protocol (ResearchDX, Irvine, CA).

Statistical Analysis

Fisher's exact and Wilcoxon rank-sum tests were used to compare categorical and continuous variables, respectively. All reported *P* values are two-sided hypothesis tests conducted at the .05 level, and no adjustments were made for multiple comparisons.

RESULTS

Patient Characteristics

To determine the frequency of *MET* exon 14 mutations in various cancer types, 6,376 solid and hematologic malignancies were analyzed using NGS between August 1, 2013, and May 1, 2015. Of the 1,141 lung cancers in this cohort, *MET* exon 14 mutations were identified in 28 (3.0%) of 933 nonsquamous NSCLCs (Fig 1), comprising 873 adenocarcinomas, 15 pleomorphic carcinomas, and 45 NSCLCs, including poorly differentiated carcinomas, large-cell carcinomas, and adenosquamous carcinomas. *MET* exon 14 mutations were not detected in other tumor types or other lung cancer histologic subtypes, including 132

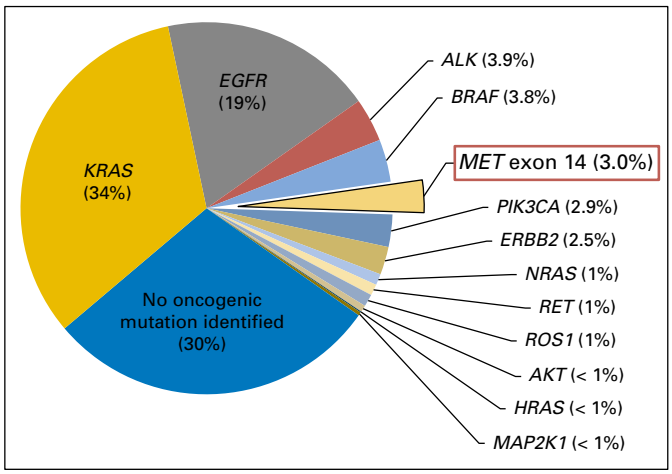

Fig 1. Distribution of genotypes among 933 patients with nonsquamous non–small-cell lung cancer (NSCLC). Results from next-generation sequencing of these 933 patient cases are shown. In this prevalence cohort, *MET* exon 14 mutations were detected in 28 patients with nonsquamous NSCLC (3.0%). None of these 28 patients also had an activating mutation in *KRAS*, *EGFR*, or *ERBB2* or a chromosomal rearrangement in *ALK*, *ROS1*, or *RET*. Of the 35 *BRAF* mutations detected in the cohort, 16 involved codon V600. Seven *BRAF* mutations co-occurred with other oncogenic mutations, most often as a minor subclone. Of the 27 *PIK3CA* mutations detected in the cohort, 14 co-occurred with another oncogenic mutation, including *KRAS*, *EGFR*, and *MET* exon 14. *NRAS* Q61L mutations co-occurred with *KRAS* mutations in two patients, and a *BRAF* exon 11 mutation occurred in one patient. A *MAP2K1* D67N variant co-occurred with a *KRAS* and *AKT1* mutation in one patient each. A single patient had both *EGFR* L858R and *ERBB2* extracellular domain (S310F) mutations. The figure represents the percentage of patients in the overall cohort with a functional variant in each gene; because of co-occurring mutations, the percentages total more than 100%.

squamous cell carcinomas, 41 SCLCs, 20 typical or atypical carcinoid tumors, and 15 neuroendocrine carcinomas, including large-cell neuroendocrine carcinomas and SCLC and large-cell neuroendocrine carcinoma combinations.

The clinical and pathologic characteristics of all 28 patients with *MET* exon 14–mutated NSCLC are listed in Table 1. Among these patients, the median age at disease onset was 72.5 years (range, 59 to 84 years), 19 (68%) were women, and 10 (36%) were never-smokers. At the time of their cancer diagnosis, 13 patients (46%) with *MET* exon 14 mutations had stage I NSCLC, two (7%) had stage II disease, four (14%) had stage III disease, and nine (32%) had stage IV disease. Histologic analysis showed that 18 patients (64%) had adenocarcinoma, four (14%) had pleomorphic (including sarcomatoid) carcinoma with an adenocarcinoma component, five (18%) had poorly differentiated NSCLC not otherwise specified, and one (4%) had adenosquamous histology. The four patients with pleomorphic or sarcomatoid histology and *MET* exon 14 mutations represented 26.7% of 15 total patients with pulmonary sarcomatoid carcinoma sequenced in our cohort, consistent with a recent report that *MET* exon 14 mutations seem to be enriched in this histologic subtype of NSCLC.[27]

We compared demographic characteristics of the cohort of patients with *MET* exon 14 mutations with those of patients with NSCLCs harboring activating mutations in *EGFR* and *KRAS* identified during the same period of time who had also consented to our institutional clinical research protocol (Table 1). Patients with *MET* exon 14–mutant NSCLC were significantly older than patients with *EGFR*-mutant ($P < .001$) and *KRAS*-mutant NSCLC ($P < .001$). Patients with *MET* exon 14 mutations were significantly more likely than those with *KRAS* mutations to be never-smokers ($P < .001$) and significantly more likely than those with *EGFR* mutations to have a history of tobacco use ($P = .03$). Asian race was only enriched in the cohort of those with *EGFR* mutations ($P < .001$), and all 28 patients in the *MET* exon 14 cohort were white, non-Hispanic. A significantly higher percentage of patients with *MET* exon 14 mutations had stage I disease compared with those with *EGFR* or *KRAS* mutations ($P < .001$).

Characterization of MET *Exon 14 Mutations*

The positions of the *MET* mutations relative to *MET* exon 14 and its flanking introns are shown in Fig 2. Genomic deletions occurred in 17 (61%) of the 28 patients with *MET* exon 14 mutations, ranging in size from a two–base pair deletion to a 193–base pair deletion, and point mutations occurred in 11 patients (39%). Of the 17 deletions, four were entirely within intron 13 but did not disrupt the intron 13 splice acceptor site, six overlapped with the intron 13 splice acceptor site, two occurred entirely within exon 14 (one in frame, one out of frame), and five involved the splice donor site of intron 14. Of the 11 point mutations, one resulted in a Y1003C amino acid substitution at the Cbl binding site, seven disrupted the splice donor site of intron 14, and three occurred in the last nucleotide of exon 14 (c.3028G>A); one of these latter three also had a splice acceptor mutation in intron 5 of unclear significance.

Table 1. Clinical Characteristics of Patients With Lung Cancers Harboring *MET* Exon 14 Versus *EGFR* or *KRAS* Mutations

Characteristic	No. (%)		
	MET Exon 14 (n = 28)	*EGFR* (n = 99)	*KRAS* (n = 169)
Median age (range), years	72.5 (59-84)	61 (30-93)	65 (42-93)
Sex			
Male	9 (32)	30 (30)	62 (37)
Female	19 (68)	69 (70)	107 (63)
Smoking history, pack-years*			
Never-smoker	10 (36)	57 (58)	6 (4)
≤ 10	3 (11)	10 (10)	11 (7)
> 10	15 (53)	28 (28)	152 (90)
Race			
White, non-Hispanic	28 (100)	79 (80)	157 (93)
Asian	0 (0)	15 (15)	0 (0)
Black	0 (0)	1 (1)	5 (3)
White, Hispanic	0 (0)	3 (3)	3 (2)
Unknown	0 (0)	1 (1)	4 (2)
Histology			
Adenocarcinoma	18 (64)	92 (93)	150 (89)
Pleomorphic with adenocarcinoma component	4 (14)	0 (0)	3 (2)
NSCLC, poorly differentiated	5 (18)	4 (4)	10 (6)
Squamous	0 (0)	2 (2)	5 (3)
Adenosquamous	1 (4)	1 (1)	1 (1)
Stage at diagnosis			
I	13 (46)	9 (9)	12 (7)
II	2 (7)	3 (3)	12 (7)
III	4 (14)	9 (9)	44 (26)
IV	9 (32)	78 (79)	101 (60)

NOTE. Percentages may not add up to 100% because of rounding.
Abbreviation: NSCLC, non–small-cell lung cancer.
*Number of smoking pack-years was not available for four patients with *EGFR* mutations.

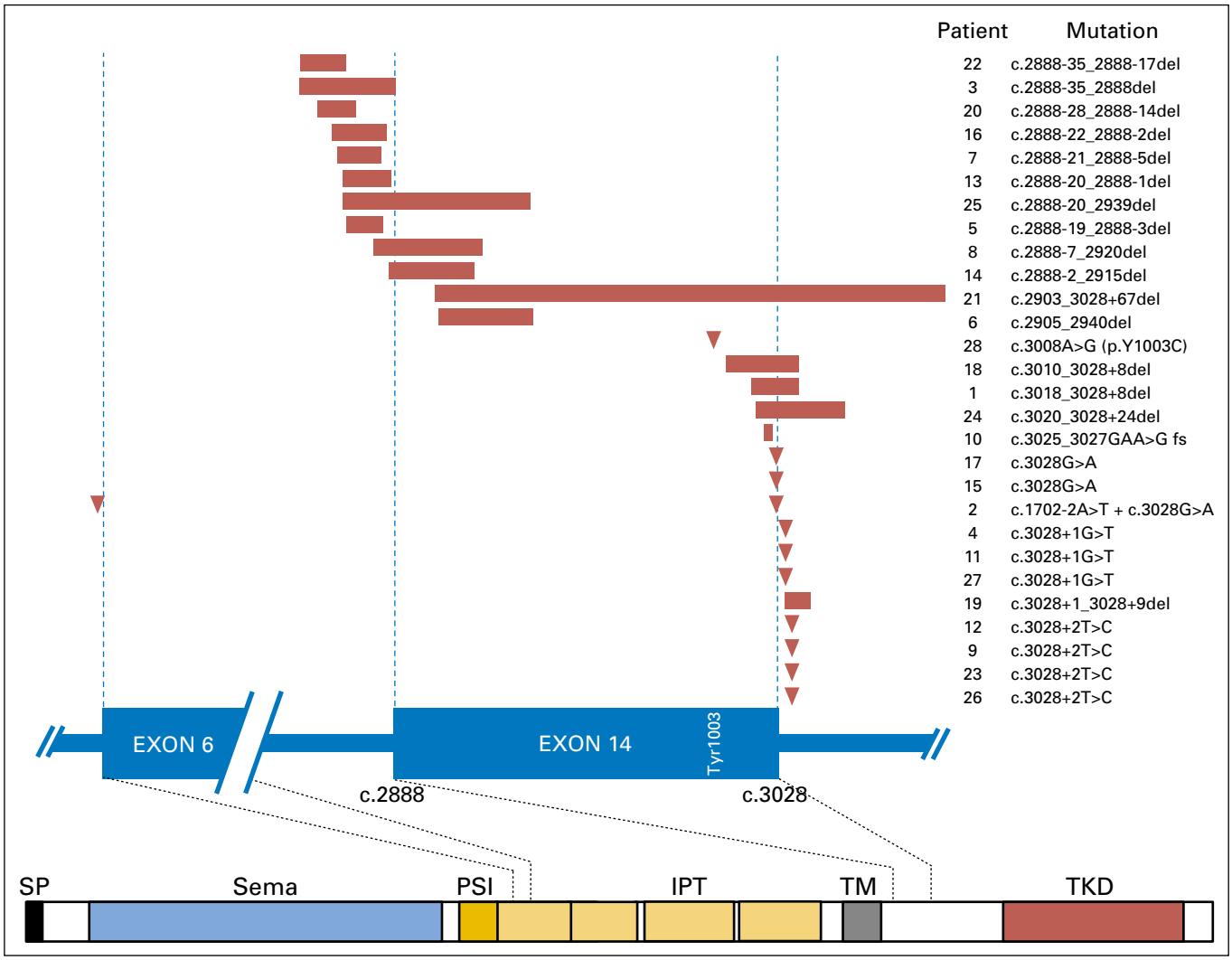

Fig 2. Locations of *MET* exon 14 genomic alterations found in 28 patients with non–small-cell lung cancer. The positions of each *MET* mutation are displayed in relation to the *MET* gene. Deletions are shown as rectangles, and point mutations are shown as triangles. The table to the right indicates the patient number and the nucleotide position of each mutation. In one patient who harbored a c3028G>A mutation, there was also a c.1702-2A>T splice acceptor site mutation in intron 5. The protein domain structure is shown at the bottom of the panel. The position of the tyrosine 1003 c-Cbl binding site is indicated. IPT, immunoglobulin-plexin-transcription domain; PSI, cysteine-rich domain found in plexins, semaphorins, and integrins; Sema, semaphorin-like domain; SP, signal peptide; TKD, tyrosine kinase domain; TM, transmembrane domain. c-Met protein diagram data adapted.[28]

Concurrent Genomic Alterations

None of the 28 patients with *MET* exon 14 mutations also had activating mutations in *KRAS*, *EGFR*, or *ERBB2* or rearrangements in *ALK*, *ROS1*, or *RET* (Figs 1 and 3). In one patient, a *MET* c3025_3027GAA>G frame-shift mutation was detected in 76% of 453 sequencing reads, and there was also a concurrent *BRAF* V600E mutation in 3% of 159 reads (Fig 3), suggesting that the *BRAF* mutation occurred in a subclonal tumor population. *EGFR* copy gain was also observed in eight patients (29%) with *MET* exon 14 mutations. Inactivating mutations in *TP53* were observed in nine patients (32%), and amplification of *MDM2*, a negative regulator of p53,[29] was observed in 13 patients (46%). *TP53* mutation and *MDM2* amplification tended to occur in a non-overlapping distribution, with a total of 20 patients (71%) having alterations in at least one of these genes (Fig 3). Concurrent *MDM2* amplification was significantly more common in *MET* exon 14–mutated lung cancers than in patients with *KRAS* (one of 315 [0.3%]; $P < .001$) or *EGFR* mutations (six [3.4%] of 178; $P < .001$). Compared with patients with *MET* exon 14 mutations, concurrent *TP53* mutations occurred at a similar frequency among those with *KRAS* mutations (113 of 315 [36%]; $P = .84$) but were more common among those with *EGFR* mutations (112 [63%] of 178; $P = .003$). Other selected co-occurring genomic mutations and copy number alterations are shown in Fig 3.

Genomic copy-number analysis demonstrated that among the 28 patients with *MET* exon 14 mutations, six (21%) had concurrent high-level *MET* copy gain, and eight (29%) showed low-level *MET* copy gain (Fig 3). In all 14 such patients, the *MET* exon 14–mutated allele seemed to be selectively amplified over the *MET*–wild-type allele on the basis of read count bias toward the mutant allele. Stage IV NSCLCs with *MET* exon 14 mutations had a significantly higher ratio of *MET* to chromosome 7 (mean ratio, 4.3) than stage I to III

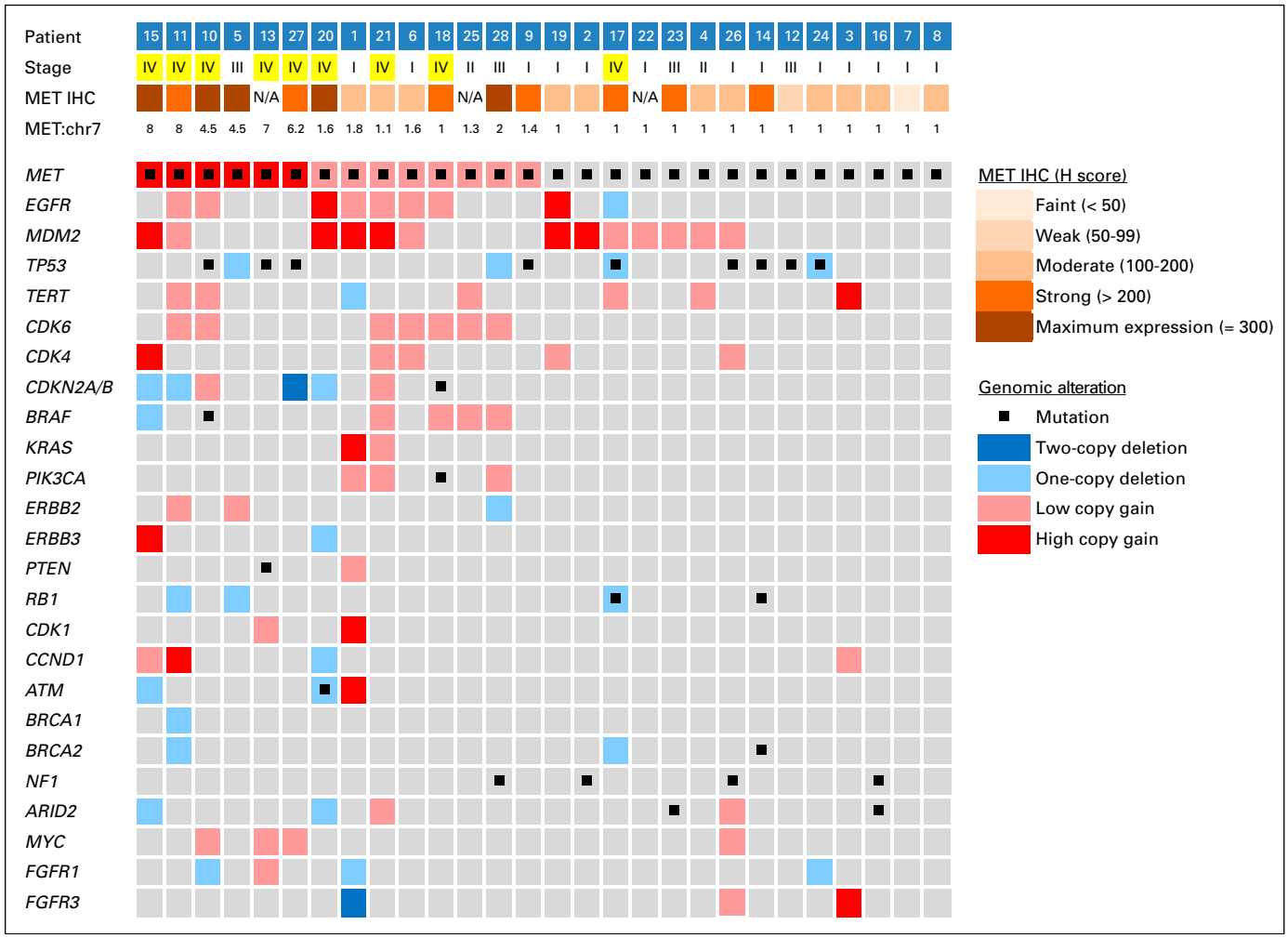

Fig 3. c-Met immunohistochemistry (IHC), co-occurring mutations, and copy-number alterations in tumors harboring *MET* exon 14 mutations. Patient numbers are shown in the top row. Stage at presentation is indicated, with patients with stage IV disease highlighted in yellow. c-Met IHC staining is shown in shades of brown reflecting the H score, which is the product of percent of positively staining cells (from 0 to 100) and the intensity of staining (0, 1+, 2+, 3+), with a maximum H score of 300. The copy-number ratios of *MET* to chromosome 7 (*MET*:chr7) are shown. Mutations are shown as black squares within each box. High-level amplification (*MET*:chr7 ratio ≥ 3), red. Low-level amplification (*MET*:chr7 ratio > 1 and < 3), pink. One-copy deletion, light blue. Two-copy deletion, blue.

NSCLCs with *MET* exon 14 mutations (mean ratio of *MET* to chromosome 7, 1.4; $P = .007$) and than 67 stage IV NSCLCs that lacked *MET* exon 14 mutations (mean ratio of *MET* to chromosome 7, 1.2; $P < .001$; Fig 4A).

c-Met Expression by IHC

Sufficient tissue to perform c-Met IHC was available for 25 of 28 patients (Fig 3). c-Met staining in the cohort of those with *MET* exon 14 mutations varied from weak expression (H score, < 50) to maximum expression (H score, 300). Stage IV NSCLCs with *MET* exon 14 mutations had a significantly higher H score (mean, 253) than stage I to III NSCLCs with *MET* exon 14 mutations (mean H score, 155; $P = .002$) and than 82 stage IV NSCLCs that lacked *MET* exon 14 mutations (mean H score, 142; $P < .001$; Fig 4B).

Representative histologic stains and genomic copy-number plots from a patient with stage I and a patient with stage IV NSCLC are shown in Figs 4C and 4D, respectively. Fig 4C shows patient 2, a never-smoker with stage I minimally invasive lung adenocarcinoma, lepidic predominant, with no *MET* genomic amplification (ratio of *MET* to chromosome 7, 1.0), moderate c-Met expression (H score, 120), focal low-level *MDM2* amplification, and few other genomic alterations. In contrast, Fig 4D shows patient 20, a patient with a 40–pack-year smoking history with stage IV high-grade solid adenocarcinoma with high-level c-Met protein expression. This tumor showed marked genomic instability, with numerous copy-number alterations, including low-level amplification of *EGFR* and *MET* (ratio of *MET* to chromosome 7, 1.6), low-level gain of chromosome 8q, homozygous deletion of *CDKN2A/B*, and *MDM2* amplification.

MET Splicing Analysis

We performed a qRT-PCR–based assay in patient samples where enough RNA was available for analysis. Exon 14 skipping was observed in 23 (96%) of 24 samples (Appendix Fig A1A, online only),

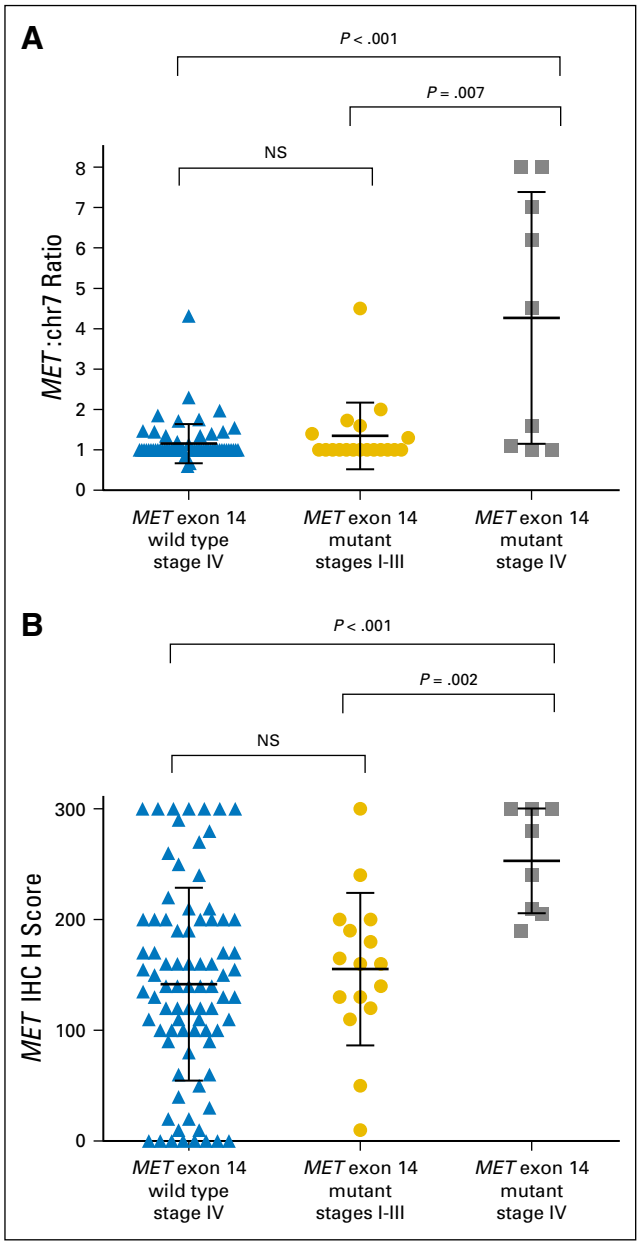

Fig 4. Histopathologic and genomic correlations with stage at presentation in patients with *MET* exon 14 mutations. Stage IV non–small-cell lung cancers (NSCLCs) with *MET* exon 14 mutations have significantly greater (A) *MET* amplification and (B) c-Met immunohistochemical (IHC) expression than stage IA to IIIB NSCLCs with *MET* exon 14 mutations and stage IV *MET*–wild-type NSCLCs. Error bars show mean values ± one standard deviation. chr7, chromosome 7; NS, not significant.

including those that showed intronic deletions upstream of the intron 13 splice acceptor site, one with an in-frame deletion within exon 14, and those with a c.3028G>A point mutation in the last nucleotide of exon 14. Exon skipping was not detected in any of the four control patients with lung cancer who lacked *MET* exon 14 mutations. In the one patient (patient 28) with a Y1003C substitution in the c-Cbl binding site, exon 14 skipping was not observed (Appendix Fig A1A); this patient's tumor displayed low-level *MET* genomic amplification (ratio of *MET* to chromosome 7, 2) and maximal c-Met expression by IHC, with an H score of 300 (Fig 3; Appendix Fig A1B).

Case Report: Response to Crizotinib

A 64-year-old female never-smoker (patient 15) was diagnosed with stage IV NSCLC with poorly differentiated carcinoma histology, favoring adenocarcinoma. Her tumor underwent NGS at our institution, which showed no activating mutations in *KRAS*, *EGFR*, or *BRAF* and no genomic rearrangements in *ALK* or *ROS1*. A c.3028G>A mutation was identified in *MET* exon 14 in 94% of 867 reads (Fig 5A), in association with high-level *MET* amplification (Fig 5B). Exon 14 skipping was detected using qRT-PCR (Fig 5C). After disease progression during first-line chemotherapy, the patient started crizotinib 250 mg orally twice per day, and repeat imaging 8 weeks later showed dramatic improvement in multiple lesions throughout her body (Figs 5D and 5E), with an ongoing response at 8 months.

DISCUSSION

Lung cancer remains the leading cause of cancer-related death worldwide, and detection of targetable genomic mutations within tumors will continue to improve outcomes for patients with NSCLC. Here we present the largest, to our knowledge, single-institution cohort of patients with NSCLC harboring *MET* exon 14 splicing mutations. These mutations occurred in 3.0% of nonsquamous NSCLCs, similar in prevalence to *ALK* translocations in our cohort and more common than rearrangements in *ROS1*, *RET*, and *NTRK1* both in our cohort and in comparison with previously reported frequencies for these genomic alterations.[30-35]

Two recent reports have focused attention on *MET* exon 14 mutations as a targetable alteration in lung cancer. Paik et al[13] described eight patients with *MET* exon 14 mutations, four of whom were treated with either crizotinib or cabozantinib, and partial responses were observed in some of these patients. Comparisons of clinical characteristics of these eight patients with those of patients with other molecular NSCLC subtypes were not provided given the sample size. Frampton et al[12] impressively screened more than 38,000 tumors and identified 221 patient cases with *MET* exon 14 mutations; however, limited clinical and histopathologic data were available in this study. Here we describe detailed clinical, pathologic, and genomic features of 28 lung cancers harboring *MET* exon 14 mutations.

Uniquely, the *MET* exon 14 mutation seems to occur in older adults, with a median age of 72.5 years. This is the first time that a lung cancer mutation has been identified specifically in an older population and is in contrast to *ALK* and *ROS1* rearrangements, which tend to occur at younger ages of 50 to 60 years,[30,36-38] and to *KRAS*, *EGFR*, and *BRAF* mutations, which tend to occur at the ages of 61 to 66 years.[38,39] Older patients may not be able to receive full-dose chemotherapy because of comorbidities; therefore, successful identification of targetable mutations in this population may improve treatment tolerability.

EGFR-, *ALK*-, and *ROS1*-driven cancers tend to occur in light or never-smokers.[30,36-38] However, in our cohort, 64% of patients with *MET* exon 14 mutations had a history of tobacco use. We also found that although *MET* exon 14 mutations occurred predominantly in adenocarcinomas, 14% of patients with *MET* exon 14 mutations had pleomorphic (including sarcomatoid) histology, which is a higher-than-expected rate compared with historical case series.[40] These findings highlight the need for comprehensive

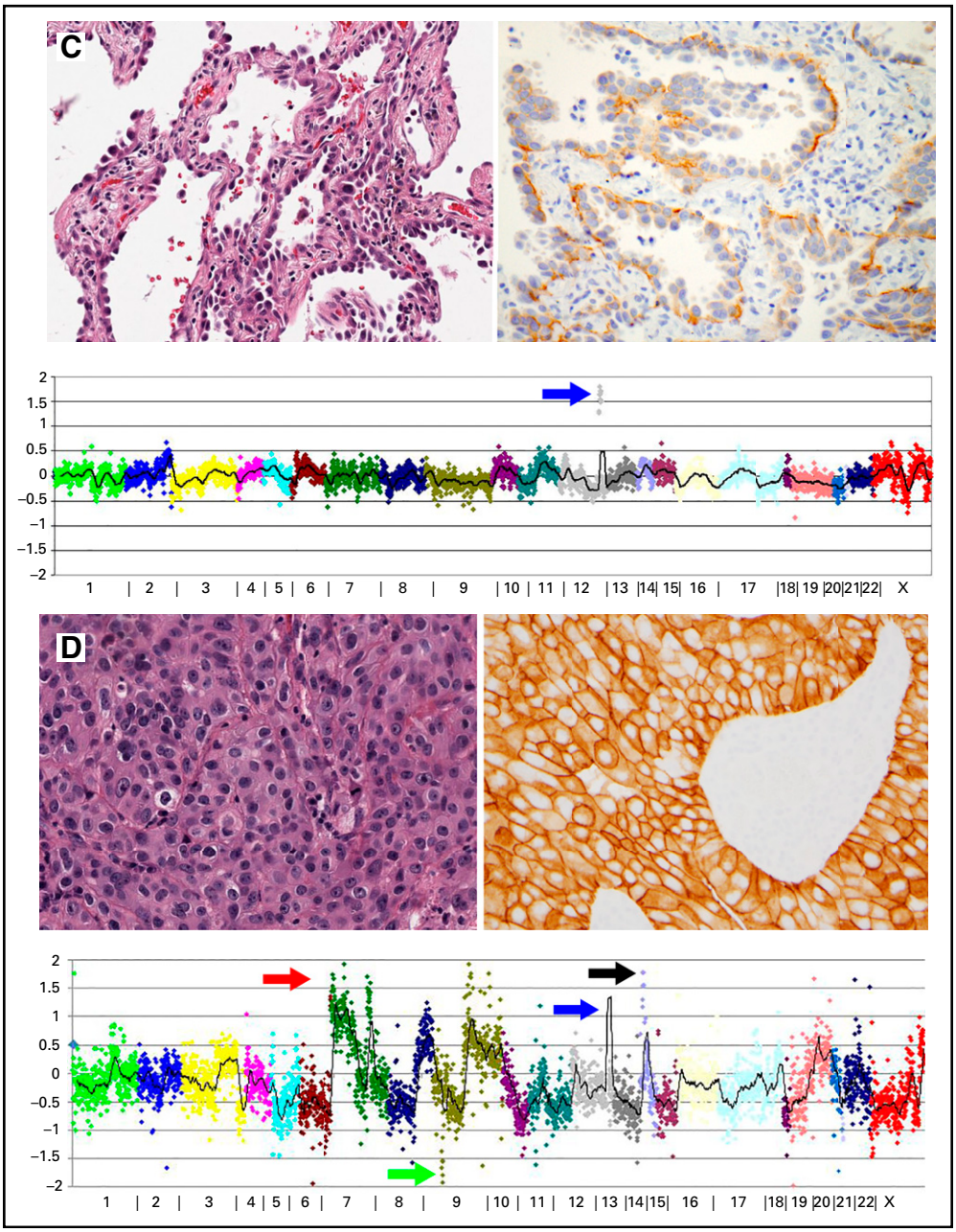

Fig 4. (continued) Representative (C) stage I and (D) stage IV cancers are shown. (C) Stage I minimally invasive lung adenocarcinoma (top left) associated with low-level c-Met protein expression (top right), focal amplification of *MDM2* (bottom; arrow), and no other significant amplifications or deletions. (D) A small insertion-deletion event at the 3′ end of *MET* exon 14 was found in the context of a high-grade solid adenocarcinoma (top left) with high-level c-Met protein expression (top right). Notably, this tumor showed evidence for significant genomic instability (bottom) marked by numerous copy-number alterations, including low-level amplification of *EGFR* and *MET* (estimated at approximately six copies; red arrow), low-level gain of 8q (dark blue shading of points on chromosome 8q), homozygous deletion of *CDKN2A/B* (green arrow), *MDM2* amplification (blue arrow), and *NKX2-1* amplification (black arrow).

molecular profiling in all patients with NSCLC regardless of histology or clinical characteristics.

Nearly half of the patients with *MET* exon 14–mutated cancer in our study presented with stage I disease; this is in contrast to *ALK*-rearranged lung cancer, for example, which is rare in early-stage NSCLC.[36] In The Cancer Genome Atlas study of lung adenocarcinoma[41] and in a separate Japanese study,[42] *MET* amplification and *MET* exon 14 mutations seemed to be mutually exclusive in NSCLC; however, both of these studies used surgically resected (early-stage) lung tumors for analysis. Compared with patients presenting with *MET* exon 14–mutated stage IA to IIIB NSCLC, patients with *MET* exon 14 mutations with stage IV disease in our study were significantly more likely to have concurrent *MET* genomic amplification and strong c-Met expression. Our findings suggest that a *MET* exon 14 mutation may be an early event in lung tumorigenesis, and the stepwise addition of *MET* amplification and/or overexpression may contribute to a more aggressive clinical phenotype, but comparisons of serial tumor samples from the same patient will be necessary to validate this hypothesis.

Several different mutation types in *MET* exon 14 and its flanking introns were detected in our study, and this large degree of variation will have to be taken into account when designing clinical diagnostic sequencing assays to capture all possible activating *MET* mutations. Although some mutations affected splice acceptor and splice donor sites, which would easily be predicted to affect splicing, others occurred deeper within intron 13 and did not overlap with the splice acceptor site. In addition, a recurrent point mutation at the last

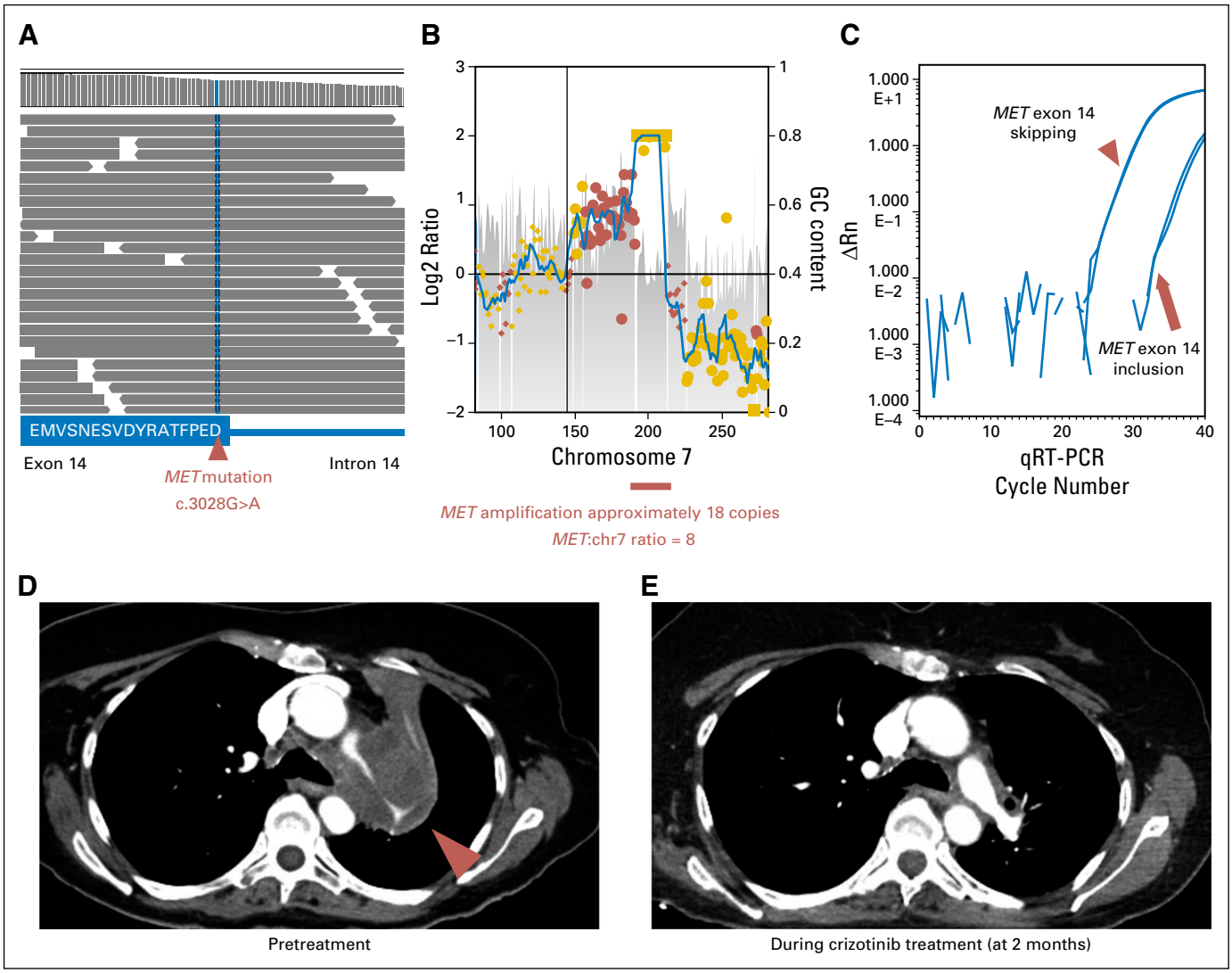

Fig 5. Response to crizotinib in a patient with *MET* exon 14 mutation and concurrent *MET* amplification. A 64-year-old female never-smoker (patient 15) with poorly differentiated stage IV lung adenocarcinoma was found by next-generation sequencing to have (A) a *MET* exon 14 c.3028G>A mutation, (B) high-level *MET* amplification with a ratio of *MET* to chromosome 7 (chr7) of 8, and (C) exon 14 skipping (arrowhead), as detected by a real-time polymerase chain reaction (RT-PCR) –based assay. (C) Amplification of the wild-type *MET* transcript was also detected but at a higher RT-PCR cycle number (arrow). A chest computed tomography scan (axial view) at the level of the carina and left main pulmonary artery is shown (D) before and (E) after treatment with the c-Met inhibitor crizotinib.

nucleotide of exon 14 (c.3028G>A) was detected in three patients; G>A mutations in the last nucleotide of exons have been reported to cause alternative splicing in a number of other diseases.[43-45] Robust exon 14 skipping was observed in 23 of 24 samples from patients with *MET* exon 14–mutant NSCLC tested using a qRT-PCR–based assay, demonstrating that a variety of sequence changes in this region can affect precursor mRNA processing. The only patient in whom exon 14 skipping was not demonstrated was the one patient with a c.3008A>G point mutation, resulting in a Y1003C amino acid substitution. Tyrosine 1003 is necessary for the binding of c-Cbl to c-Met and is required for receptor ubiquitination and degradation.[16] In vitro, a Y1003F substitution mutation has been shown to transform fibroblasts in the absence of ligand, promote epithelial-to-mesenchymal transition, and lead to cell dispersal.[16] In this particular patient, the Y1003C mutation, rather than exon 14 skipping, may have been the main mechanism of c-Met activation.

We describe a patient with a c.3028G>A mutation causing *MET* exon 14 skipping who also had concurrent high-level amplification of the mutated *MET* allele and experienced a major partial response to the c-Met inhibitor crizotinib; no rearrangements in *ALK* or *ROS1*, other targets of crizotinib, were detected in this patient. Whether sensitivity to crizotinib in this patient was conferred more by the *MET* exon 14 mutation or by *MET* amplification is unclear; however, responses to c-Met inhibitors have been reported in patients with *MET* exon 14 mutations without *MET* amplification.[12,13] Prospective clinical trials will be necessary to determine if certain *MET* exon 14 mutations are more responsive to c-Met inhibition than others and whether concurrent *MET* amplification predicts for increased

sensitivity to c-Met inhibitors. Studying how these initially responsive cancers acquire resistance to c-Met inhibitors will also be critical for the development of therapeutic strategies to overcome resistance.[46]

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

MET Exon 14 Mutations in Non–Small-Cell Lung Cancer Are Associated With Advanced Age and Stage-Dependent _MET_ Genomic Amplification and c-Met Overexpression

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Appendix

Methods

Next-Generation Sequencing

Manual macrodissection from unstained tissue sections was performed to enrich for areas containing ≥ 20% tumor nuclei. DNA was isolated following standard extraction protocols (Qiagen, Valencia, CA) and quantified using PicoGreen-based dsDNA detection (Life Technologies, Carlsbad, CA). Sequencing libraries were prepared from 50 ng of DNA using Illumina TruSeq LT reagents (Illumina, San Diego, CA) and enriched for exons and select introns in 282 genes implicated in cancer biology (Appendix **Table A1**) through solution-based hybrid capture using an Agilent SureSelect custom RNA bait set (Agilent Technologies, Santa Clara, CA). Massively parallel sequencing performed on Illumina HiSeq2500 with 100 × 100 paired-end reads achieved an average mean target coverage of 187× per sample.

Pooled sample reads were demultiplexed and sorted, and duplicate reads were removed using Picard. Reads were aligned to the reference sequence b37 edition from the Human Genome Reference Consortium using BWA software (Li H, et al: Bioinformatics 25:1754-1760, 2009). Mutation analysis for single-nucleotide variants was performed using MuTect (version 1 0.27200; Cibulskis K, et al: Nat Biotechnol 31:213-219, 2013) and annotated using Oncotator software (http://www.broadinstitute.org/oncotator). Insertions and deletions were called using Indelocator software (https://www.broadinstitute.org/cancer/cga/indelocator). Gene rearrangements were identified using BreaKmer (Abo RP, et al: Nucleic Acids Res 43:e19, 2015). Integrative Genomics Viewer (version 2.0.16 or later; https://www.broadinstitute.org/igv) was used for visualization and interpretation. Variants present at a population frequency of greater than 0.1% in the Exome Sequencing Project database were filtered out as germline polymorphisms.

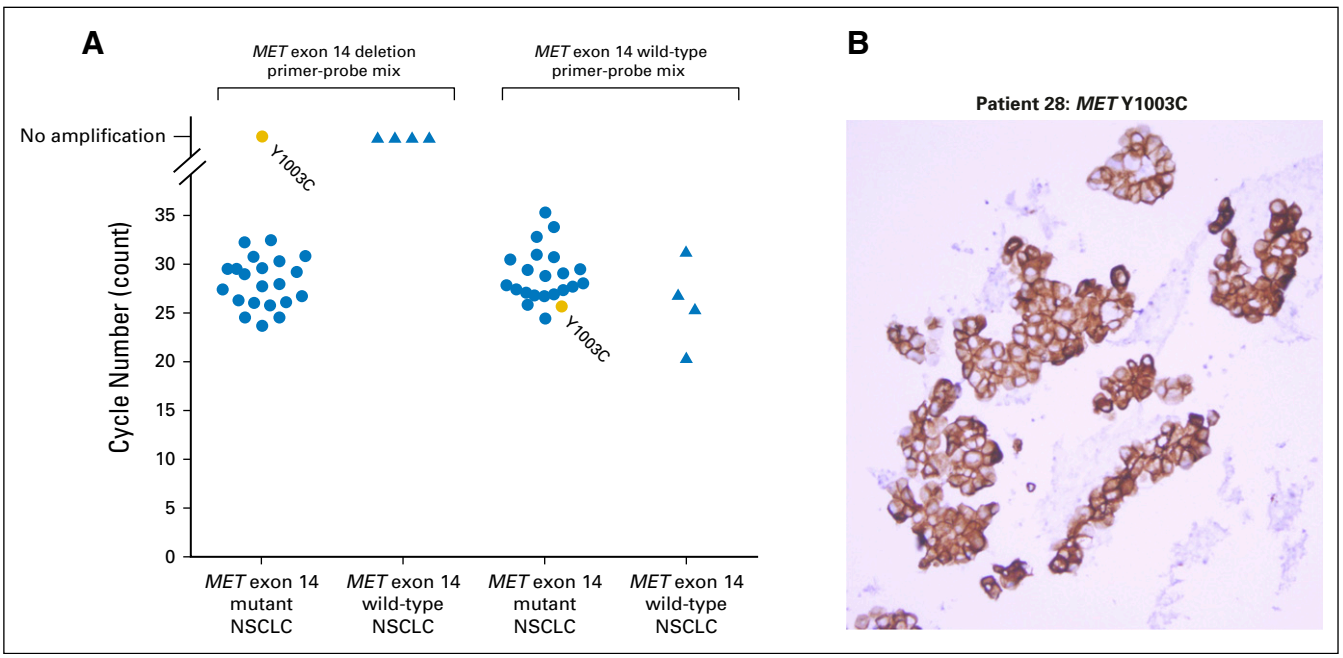

Fig A1. Of the 28 patients in this cohort with *MET* exon 14 mutations, 24 had RNA available for testing. *MET* exon 14 skipping was detected in 23 (96%) of 24 patients using a qualitative real-time polymerase chain reaction–based assay. (A) Amplification cycle number for the 24 available patients with *MET* exon 14 mutations as well as for four patients with *MET* wild type using primer–probe mixes designed to detect either *MET* exon 14 deletion or *MET* exon 14 inclusion. For samples that test positive for *MET* exon 14 skipping, the cycle number using the *MET* exon 14 deletion primer–probe mix should be in the range of 16 to 35. Samples that test positive for *MET* exon 14 inclusion should amplify at a cycle number of 16 to 30 using the *MET* exon 14 wild-type primer–probe mix, according to the product insert. Patient 28 had a Y1003C mutation at the c-Cbl binding site; (A) exon 14 skipping was not detected (gold circles), but (B) maximal c-Met expression (H score, 300) was demonstrated using immunohistochemistry. NSCLC, non–small-cell lung cancer.

Table A1. List of Genes Analyzed Using Next-Generation Sequencing

Gene									
ABL1	*BRCA1*	*CEBPA*	*ESR1*	*GATA3*	*LMO1*	*NBN*	*PRAME*	*SDHAF2*	*TCF3*
AKT1	*BRCA2*	*CHEK2*	*ETV1*	*GATA4*	*LMO2*	*NF1*	*PRDM1*	*SDHB*	*TCF7L1*
AKT2	*BRD4*	*CIITA*	*ETV4*	*GATA6*	*LMO3*	*NF2*	*PRF1*	*SDHC*	*TCF7L2*
AKT3	*BRIP1*	*CREBBP*	*ETV5*	*GLI1*	*MAP2K1*	*NFE2L2*	*PRKAR1A*	*SDHD*	*TERT*
ALK	*BUB1B*	*CRKL*	*ETV6*	*GLI2*	*MAP2K4*	*NFKBIA*	*PRKCI*	*SETBP1*	*TET2*
ALOX12B	*CARD11*	*CRLF2*	*EWSR1*	*GLI3*	*MAP3K1*	*NFKBIZ*	*PRKCZ*	*SETD2*	*TNFAIP3*
APC	*CBL*	*CRTC1*	*EXT1*	*GNA11*	*MAPK1*	*NKX2-1*	*PRKDC*	*SF1*	*TRA*
AR	*CBLB*	*CRTC2*	*EXT2*	*GNAQ*	*MCL1*	*NOTCH1*	*PRPF40B*	*SF3B1*	*TRB*
ARAF	*CCND1*	*CTNNB1*	*EZH2*	*GNAS*	*MDM2*	*NOTCH2*	*PRPF8*	*SH2B3*	*TRG*
ARID1A	*CCND2*	*CUX1*	*FAM46C*	*GPC3*	*MDM4*	*NPM1*	*PSMD13*	*SMAD2*	*TSC1*
ARID1B	*CCND3*	*CYLD*	*FANCA*	*GSTM5*	*MECOM*	*NRAS*	*PTCH1*	*TP53*	*TSC2*
ARID2	*CCNE1*	*DDB2*	*FANCC*	*H3F3A*	*MEF2B*	*NTRK1*	*PTEN*	*TRA*	*U2AF1*
ASXL1	*CD274*	*DDR2*	*FANCD2*	*HNF1A*	*MEN1*	*NTRK2*	*PTK2*	*SMARCB1*	*VHL*
ATM	*CD58*	*DICER1*	*FANCE*	*HRAS*	*MET*	*NTRK3*	*PTPN11*	*SMC1A*	*WRN*
ATRX	*CD79B*	*DIS3*	*FANCF*	*ID3*	*MITF*	*PALB2*	*SMAD4*	*SMC3*	*WT1*
AURKA	*CDC73*	*DMD*	*FANCG*	*IDH1*	*MLH1*	*PARK2*	*SMARCA4*	*SMO*	*XPA*
AURKB	*CDH1*	*DNMT3A*	*FAS*	*IDH2*	*MLL*	*PAX5*	*RAD21*	*SOCS1*	*XPC*
AXL	*CDK1*	*EGFR*	*FBXW7*	*IGF1R*	*MLL2*	*PDCD1LG2*	*RAF1*	*SOX2*	*XPO1*
B2M	*CDK2*	*EP300*	*FGFR1*	*IgH*	*MPL*	*PDGFRA*	*RARA*	*SOX9*	*ZNF217*
BAP1	*CDK4*	*EPHA3*	*FGFR2*	*IgL*	*MSH2*	*PDGFRB*	*RB1*	*SRC*	*ZNF708*
BCL2	*CDK5*	*EPHA5*	*FGFR3*	*IKZF1*	*MSH6*	*PHF6*	*RBL2*	*SRSF2*	*ZRSR2*
BCL2L1	*CDK6*	*EPHA7*	*FGFR4*	*IKZF3*	*MTOR*	*PHOX2B*	*REL*	*STAG1*	
BCL2L12	*CDK9*	*ERBB2*	*FH*	*JAK2*	*MUTYH*	*PIK3C2B*	*RET*	*STAG2*	
BCL6	*CDKN1A*	*ERBB3*	*FKBP9*	*JAK3*	*MYB*	*PIK3CA*	*RFWD2*	*STAT3*	
BCOR	*CDKN1B*	*ERBB4*	*FLCN*	*KDM6A*	*MYBL1*	*PIK3R1*	*RHPN2*	*STAT6*	
BCORL1	*CDKN1C*	*ERCC2*	*FLT1*	*KDM6B*	*MYC*	*PIM1*	*ROS1*	*STK11*	
BLM	*CDKN2A*	*ERCC3*	*FLT3*	*KDR*	*MYCL1*	*PMS1*	*RPL26*	*SUFU*	
BMPR1A	*CDKN2B*	*ERCC4*	*FLT4*	*KIT*	*MYCN*	*PMS2*	*RUNX1*	*SUZ12*	
BRAF	*CDKN2C*	*ERCC5*	*FUS*	*KRAS*	*MYD88*	*PNRC1*	*SBDS*	*SYK*	

Table A2. Immunohistochemical Antibodies Used in This Study

Antibody	Clone (Company)	Dilution	Antigen Retrieval	Detection System
MET	SP44 (Spring Bioscience, Pleasanton, CA)[23]	1:100	Citrate buffer/pressure cooker	Leica Novolink
ALK	5A4 (Leica Biosystems, Nussloch, Germany)[24,25]	1:75	Citrate buffer/pressure cooker	Leica Novolink
ROS1	D4D6 (Cell Signaling Technology, Danvers, MA)[26]	1:400	EDTA/pressure cooker	Leica Novolink