original reports abstract

Landscape and Clonal Dominance of Co-occurring Genomic Alterations in Non–Small-Cell Lung Cancer Harboring *MET* Exon 14 Skipping

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PURPOSE *MET* exon 14 skipping alterations (*MET*ex14) comprise a diverse set of actionable oncogene drivers in non–small-cell lung cancer (NSCLC). Recent studies have established the efficacy of tyrosine kinase inhibitors for this patient population. The landscape of co-occurring genetic alterations in *MET*ex14 NSCLC and their potential impact to therapeutic sensitivities has not yet been fully described.

MATERIALS AND METHODS *MET*ex14 NSCLC cases were collected from three cohorts: the VISION trial, and data sets from Guardant360 and GenePlus. Clinicopathologic characteristics and *MET*ex14 mutation sites were analyzed and compared across data sets. Co-occurring genetic alterations and the clonality relationships to *MET*ex14 were evaluated.

RESULTS Of 40,824 NSCLCs, 692 *MET*ex14 cases (1.7%) were identified, including 332 in Guardant360, 188 in VISION, and 172 in GenePlus. The demographics and mutation type and/or sites were similar in the Asian versus Western cohorts. *MET* amplification, which were found to be associated with sensitivity to MET kinase inhibitors, co-occurs in 7.6%-13.8% of cases, whereas kinase domain secondary mutation of *MET* co-occurs in 5%-6%. When co-occurring with *MET*ex14, *EGFR* mutations were often identified as the dominant clone (78%, 7 of 9), whereas when co-occurring, *MET*ex14 (39%, 7 of 18) and *KRAS* (44%, 8 of 18) had similar rates of clonal dominance. *PIK3CA and PTEN* mutations were almost always subclones (89%, 16 of 18) to *MET*ex14. Moreover, *RET-CCDC6* fusion and *EGFR* mutation were detected following crizotinib treatment in two patients, suggesting novel mechanisms of resistance.

CONCLUSION *MET*ex14 mutations frequently co-occur with other potential driver oncogenes with differing patterns of clonal dominance observed among the drivers. This cellular context can provide insights into whether *MET*ex14 is acting as a primary oncogenic driver or resistance mechanism and help guide treatment choices.

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BACKGROUND

MET exon 14 skipping alterations (*MET*ex14) have recently been established as an actionable oncogene driver in non–small cell lung cancer (NSCLC).[1] Small molecule *MET* tyrosine kinase inhibitors (TKIs) have shown efficacy in patients with *MET*ex14 NSCLC, with objective response rate ranging from 25% to 68% and median progression-free survival at 7.6-13.8 months.[2-7] From 2020 to 2021, capmatinib and tepotinib received US Food and Drug Administration approval for *MET*ex14 NSCLC, representing a significant milestone in MET TKI development.

Obtaining a full understanding of co-occurring alterations with *MET*ex14 could be crucial in providing novel insights to increase our understanding of treatment sensitivity and resistance in *MET*ex14 NSCLC, and thus, guide future therapeutic strategy development. Acquired *MET* kinase domain (KD) mutations in residues D1228 and Y1230 have been shown to cause MET TKI resistance.[8] Recent studies also indicate that some co-occurring alterations are detected in TP53, RAS-MAPK, and PI3K pathways.[9] Gene amplifications (eg, *EGFR*, *MDM2*, and *CDK4*) were also observed in 6%-35% of *MET*ex14 NSCLC.[10] Some of these genomic alterations have been confirmed as mechanisms of MET TKI resistance, especially *RAS-MAPK* and *PI3K* pathways.[9,11-14] Furthermore, the efficacy of immunotherapy for patients with *MET*ex14 NSCLC was low despite high programmed death-ligand 1 expression.[15] However, comprehensive landscape

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

To evaluate the mutational profile and co-occurring genetic alteration landscape of non–small-cell lung cancer harboring *MET* exon 14 skipping (*MET*ex14) and the clonality relationship between the *MET*ex14 and co-occurring mutations inferred from the variant allele frequency and dissect the potential resistance mechanisms in cases with longitudinal biopsies.

Knowledge Generated

The demographics and mutation type and/or sites of *MET*ex14 were similar in the Asian versus Western cohorts. *MET*ex14 mutations frequently co-occur with other potential driver oncogenes with differing patterns of clonal dominance observed among the drivers, and usually nondominant subcloned when co-occurred with *EGFR* and *ERBB2*.

Relevance

*MET*ex14 can act as a primary oncogenic driver or resistance mechanism, suggesting that appropriate treatment choices can be potentially guided by co-occurring alterations.

description of co-occurring mutations with *MET*ex14 in NSCLC is still missing.

Here, we leveraged three cohorts of *MET*ex14 NSCLCs and aimed to evaluate the mutational profile and co-occurring genetic alteration landscape of *MET*ex14 NSCLC across countries. We evaluated clonaility relationship between the *MET*ex14 and co-occurring mutations inferred from the variant allele frequency (VAF) and dissected the potential resistance mechanisms in cases with longitudinal biopsies.

MATERIALS AND METHODS

Study Population and Platform

Three data sets were queried for *MET*ex14 NSCLC: Guardant360 (July 2019 to July 2020), GenePlus (both circulating tumor DNA [ctDNA] and tissue, February 2017 to April 2020), and VISION trial ctDNA cohort (NCT02864992) detected by Guardant360. The Data Supplement shows Guardant360 ctDNA 74 gene and VISION ctDNA 73 gene (without *CDK12*) panels. The Data Supplement also shows GenePlus 1,021 and 59 gene panels for ctDNA or tissue. The panels used in tissue or blood samples from GenePlus were summarized in the Data Supplement. There were 48 genes covered in all patients across the three data sets (Data Supplement).

*MET*ex14 Detection

For Guardant360 (also VISION), single-nucleotide variant or indel variant that overlaps any of the two splice regions of MET exon 14 (chromosome 7:116411902-116412043; human genome [hg19]) defined as eight bp into the intron or three bp into the exon was identified with the Guardant360 assay. Detection of indels larger than 50 bp is described in previous publication.[16] For GenePlus, the regions defined as *MET*ex14 were the same to Guardant360. Additionally, variants that affect bases as far as 26 bp into the intron were also identified as *MET*ex14.

Actionable Mutation Determination

The actionability of each mutation was determined when it was considered as pathogenic by Catalogue Of Somatic Mutations In Cancer (COSMIC) Score.[17]

Estimation of Mutation Clonality

Variant clonality was determined by normalizing VAF to the maximum somatic VAF in a sample. Variants were classified as clonal if the normalized value was ≥ 0.5, subclonal for values < 0.5 but ≥ 0.05, and subclonal minor if < 0.05.

Statistical Analysis

Group comparisons were performed using a 2-tailed chi-square test, with significance threshold of P value $< .05$. Analyses were performed using GraphPad Prism 8.0.

RESULTS

Clinicopathologic Characteristics of ctDNA Detected *MET*ex14 NSCLC

A total of 692 patients with NSCLC with *MET*ex14 were identified from three independent data sets of a combined total of 40,824 patients with NSCLC with an overall incidence of 1.7%, including Guardant360 (332 of 20,987, 1.6%), GenePlus (172 of 14,657, 1.2%), and VISION trial (188 of 5,180, 3.6%). Patient demographics and tumor characteristics were summarized in the Data Supplement. In all three data sets, *MET*ex14 occurred with higher frequency in adenocarcinoma, with increasing age and equal sex distribution.

When the GenePlus ctDNA cohort (n = 37) was compared with the Western data sets (Guardant360 plus VISION, n = 520), there were no differences in age (median 70.5 *v* 73 years) and sex distribution (female 54% *v* 57%), and similar patient demographic characteristics were noted in both Asian and Western data sets. Prevalence of *MET*ex14 was higher in the VISION trial (3.6%) than the other two real-world cohorts (Guardant360 [1.6%] and GenePlus [1.2%]), likely because VISION trial excluded *EGFR* and *ALK*-positive patients at initial screening.

Mutation Characteristics of *MET*ex14 in NSCLC

Next, we characterized the mutational landscape of *MET*ex14 NSCLC from the three data sets. The positions of *MET* mutations and the prevalence by functional alterations

are shown in Figure 1 and Table 1. The functional sites of *MET* mutations were similar across the three data sets, allowing the differences among platforms and *MET*ex14 detection methods. In Guardant360, the prevalence by functional alteration sites were as follows: donor (44.3%), acceptor (30.4%), D1010 (20.5%), and Y1003 (3.9%). The most frequent mutation type was base substitution (55.7%), followed by indel (43.4%). Both the VISION and the GenePlus data sets revealed a remarkably similar pattern in terms of prevalence by functional alteration sites and most frequent mutation type (Fig 1, Table 1). Regarding acceptor versus donor sites and SNVs versus indels, there was no significant difference between the Asian and Western data sets.

Co-occurring *MET* Amplification (*MET*amp) With *MET*ex14 in NSCLC

The frequency of *MET*amp co-occurred with *MET*ex14 was 8.4% (Guardant360), 13.8% (VISION), and 7.6% (GenePlus), respectively (Data Supplement). The mean VAF of *MET*ex14 in cases concomitant with *MET*amp was significantly higher than those without *MET*amp across three data sets ($P < .001$, Data Supplement). The distribution of gene copy number for Guardant360 and GenePlus is displayed in the Data Supplement. Most of the patients had an *MET* gene copy number between 2 and 4 (24 of 28 in Guardant360; 9 of 13 in GenePlus).

In VISION trial, four in five co-occurring *MET*ex14 and *MET*amp cases (80%) had a partial response and one had target lesion tumor reduction, but progressive disease because of a new lesion. The response rate was numerically higher than in patients whose tumor did not have co-occurring *MET*amp (4 of 5, 80% *v* 28 of 61, 46%), suggesting co-occurring *MET*amp might be associated with responsiveness to targeted therapy,[4] although the number in the *MET*amp group was too small for a statistical comparison.

Secondary Mutations Within *MET* in *MET*ex14 NSCLC

Secondary mutations located in *MET* KDs, such as D1228 and Y1230, have been reported to be associated with resistance to MET TKI.[8,11] In Guardant360, 46 (13.9%) had *MET* secondary mutations including 17 (5%) having at least one secondary mutation in the KDs, including H1094C/Y, D1228H/N, and Y1230C/H (Fig 2B). In GenePlus, secondary mutations were detected in 29 (16.9%) patients, including 11 (6%) in the KDs (Fig 2B). Most of the KD mutations were deemed pathogenic on the basis of COSMIC prediction score > 0.95. The VISION data set only included TKI-naive (part of trial eligibility criteria) patients and had no secondary mutations.

Co-ocurring Genetic Alterations in Bypass Pathways in *MET*ex14 NSCLC

Next, we evaluated other co-occurring genentic alterations with *MET*ex14 in NSCLC. For a fair comparison, we focused only on the 48 cancer genes tested in all patients across the three data sets. First, we compared whether any comutations were enriched in *MET*ex14 NSCLC compared with NSCLC without *MET*ex14. In the Guardant360 and GenePlus cohorts, no gene alterations were enriched for co-occurring with *MET*ex14. *EGFR*, *KRAS*, and *TP53* were significantly enriched in the non-*MET*ex14 tumors in both cohorts (Figs 3A and 3B), consistent with the notion that *MET*ex14 is a de novo oncogenic driver.

We then focused on comutations in key bypass pathways, including RAS-MAPK, EGFR and ERBB2, PI3K and AKT, and cell cycle (*CDK4/6*) pathways (Data Supplement). In Guardant360, 136 (41%) had at least one co-occurring alteration in those pathways, including *EGFR* (24 of 332), *ERBB2* (13 of 332); *KRAS* (20 of 332), *BRAF* (12 of 332), *NRAS* (7 of 332), *NF1* (3 of 332), and *PIK3CA* (33 of 332), *PTEN* (7 of 332), and *AKT1* (1 of 332) (Data Supplement). In the VISION trial, *EGFR* driver mutations were specifically excluded. Fifty-one (27%) patients had at least one co-occurring alteration; *ALK* and *RET* fusions, each occured once (Data Supplement). In GenePlus data set, 49 of 172 (28%) *MET*ex14 cases had at least one co-ocurring alteration (Data Supplement), with similar distribution to Guardant360 data set.

Clonality Relationship Between *MET*ex14 and Other Driver Oncogenes in NSCLC

Oncogene variant clonality can be deduced from VAF to infer dominant versus nondominant clonal relationship. We annotated comutations using COSMIC score to identify the potential activating mutations and found that 76 unique cases (23%) had at least one activating comutation in Guardant360 data set (Fig 3C). We then evaluated the relative clonality inferred by VAF for *MET*ex14 and the second activating mutations to dissect potential resistance mechanisms. In the *EGFR* cases, most cases (7 of 9, 78%) had *EGFR* as the dominant clone, as was the one case with *ERBB2* mutation (A775_G776insYVMA, VAF 31.4%) being dominant compared with 0.56% in *MET*ex14 (GH#128, Fig 3C and Data Supplement). ALK fusions also had high VAF in both of two co-occurring cases (GH#201 and GH#213, Fig 3C). For *PIK3CA* and *PTEN* mutations, the clonality of *MET*ex14 was higher in 16 of 18 (89%) cases. Interestingly, for *KRAS* and *MET*ex14 co-occurring cases, 44% (8 of 18) had *KRAS* as the dominant clone, and 39% of cases were *MET*ex14-dominant. *KRAS* G12C and *BRAF* V600E were both detected in one single patient (GH#89, Fig 3C), and *MET*ex14 was the dominant mutation.

Eighteen out of 188 (9.6%) patients in the VISION cohort had co-occurring alterations and the dominant clonality of *MET*ex14 was observed in 2 of 5 (40%) patients with co-occurring in *EGFR* and *ERBB2*, 0 of 1 in *ALK* (*EML4-ALK* fusion), 3 of 4 (75%) in *K/NRAS*, and 7 of 8 (87.5%) in *PIK3CA* and *PTEN* (Data Supplement). In GenePlus, similar pattern was seen (Data Supplement). In summary, when co-

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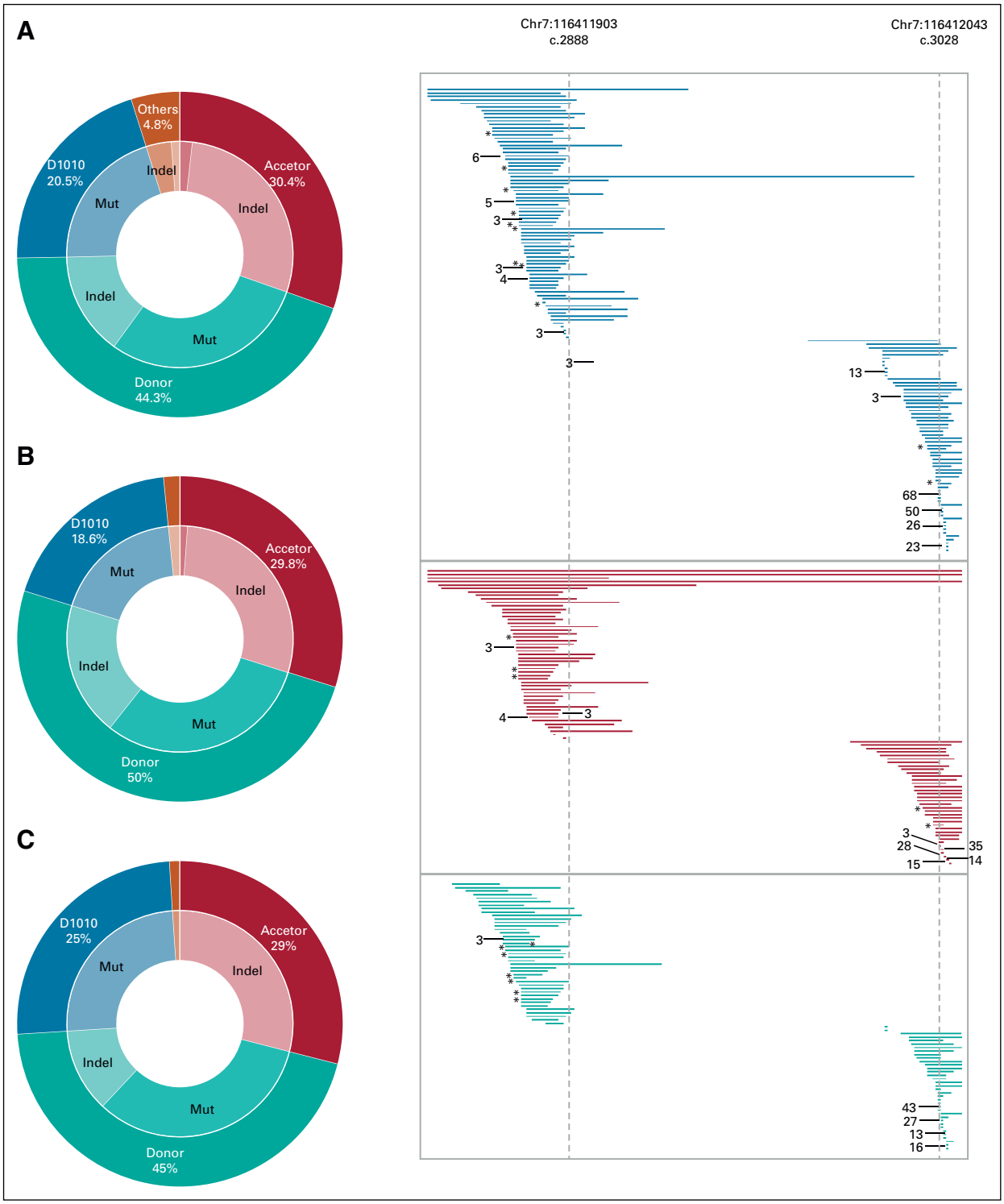

FIG 1. MET exon 14 skipping alterations mutation distribution in the three data sets: (A) Guardant360, (B) VISION, and (C) GenePlus. Genomic positions with alterations occurring in more than one case are indicated with an asterisk (*) for two and the number of cases is greater than two. Mut, mutation.

occurring with *EGFR* and *ERBB2* mutations, *MET*ex14 alterations were more frequently observed as a subclone, whereas in the *KRAS* and *BRAF* co-occurring cases, *KRAS* and *MET*ex14 had similar frequency of being clonal.

We next explored the potential impact of clonality of *MET*ex14 and coalterations on response to *MET* inhibitors, leveraging the VISION cohort. In the 62 patients with outcome data, 52 had tumors with clonal *MET*ex14 and 10

TABLE 1. Patient Characteristics and METex14 Functional Site by Data Set

Characteristics	Guardant360	Vision	GenePlus
No. of cases tested = 40,824	20,987	5,180	14,657
No. of METex14 = 692	332	188	172
Frequency, %	1.6	3.6	1.2
Age at tested, years			
Median (range)	73 (53-81)	72 (49-89)	69 (36-95)
Sex, No. (%)			
Female	197 (59)	101 (54)	83 (48)
Male	135 (41)	87 (46)	89 (52)
Race, No. (%)			
White	—	136 (72)	0
Asian	—	32 (17)	172 (100)
NA	332	20	—
Smoking history, No. (%)			
No	—	87 (46)	73 (42)
Yes	—	83 (44)	36 (21)
NA	332	18 (9)	63 (37)
Histologic subtype, No. (%)			
Adenocarcinoma	268 (81)	121 (64)	105 (61)
Squamous	34 (10)	16 (9)	9 (5)
Others	30 (9)	51 (27)	58 (33)
Functional site, No. (%)			
Acceptor site	101 (30.4)	56 (29.8)	50 (29)
Indel	95 (28.6)	54 (28.7)	50 (29)
Base substitution	6 (1.8)	2 (1.1)	0
Donor site	147 (44.3)	94 (50)	77 (45)
Indel	49 (14.7)	36 (19.1)	20 (12)
Base substitution	98 (29.5)	58 (30.8)	57 (33)
Y1003	13 (3.9)	—	2 (1)
D1010	68 (20.5)	35 (18.6)	43 (25)
Whole exon 14 deletion	0	3 (1.6)	0
Others	3 (0.9)	0	0

Abbreviation: *MET*ex14, MET exon 14 skipping alterations.

had subclonal. The response rate for clonal group was 46.1% and subclonal group was 50% (Data Supplement), suggesting similar responses to tepotinib regardless of clonality; however, this analysis was underpowered because of the low number in the subclonal cohort.

Of the 18 patients with concomitant alterations, seven had clinical outcome information with tepotinib (Data Supplement). One case with both *MET*ex14 and *ERBB2* (L796_V797del) had a reduction in tumor size. The *MET*ex14 was not the clonal mutation for this patient. No response was seen in the remaining six patients with point mutations in *PI3KCA* (n = 2), *K/NRAS* (n = 2), and *PTEN* (n = 2) at baseline, regardless of the clonality of *MET*ex14.

Longitudinal ctDNA Analysis for *MET*ex14 NSCLC

In Guardant360, 23 cases had more than one blood sample collected at different time points. Two cases had acquired secondary mutations in the *MET* KDs (Fig 3D), Y1230C (GH#070) and D1228N (GH#146). In GH#146 (Data Supplement) with *MET*ex14 (VAF 9.9%) and an acquired *MET* D1228N (VAF 1.5%), an *EGFR* exon 20 insertion (A767_V769dup, VAF 3.9%) and *EGFR* amplification were also observed, all following therapy with crizotinib, suggesting that alterations in *EGFR* can mediate polyclonal resistance to MET TKI therapy. Conversely, *EGFR* driver alterations (defined with high VAF) were detected in 3 cases (GH#127, #134, and #162, Fig 3D), suggesting *MET*ex14 could be a resistance mechanism to EGFR TKI, consistent with previous publications.[18,19] In GH#162, nine different ctDNA samples was taken over three years, and *MET*ex14 was acquired at the fifth ctDNA. *EML4-ALK* and *BRAF* V600E were also observed over the course of treatment, suggesting a high heterogeneity of resistance mechanisms. In GH#107, *RET-CCDC6* fusion was acquired, following crizotinib and chemoimmunotherapy, with VAF of 1.53% relative to *MET*ex14 VAF 16.5%. The emergence of *RET-CCDC6* was also confirmed in tumor tissue by FISH assay, appearing only after crizotinib, indicating *RET* fusion as an acquired potential resistant mechanism to *MET* TKI.

In the GenePlus data set, six patients had samples collected at different time points. Five were collected before and after crizotinib treatment, and one was taken before and after afatinib. *MET* D1228N was identified in two post-crizotinib cases (GP#52 and #53, Fig 3D and Data Supplement). In GP# 46, *EGFR* exon 20 insertion (S768_D770dup, dominant clone), *MET*ex14, and *EGFR* amplification were present before treatment. Following afatinib treatment, *EGFR* exon 20 and *MET*ex14 remained detectable in ctDNA, with loss of *EGFR* amplification. Taken together, we confirm secondary *MET* mutations as a consistent resistance mechanism to MET TKI therapy, and provide evidence of novel resistance mechanisms, such as acquired *RET* fusion or *EGFR* mutation.

Tissue and Liquid Biopsy Concordance for *MET*ex14 NSCLC

Ten cases from GenePlus cohort had both tissue and ctDNA profiling at the same time from the same patients (Data Supplement). *MET*ex14 were identified both in tissue and ctDNA at identical functional sites for all, demonstrating perfect concordance. Among them, four had identical comutation results. The remaining six had different co-ocurring genomic alterations in *TP53* (ctDNA only), *MDM2* amplification (ctDNA only), *PTEN* mutation (tissue only), and four genomic mutations (*EGFR*, *NF1*, *TP53*, and *RB1*, tissue only, Data Supplement).

DISCUSSION

In this retrospective multicohort study, we identified 692 cases of *MET*ex14 NSCLC, including 557 ctDNA cases,

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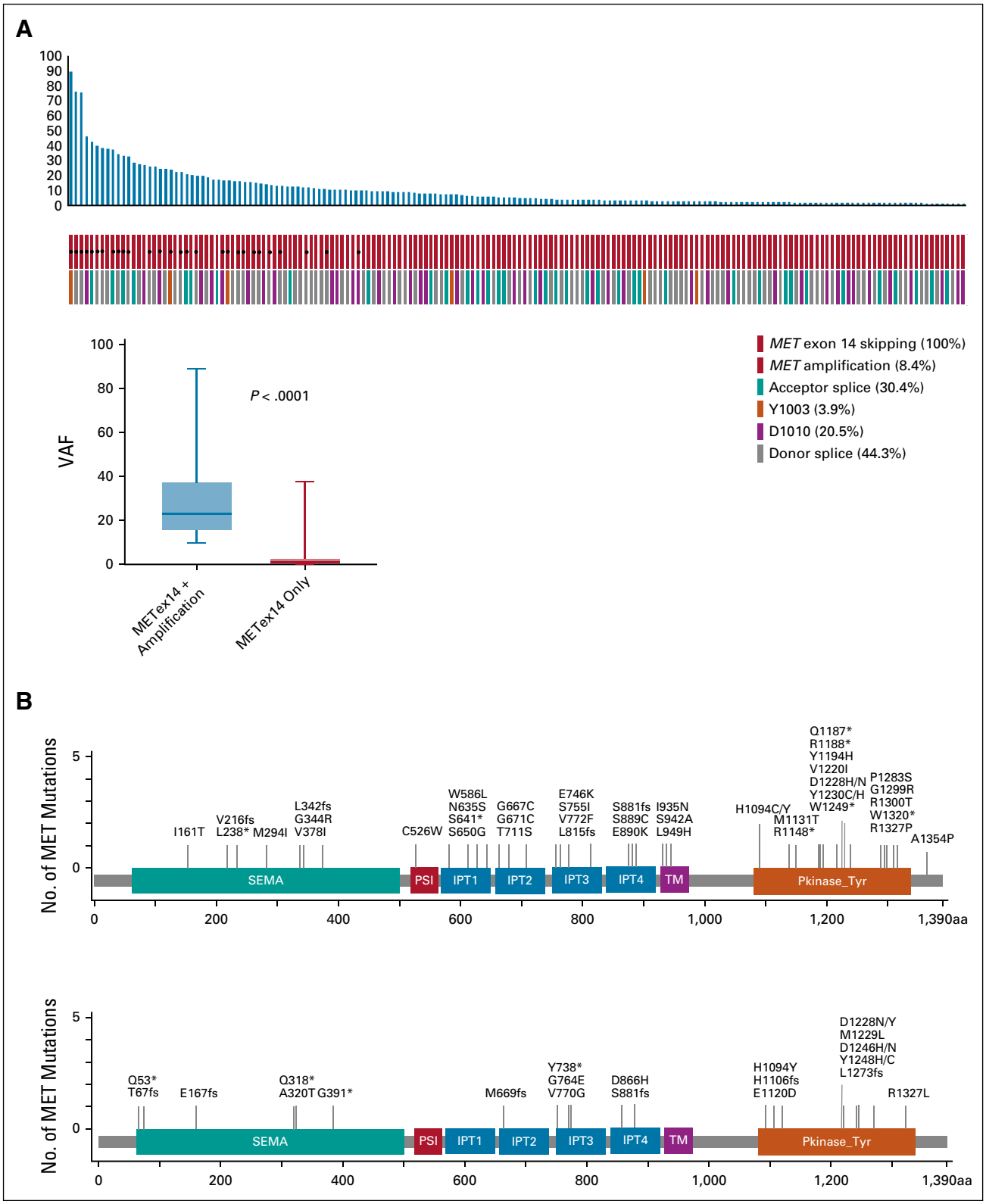

FIG 2. Co-occurring *MET* amplification and secondary mutations. (A) VAF of *MET*ex14 concurrent with *MET* amplification versus *MET*ex14 only in Guardant360. (B) Second site MET mutations in patients with *MET*ex14 non–small-cell lung cancer in Guardant360 and GenePlus data sets. IPT, immunoglobulin plexins transcription domains; *MET*ex14, MET exon 14 skipping alterations; PSI, plexins-semaphorin-integrin domain; TM, transmembrane domain; VAF, variant allele frequency.

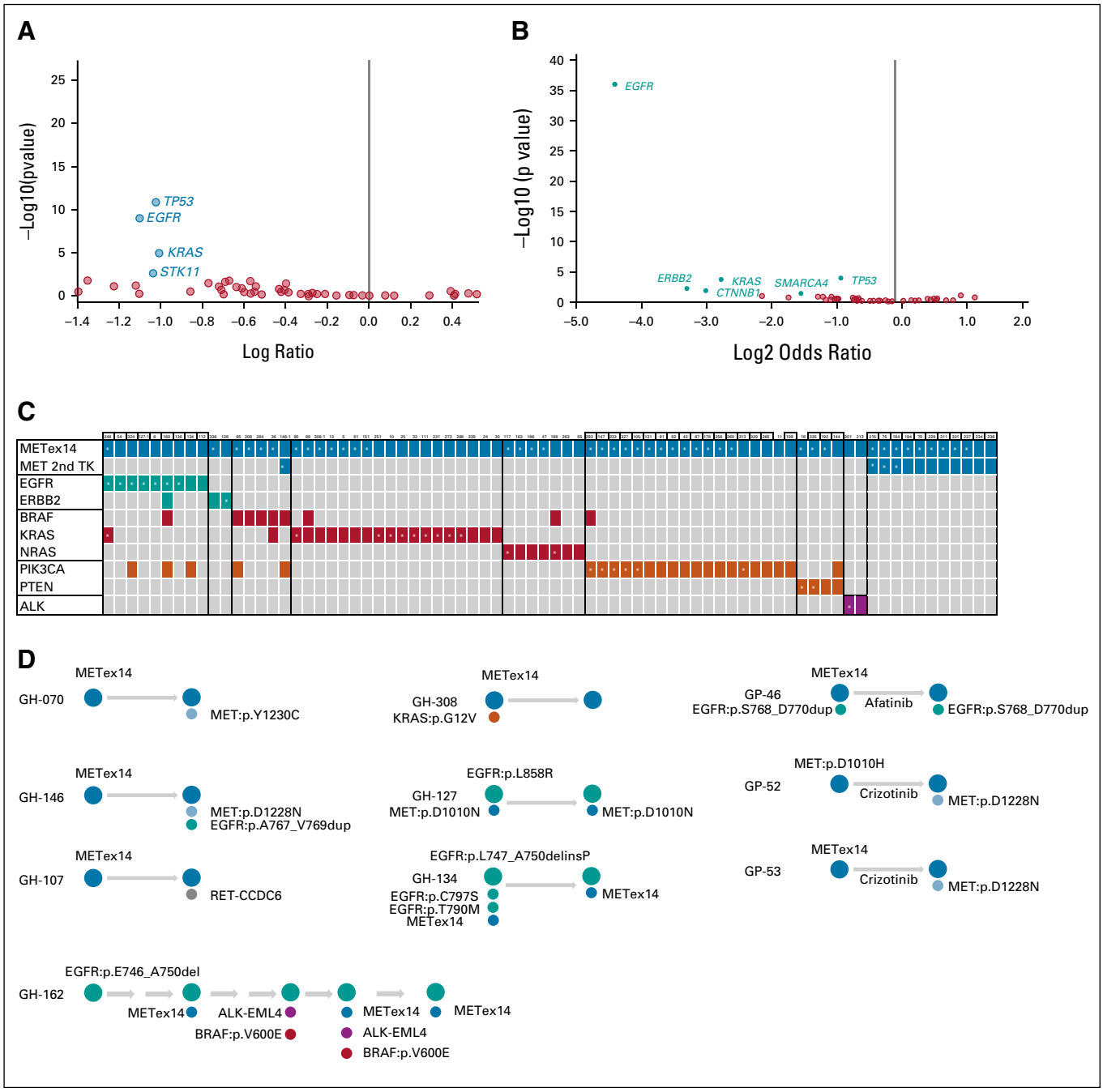

FIG 3. Co-occuring mutations with *MET*ex14 and longitudinal ctDNA cases. (A and B) Volcano plots showing the difference of co-occurring alterations between *MET*ex14 NSCLC and non-*MET*ex14 NSCLC in (A) Guardant360 and (B) GenePlus data sets. (C) Cases with co-occurring alterations with *MET*ex14 were shown, and the gene with dominant clone was labeled by star. (D) Genomic alterations of longitudinal cases at different time for patients with *MET*ex14 NSCLC in Guardant360 (GH) and GenePlus (GP) data sets. *MET*ex14, MET exon 14 skipping alterations; NSCLC, non–small-cell lung cancer; TK, tyrosine kinase domain.

which is, to our knowledge, the largest ctDNA *MET*ex14 NSCLC cohort reported to date. Our cohort confirms that patients with *MET*ex14 NSCLC are older with equal sex distribution,[20-22] with a relatively high incidence in non-adenocarcinoma pathology. We separately confirmed these clinicopathologic features in both the Asian cohort and the predominantly Western data sets. The incidence of *MET*ex14 in GenePlus data set was 1.17% in 14,657 cases, similar to prior reports of 0.9% in 1,296 Chinese lung cancer cases,[23,24] also similar to the Guardant360 real-world data set with an incidence of 1.6%. Furthermore, the types of *MET*ex14 alterations and the location were similar (Table 1), supporting that there are no significant differences between Asian and Western patients with *MET*ex14 NSCLC.

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Co-occuring genetic alterations with an oncogene driver can associate with clinical response or resistance. We found *MET*amp as the most frequent co-occuring alterations in *MET*ex14 NSCLC, at approximately 8%. A number of recent studies have reported on the outcomes of patients with *MET*ex14 and *MET*amp NSCLC to MET TKI, including four of the five patients achieving partial response in the VISION study[4] and 75%-80% partial response in cohort 4 and 5b in GEOMETRY mono-1 study.[3] With the small sample sizes acknowledged, these data indicate that *MET*ex14 concurrent with *MET*amp may have higher sensitivity to MET TKI. In our analysis, we found significantly higher VAF for *MET*ex14 when *MET*amp is detected. Although it is likely that the high VAF of *MET*ex14 with *MET*amp was related to increased copy number, as previously demonstrated in *EGFR*-mutant NSCLC,[25] *MET*ex14 and *MET*amp tumors represent a subgroup that are deeply addicted to aberrant *MET* signaling for tumorigenesis, and thus, a subgroup where the benefit of MET TKI is likely to be most pronounced. By contrast, secondary mutations in the *MET* KD are resistant mechanisms to MET TKI. In our study, a number of KD mutations, including D1228N in both Guadarnt360 and GenePlus, were detected, at a rate of 5%-6%, representing potential resistance mechanisms.

Using relative VAF to infer clonality, we analyzed other functional oncogenic alterations co-occurring with *MET*ex14. In the Guardant360 data set, most of the *EGFR* (9) and *ERBB2* (1) mutations had higher clonality than *MET*ex14, indicating that *EGFR* and *ERBB2* mutations were the dominant oncogene drivers, and suggesting that *MET*ex14 alterations were the potential drivers of resistance. This is consistent with the established notion that *MET*ex14 and *MET*amp are resistance mechanisms to EGFR TKI for *EGFR*-mutant NSCLC.[18,19,26]

KRAS-activating mutations were found in only three cases in GenePlus cohort; however, 18 cases were identified in the Guardant data set,[10,12,24] consistent with prior reports showing RAS alterations are more common in Western lung cancer populations.[27] *KRAS* mutations and *KRAS* and *BRAF* amplification constitute a cause of resistance to MET TKI on the basis of previous clinical and preclinical studies.[9,12,28] We found that *KRAS* mutation and *MET*ex14 demonstrate similar tendency to be the dominant clone when co-occurring, as opposed to *EGFR* and *ERBB2*, which are usually the dominant driver. Now that both *KRAS* G12C[29] and *MET*ex14 have available targeted therapy options, it is of great importance to recognize the dominant clone to prioritize treatment: ultimately, it is likely that dual inhibition of KRAS and MET pathways may be needed for these cases.

Through longitudinal ctDNA analysis, we identified, to our knowledge, the first reported case of acquired *RET-CCDC6* fusion co-occurring with *MET*ex14 (GH #107; Fig 3C). Acquired *RET* fusion has been reported in osimertinib-resistant *EGFR*-mutant NSCLC, where acquired resistance was overcome by *EGFR* plus *RET* inhibition.[30] Similar to *ALK* fusion concurrent with *MET*ex14, either combination therapy or a multikinase inhibitor would merit investigation as a therapeutic option.

Our study analyzed data sets from various sequencing platforms used in real-world and a clinical trial, which brings strengths as well as weaknesses. The addition of GenePlus cohort allowed a general comparison between Asian and Western populations, but the conclusion is limited by the heterogeneity of laboratory assays and sample source. Furthermore, the comutation and clonality analysis were only comparable across cohorts in the mutually tested 48 genes in all panels. Although they covered key oncogenic pathways in cancer, other genes of potential interest, such as *SMAD4* and *EZH2*, cannot be compared because they were only tested in Guardant360 and GenePlus 1,021 platforms. One important inherited limitation of real-world study is the incomplete data. In our study, for example, we did not have clinical outcome data to different therapies in the Guardant360 and GenePlus cohorts. As such, future studies, both experimental and clinical, are warranted to validate these provocative genomics findings and their clinical implications.

In conclusion, we describe a large cohort of *MET*ex14 NSCLC, mostly identified through ctDNA detection. We demonstrate that co-occurring *MET*amp is associated with high *MET*ex14 VAF and potential targeted therapy benefit, whereas *MET* KD secondary mutations are associated with targeted therapy resistance. When *MET*ex14 co-occurs with *EGFR* and *ERBB2* mutations, *MET*ex14 most commonly serves as a nondominant subclone and is a potential mediator of EGFR TKI resistance. Finally, we reveal emerging novel resistance mechanisms to MET TKI, such as *RET* fusion, which warrant future translational and therapeutic studies to overcome resistance.

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