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ORIGINAL RESEARCH ARTICLE

Mutational Landscape and Expression of PD-L1 in Patients with Non-Small Cell Lung Cancer Harboring Genomic Alterations of the MET gene

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Abstract

Background Mesenchymal-to-epithelial transition *(MET)* exon 14 skipping mutations and *MET* gene amplification occur in 3–5% of non-small cell lung cancer (NSCLC) patients. Tyrosine kinase inhibitors (TKIs) targeting *MET* alterations have shown promising results in these patients.

Objective The aim of this study was to describe the genomic profile, PD-L1 expression and clinicopathological features of *MET* dysregulated NSCLC.

Patients and Methods We identified 188 patients with advanced-stage NSCLC with data on MET expression by immunohistochemistry (IHC). IHC for PD-L1 expression was performed in 131 patient samples, and next-generation sequencing (NGS) analysis was performed in 109 patient samples.

Results *MET* exon 14 skipping alterations were identified in 16 (14.7%) samples, *MET* amplifications with cut-off ≥4 copy number variations were identified in 11 (10.1%) samples, and an oncogenic *MET* mutation (MET p.D1228N) was identified in 1 (0.9%) sample. 12/15 tumors (80.0%) harboring *MET* exon 14 alterations and 7/11 (63.6%) *MET*-amplified tumors expressed PD-L1 in ≥1% of tumor cells. Tumors harboring *MET* exon 14 skipping alterations expressed PD-L1 more frequently than *MET* wild-type IHC-positive tumors ($p = 0.045$). Twenty-five percent of *MET* exon 14-altered cases and 33% of *MET*-amplified cases harbored potentially targetable oncogenic co-mutations in *KRAS*, *BRAF,* and *EGFR.* The most frequent co-occurring mutations in all *MET*-altered tumors were *TP53*, *KRAS*, *BRAF,* and *CDK4*.

Conclusions We demonstrated that *MET* exon 14 skipping alterations and *MET* amplification are not mutually exclusive to other oncogenic co-mutations, and report the association of genomic *MET* alterations with PD-L1 expression. Since genomic *MET* alterations are emerging targets requiring upfront treatment, optimal understanding of the co-mutational landscape for this patient population is needed.

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Key Points

Understanding the co-mutational profile of mesenchymal-to-epithelial transition (*MET*)-altered tumors is crucial as MET-directed tyrosine kinase inhibitors have emerged as treatment options.

In 188 patients with advanced-stage non-small cell lung cancer, *MET* exon 14 skipping alterations were associated with PD-L1 expression.

Several *MET* exon 14-altered and *MET*-amplified tumors harbored oncogenic co-mutations in *KRAS*, *BRAF,* and *EGFR*.

1 Introduction

The mesenchymal-to-epithelial transition (*MET)* proto-oncogene on chromosome 7 encodes for the membrane-spanning tyrosine kinase receptor MET and is activated by the hepatocyte growth factor (HGF) [1, 2]. HGF and MET signaling induce proliferation, epithelial-mesenchymal transition, scattering, and invasion of epithelial cells, and promote anti-apoptotic responses in the tumor microenvironment [3–5]. Aberrant MET and HGF signaling have been demonstrated in a wide range of carcinomas, including carcinomas of the gastrointestinal tract, head and neck, and lung [6–9].

MET alterations in non-small cell lung cancer (NSCLC) can occur at the genomic level through mutations, amplifications, or gene fusions. Mutations within the juxtamembrane domain of *MET* encoded by exon 14 occur in 3–4% of NSCLCs [10–13]. Mutations within splice sites flanking *MET* exon 14 result in *MET* exon 14 skipping. In this case, degradation of the MET receptor is hampered, whereas MET activation and signaling are sustained, resulting in oncogenic transformation. To investigate such alterations RNA sequencing can be applied to detect fusions between exon 13 and exon 15 (resulting in *MET* exon 14 skipping), whereas DNA sequencing detects mutations predicted to result in *MET* exon 14 skipping. Differences in the detection rate of *MET* exon skipping events between RNA- and DNA-based sequencing were reported [14, 15]. High-level amplification of the *MET* gene, present in about 3–5% of lung adenocarcinomas, may also lead to MET-dependent oncogenesis [16–20]. *MET* gene copy numbers are assessed by either fluorescence *in situ* hybridization (FISH) or next-generation sequencing (NGS) gene panels. At the protein level, MET overexpression is detected by immunohistochemistry (IHC) in approximately 22–24% of NSCLCs and may occur in the absence of genomic *MET* alterations [10, 16, 20–23]. Thus, it is not used as a screening tool for detecting *MET* amplification or *MET* exon 14 skipping. Importantly, available MET tyrosine kinase inhibitors (TKIs) have shown little success in tumors overexpressing MET in the absence of mutations within the *MET* gene [24–27]. Several TKIs in trials for *MET*-driven cancers inhibited cancer growth in *MET*-amplified as well as *MET* exon 14-mutated tumors [28–34]. Still, resistance to TKIs develops in the course of treatment and even pre-exists in certain tumors, and the mechanisms of such primary resistance remain unclear [35–39]. Earlier reports based on large NGS panels [11] and smaller polymerase chain reaction (PCR)-based gene panels [12, 40] stated that *MET* exon 14 alterations are mutually exclusive to other oncogenic driver mutations. Some studies indicated an association of *MET* dysregulation with PD-L1 expression [41, 42]. In order to characterize the genomic and clinical features of *MET* dysregulated tumors in patients with advanced NSCLC, we analyzed the genomic profile, PD-L1 expression, and clinicopathological features in relation to *MET* dysregulation.

2 Methods

2.1 Study Population

In this retrospective study, patients with advanced stage IV NSCLC treated at the Comprehensive Cancer Centre Zurich (C3Z), University Hospital Zurich between January 2011 and August 2020 with either c-MET staining performed at the time of diagnosis or available tissue and clinical follow-up data were included. Exclusion criteria were non-availability of tumor tissue for c-MET staining, rejection of general consent, or the lack of or incomplete clinical data. A total of 188 patients were included in this analysis and all medical records reviewed. Response Evaluation Criteria in Solid Tumors (RECIST) were applied to evaluate treatment response. Overall survival was collected from the medical records; when data were not available, patients or relatives were contacted by phone.

This study was conducted according to the law and regulations of the local Ethics Commission under reference number KEK ZH-2021-00381.

2.2 Immunohistochemistry and Fluorescence In Situ Hybridization

Formalin-fixed, paraffin-embedded 4 µm sections from tumor blocks were stained by IHC using rabbit anti-c-MET monoclonal antibody (clone SP44, dilution 1/50; Abcam, Cambridge, UK) and rabbit anti-PD-L1 monoclonal antibody (clone E1L3N, dilution 1/100; Cell Signaling

Technology, Danvers, MA, USA). Staining was performed with an automated immunostainer (DiscoveryUltra; Roche Ventana). MET overexpression was scored semi-quantitatively as described by Spigel et al. [26], corresponding to an immunoscore of 3+ (≥ 50% of tumor cells stained exhibiting strong staining intensity) or 2+ (≥ 50% of tumor cells with moderate or higher staining intensity but < 50% strong intensity). PD-L1 expression was scored as described previously by Lacour et al. PD-L1 positivity was defined as ≥ 1% of tumor cells with stained membrane [43]. Scoring was performed by experienced pathologists.

For FISH analysis, *MET* was labeled with Abbott Molecular/Vysis MET SpectrumRed Probe (7q31.2) and the *CEP7* region with Abbott Molecular/Vysis CEP (D7Z1) SpectrumGreen Probe (7p11.1-q11.1) [Abbott Molecular, Baar, Switzerland]. Staining with the fluorescent probes was performed according to the manufacturer's protocol. The *CEP7*-labeled region was used as a reference for *MET* amplification. For scoring purposes, 100 tumor cells were evaluated and the mean *MET/CEP7* ratio was calculated. Amplification of the *MET* gene was defined as a ratio of *MET/CEP7* ≥ 2 according to the University of Colorado Cancer Center criteria [19].

2.3 Next-Generation Sequencing (NGS)

NGS was performed using the commercially available Oncomine Focus Assay (OFA) panel (Thermo Fisher Scientific, Carlsbad, CA, USA), enabling detection of variants in 52 genes, or with the DNA part of the Oncomine Comprehensive Assay (OCA) panel (Thermo Fisher Scientific), enabling detection of variants in 161 genes. Samples were analyzed according to the manufacturer's protocol. Briefly, DNA and RNA were isolated from formalin-fixed, decalcified, paraffin-embedded tumor blocks with a Maxwell 16 FFPE Tissue LEV DNA/RNA Purification Kit (Promega, Fitchburg, WI, USA). Sequencing was performed on the Ion S5™ System using the Ion 540 Sequencing Kit (Thermo Fisher Scientific). Ion Reporter software 5.10 (Thermo Fisher Scientific) was used for alignment (hg19/GRChr37), variant calling and annotations (Oncomine Focus w2.4, DNA/fusions, single sample; filter chain: Oncomine 5% CI, copy number variation (CNV), ploidy greater than or equal to a gain of 2 over normal; and Oncomine Comprehensive v3—w3.2, DNA, single sample; filter chain: Oncomine 5% CI, CNV, ploidy greater than or equal to a gain of 2 over normal OR ≥ 0.5 over normal). *MET* amplification was reported at a cut-off of ≥ 4 CNVs and *MET* exon 14 skipping at a cut-off of 0.02% skipping reads in relation to total mapped fusion panel reads. For a number of patients, FoundationOne CDx™ (Foundation Medicine, Cambridge, MA, USA) was performed according to the manufacturer's protocol and results incorporated in this study.

2.4 Statistics

Descriptive statistics were used to describe tumor and patient characteristics. The Fisher's exact test was applied to test for significance and Kaplan–Meier survival curves were applied for survival analysis. P values < 0.05 were considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics 26.0 (IBM Corporation, Armonk, NY, USA).

3 Results

3.1 Patient Characteristics

Overall, 188 patients with advanced-stage NSCLC (94.3% stage IV, 5.7% stage III) were included in this single-center, retrospective cohort study. Patient characteristics are summarized in Table 1. Median age at diagnosis was 62 years (range 33–89). The majority of patients were men (106/188 [56.4%]) and 156/187 patients were former or current smokers (83.4%; one patient smoking status was not available).

3.2 Immunohistochemistry

Tumor specimens of all patients with available tissue were stained for c-MET. Of 188 patient samples, 94 (50.0%) were positive for MET IHC. PD-L1 expression, either at diagnosis or for the purpose of this study, was available in 131 patients, of whom 69 (52.7%) were positive (cut-off 1% PD-L1 positivity on tumor cells). PD-L1 expression < 1% was detected in 62 patients (47.3%), expression between 1% and 49% was detected in 40 patients (30.5%), and expression ≥ 50% was detected in 29 patients (22.1%).

3.3 Characterization of MET Alterations

NGS data were available for 109 (58.0%) patients. Eighty-three (44.1%) patient samples were sequenced with the OFA, thereof 19 samples were additionally sequenced with the OCA, and 26 (13.8%) patient samples were analyzed using FoundationOne CDx™. From these cases, 28/109 (25.7%) displayed genomic *MET* alterations. Sixteen cases (14.7%) harbored *MET* exon 14 skipping alterations and one case displayed a concurrent *MET* amplification (Fig. 1). Eleven cases were found with *MET* amplifications alone (10.1%) and a single case (0.9%) with an oncogenic *MET* mutation (MET p.D1228N) not leading to *MET* exon 14 skipping (Fig. 1). 26/28 (92.9%) cases with genomic *MET* alterations overexpressed c-MET. In two cases with *MET* exon 14 skipping alterations, MET expression was negative (one patient with squamous cell carcinoma and one patient with pleomorphic carcinoma). Fifty-one MET IHC-negative cases

Table 1 Patient characteristics

	MET E14S [$n = 16$]	*MET* amplified [$n = 11$]	*MET* non-E14S mutation [$n = 1$]	*MET* wt^{IHCpositive} [$n = 68$]	*MET* wt^{IHCnegative} [$n = 92$]	ALL [$n = 188$]
Age at diagnosis of metastatic disease, years						
Median (range)	63 (36–81)	67 (43–73)	60	62 (40–82)	62 (33–89)	62 (33–89)
Sex						
Female	9 (56.3)	5 (45.5)	0 (0)	27 (39.7)	41 (44.6)	82 (43.6)
Male	7 (43.8)	6 (54.5)	1 (100)	41 (60.3)	51 (55.4)	106 (56.4)
Smoking history						
Smoker	11 (68.8)	8 (72.7)	1 (100)	53 (77.9)	84 (91.3)	156 (83.4)
Never smoker	5 (31.3)	3 (27.3)	0	15 (22.1)	8 (8.7)	31 (16.6)
Mean pack-years smoked (SD)	23 (28)	25 (18)	25	33 (28)	33 (27)	32 (27)
Stage						
IV	16 (100)	10 (90.9)	1 (100)	62 (91.2)	89 (96.7)	178 (94.7)
IIIB	0	0	0	3 (4.4)	1 (1.1)	4 (2.1)
IIIA	0	1 (9.1)	0	3 (4.4)	2 (2.2)	6 (3.2)
Histology						
Adenocarcinoma	13 (81.3)	10 (90.9)	1 (100)	67 (98.5)	84 (91.3)	175 (93.1)
SCC	2 (12.5)	1 (9.1)	0	1 (1.5)	6 (6.5)	10 (5.3)
Large-cell carcinoma	0	0	0	0	2 (2.2)	2 (1.1)
Pleomorphic carcinoma	1 (6.3)	0	0	0	0	1 (0.5)
Sequencing						
Oncomine focus assay	11 (68.8)	8 (72.7)	0	49 (72.1)	15 (16.3)	83 (44.1)
FoundationOne CDx™	5 (31.3)	3 (27.3)	1 (100)	12 (17.6)	5 (5.4)	26 (13.8)
None	0	0	0	7 (10.3)	72 (78.3)	79 (42.0)
PD-L1 status						
Positive	12 (75.0)	7 (63.6)	1 (100)	29 (42.6)	20 (21.7)	69 (36.7)
Negative	3 (18.8)	4 (36.4)	0	29 (42.6)	26 (28.3)	62 (33.0)
Not available	1 (6.3)	0	0	10 (14.7)	46 (50.0)	57 (30.3)

Data are expressed as *n* (%) unless otherwise specified

MET mesenchymal-to-epithelial transition, *SD* standard deviation, *SCC* squamous cell carcinoma, *MET wt^{IHCposistive}* *MET* wild-type with IHC-positive tumors, *MET wt^{IHCnegative}* *MET* wild-type with IHC-negative tumors

were tested for *MET* amplification by FISH but not NGS (at the time of diagnosis, not a diagnostic standard). No MET amplification was detected by FISH within this group. Further genomic *MET* dysregulation, in particular *MET* exon 14 mutations, cannot be excluded in those cases.

3.4 PD-L1 Expression is Upregulated in Tumors Harboring MET Exon 14 Skipping Alterations Compared with MET Wild-Type IHC-Positive Tumors

PD-L1 status was evaluated in 27/28 *MET*-altered tumors, 58/61 *MET* wild-type with IHC-positive (*MET* wt^{IHCpositive}) tumors, and 46/92 *MET* wild-type with IHC-negative (*MET* wt^{IHCnegative}) tumors. Genomic *MET* alterations were associated with PD-L1 positivity, defined as PD-L1 expression in ≥1.0% of tumor cells. 12/15 (80.0%) tumors harboring *MET* exon 14 alterations and 7/11 (63.6%) *MET*-amplified tumors were PD-L1 positive, compared with 29/58 (50.0%) *MET* wt^{IHCpositive} tumors and 20/46 (43.5%) *MET* wt^{IHCnegative} tumors. The expression of PD-L1 was statistically different between the group of tumors harboring *MET* exon 14 skipping mutations and *MET* wt^{IHCpositive} tumors ($p = 0.045$) when adjusted for multiple testing.

3.5 Co-Mutational Profile of MET Dysregulated Tumors Through NGS Analysis

In 4/16 (25.0%) *MET* exon 14-altered cases and 3/9 (33.3%) *MET*-amplified cases, mutations within other potentially targetable oncogenic driver genes such as *KRAS* (one *MET* exon 14-altered case; two *MET*-amplified cases), *EGFR* (one *MET* exon 14-altered case), *BRAF* (two *MET* exon 14-altered cases; one *MET*-amplified case), and *ALK*-Fusion (one *MET*

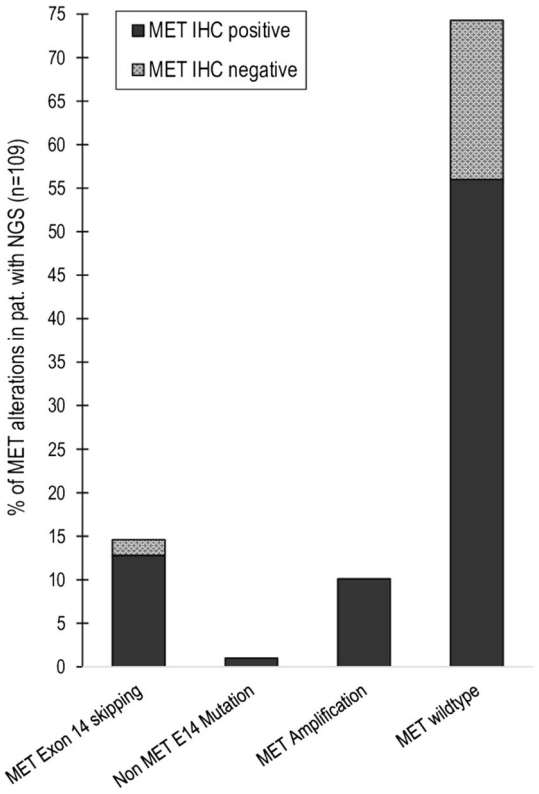

Fig. 1 Characterization of *MET* alterations. *MET* mutations resulting in *MET* exon 14 skipping were reported for seven patients with positive MET IHC: P1 MET p.D1010N, P4 MET c.2942-20TTCTTTCTCTC>T, P7 MET splicesite_3, P10 MET c.2942-18CTTTCTCTCTGTTTT>C, P12 MET c.3029C>T, P16 MET c.2888-28_2888-13>A; in MET IHC negative: P9 MET c.2888-18_2888-17insAGn. A single case with an oncogenic *MET* mutation (MET p.D1228N) not leading to *MET* exon 14 skipping was observed (non-MET E14 mutation). *MET* mesenchymal-to-epithelial transition, *IHC* immunohistochemistry, *NGS* next-generation sequencing

exon 14-altered case) were found. In two patients (7.1%), *MET* amplifications occurred as acquired resistance to therapy with EGFR TKI (patients 23 and 24) (Fig. 2a). As a consequence, these two are excluded from calculation on co-mutations. In three patients, co-occurring *EGFR* alterations (one mutation and two amplifications) were diagnosed previous to treatment with EGFR TKIs (patients 7, 12, and 25) (Fig. 2a). Three patients showed common covariant in *BRAF* (p.D945G, p.V600E, p.D469R) diagnosed previous to BRAF-targeted therapy (patients 14, 17, and 24) (Fig. 2a).

TP53 was the most frequent co-occurring mutation in tumors harboring *MET* exon 14 skipping alterations or *MET* amplification (4/16 [25.0%] and 5/9 [55.5%] patients, respectively) (Fig. 2a). Further common co-occurring mutations or CNVs in *MET*-altered tumors were *KRAS* (12.5% of *MET* exon 14-altered cases; 22.0% of *MET*-amplified cases), *BRAF* (12.5% of *MET* exon 14-altered cases; 11.1% of *MET*-amplified cases), *CDK4* (18.8% of *MET* exon 14-altered cases; 11.1% of *MET*-amplified cases), *CDKN2A* (33.3% of *MET*-amplified cases) and *TSC2* (6.3% of *MET* exon 14-altered cases; 22.2% of *MET*-amplified cases) (Fig. 2a).

MET amplification is reported at a cut-off of ≥ 4 CNVs. Patient 20, with the highest CNV in this cohort (16.4 CNVs), had no co-occurring mutations. In the group of *MET* amplification with 4 ≤ CNVs < 11, no association between the level of *MET* amplification and the number of oncogenic co-mutations was observed. Patient 8, with *MET* exon 14 skipping and *MET* amplification (6.1 CNVs), did not harbor further oncogenic driver mutations. Two *MET*-amplified tumors displayed amplification of the *HGF* gene, the ligand for the MET tyrosine kinase receptor (Fig. 2a).

In 49/61 (80.3%) *MET* $wt^{IHCpositive}$ tumors, mutations or fusions in other potentially targetable oncogenic driver genes such as *KRAS, EGFR, ALK, BRAF, ERBB2* were found. The most frequent oncogenic mutations within this group were *KRAS* (47.5%), followed by alterations in *TP53* (18.0%) and *CDKN2A* (13.1%) (Fig. 2b). The most frequent single nucleotide variants were *KRAS* p.G12C (9/61, 14.8%) and *KRAS* p.G12V (7/61, 11.5%) on exon 2. In the group of *MET* $wt^{IHCnegative}$ tumors, 13/20 (65%) *MET* $wt^{IHCnegative}$ tumors with available NGS data harbored mutations or fusions in other potentially targetable oncogenic driver genes (electronic supplementary Fig. S1). *EGFR* (35.0%) was the most frequent oncogenic mutation, followed by alteration in *KRAS* (25.0%), *CDKN2A* (15.0%), *SMARCA4* (15.0%) and *ERBB2* (10%).

3.6 Outcome of Patients with MET Dysregulation

Median overall survival (mOS) for patients harboring *MET* exon 14 skipping alterations was 64 weeks (21–336 weeks) compared with a mOS of 351 weeks (12–414 weeks) for patients with *MET* amplification (electronic supplementary Fig. S2). Fifteen patients underwent treatment with a MET TKI (11 patients received crizotinib, 1 patient received capmatinib, 2 patients received crizotinib first followed by capmatinib, and 1 patient received cabozantinib) (Fig. 3). Median progression-free survival (PFS) on MET TKI therapy was 9 weeks (4–23 weeks) and 21 weeks (18–43 weeks, excluding patient 26, with recent therapy start) for patients with *MET* exon 14 skipping alterations and *MET* amplifications, respectively. Patients 29–31 received MET TKI therapy based on high MET overexpression and below cut-off level *MET* exon 14 skipping reads. Three patients with PD-L1 expression in ≥ 50% of tumor cells and one patient with PD-L1 expression < 1% harboring *MET* alterations showed long-lasting responses to immunotherapy with immune checkpoint inhibitors, with a minimum PFS of 2 years since the start of immunotherapy**.** Between these, in patient 9, with long-lasting response to immunotherapy, a PD-L1 amplification was detected by NGS.

4 Discussion

Several MET-directed TKIs have recently emerged as treatment options for patients with oncogenic genomic alterations in the *MET* gene. Thus, it is crucial to analyze the genomic and clinical characteristics of patients harboring actionable *MET* alterations. In this study, we show that 25% of *MET* exon 14-altered cases and 33% of *MET*-amplified cases harbor potentially targetable oncogenic co-mutations. In addition, *MET* exon 14-altered tumors are significantly more likely to be PD-L1-positive. Of note, we observed long-lasting responses to immunotherapy in several patients with *MET* dysregulation, including a case of a durable response to an immune checkpoint inhibitor in a patient with MET overexpression and low-level *MET* exon 14 skipping reads.

To our knowledge, this is the first study that compares genomic profiles and PD-L1 expression in different subtypes of *MET*-dysregulated advanced-stage NSCLC. Studies on PD-L1 expression patterns in *MET*-dysregulated tumors are rare [41, 42, 44, 45]. *In vivo* studies indicated that downstream signaling of MET via AKT/GSK3β upregulates PD-L1 expression [44, 46]. In addition, *MET* activation also induces the upregulation of other immune suppressive genes such as *PDCD1LG2 (PD-L2)* and *SOCS1* [44]. MET overexpression is also significantly associated with PD-L1 status in stage I–III lung adenocarcinoma with no reports on *MET* mutations and PD-L1 expression [41]. In this study, we

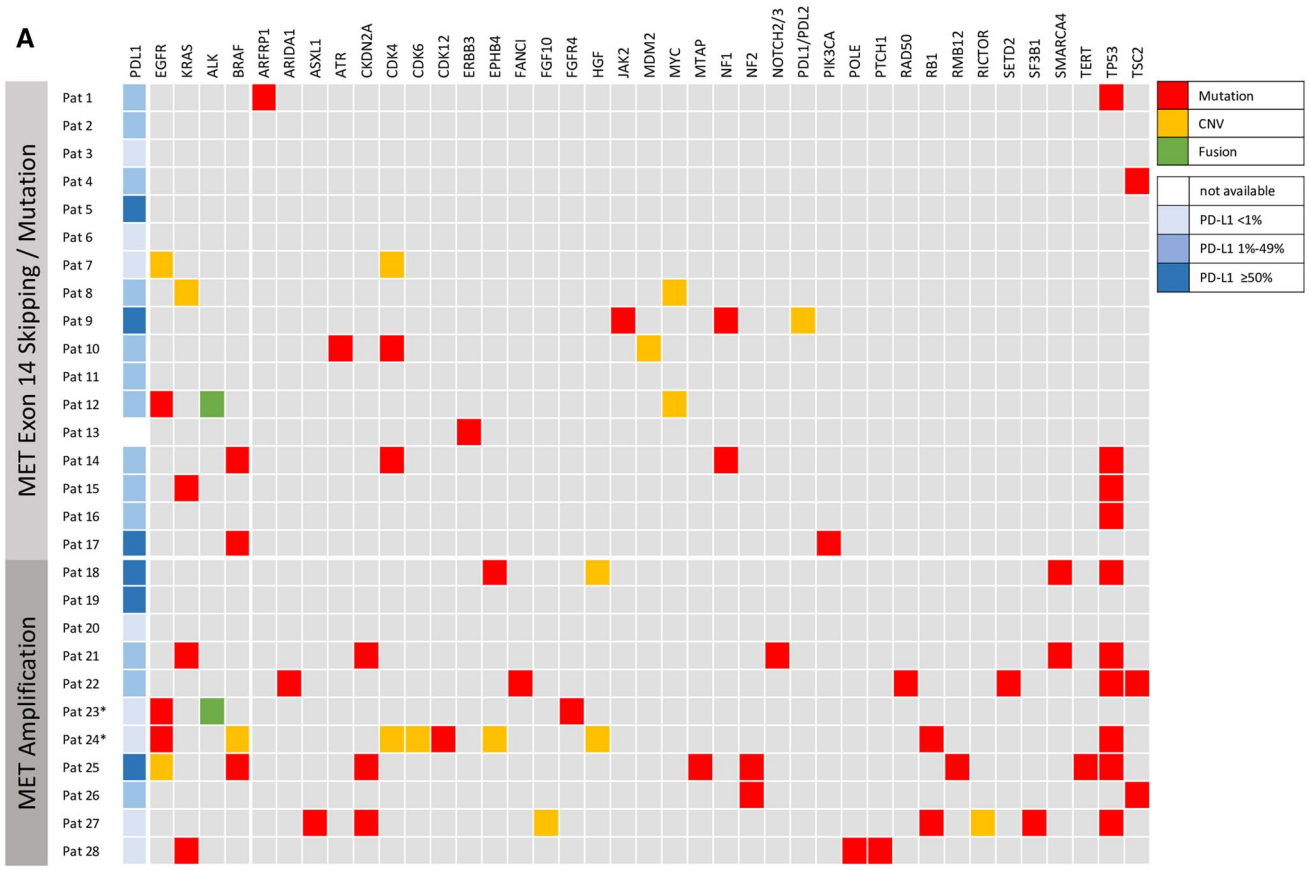

Fig. 2 **a** Co-mutational profile of *MET* exon 14-altered, *MET*-mutated (patient 2) and *MET*-amplified tumors. Specific co-mutations detected in *EGFR* (patient 7: amplification; patient 12: p.Pro772_His773insHisAla; patient 23: p.Leu858Arg; patient 24: Exon19del (A750_I759>PT); patient 25: amplification); *KRAS* (patient 8: amplification; patient 15: p.Gly12Ala; patients 16 and 21 p.Gly12Cys); *BRAF* (patient 14: p.Asp594Gly; patient 17: p.Val600Glu; patient 24: p.Gly469Arg; patient 25: amplification); *ERBB3* (patient 13: p.Val104Leu); *ATR* (patient 10: p.Leu2208Te); *ALK* (patients 12 and 23: fusion). Variants with unknown significance detected with FoundationOne CDx™ are shown in electronic supplementary Table 1. * In patients 23 and 24, *MET* amplifications occurred as acquired resistance to therapy with EGFR TKIs. **b** Co-mutations in wild-type MET IHC-positive tumors. Patients with no detected mutations in NGS are not shown (patients 32, 60, 64, 69, 75, 80, 86, 87, 88). Variants with unknown significance detected with FoundationOne CDx™ are shown in electronic supplementary Table 1. *MET* mesenchymal-to-epithelial transition, *IHC* immunohistochemistry, *EGFR* epidermal growth factor receptor, *TKIs* tyrosine kinase inhibitors, *NGS* next-generation sequencing

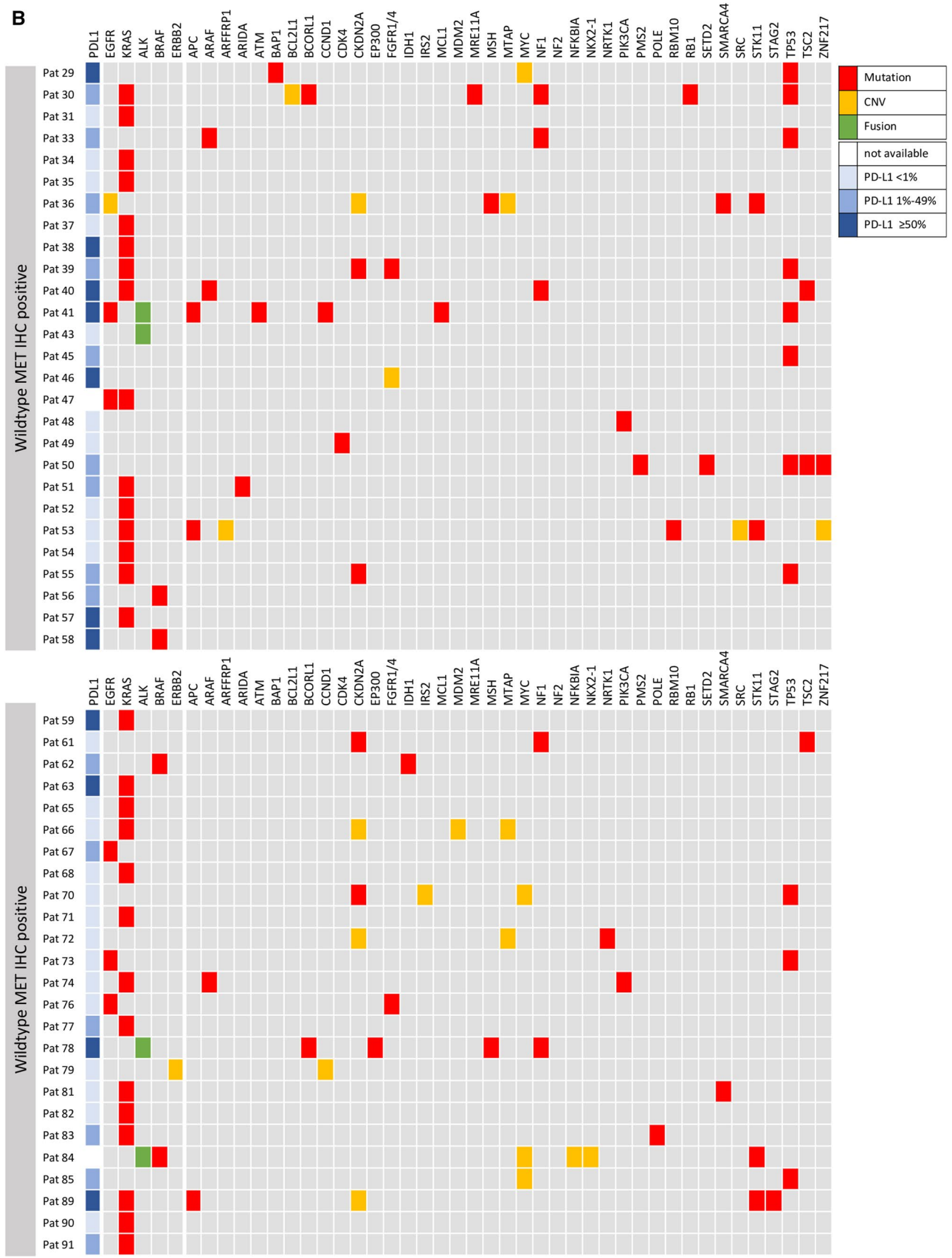

Fig. 2 (continued)

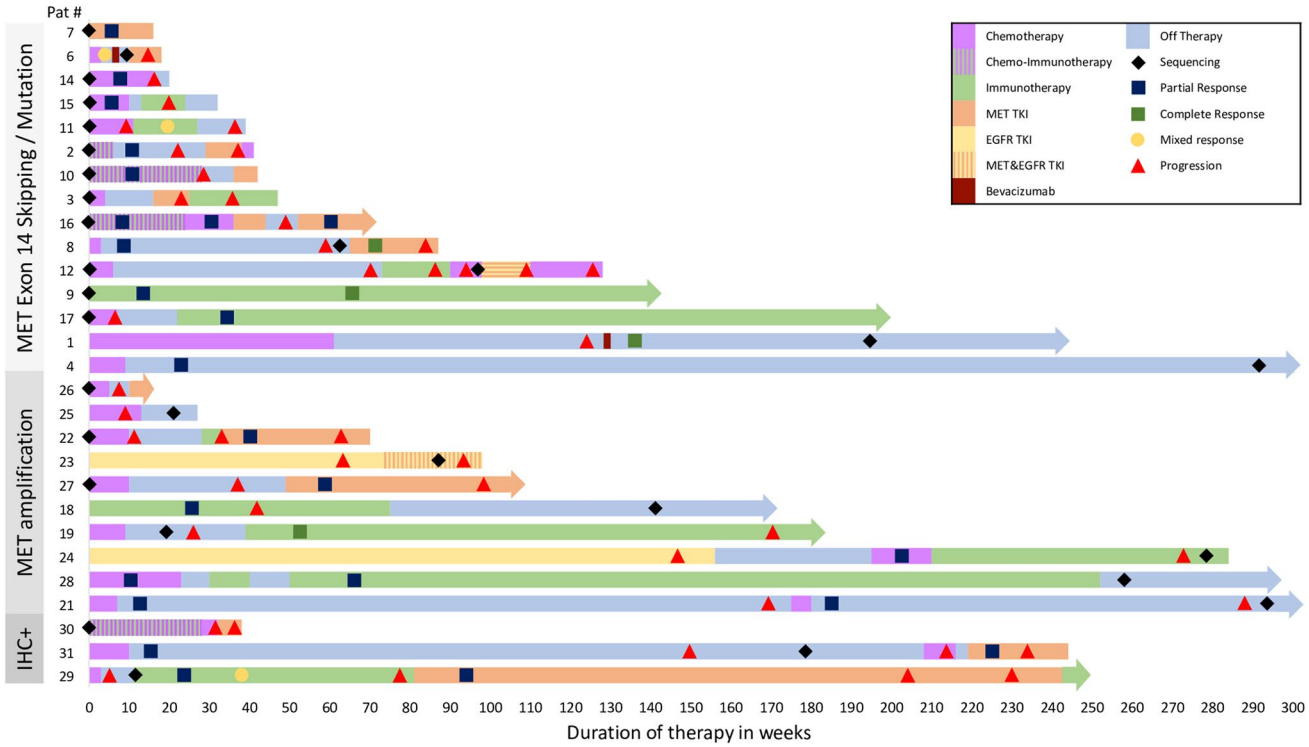

Fig. 3 Swimmer's plot showing the course of systemic treatment, length of time on specific therapy, and response in individual patients. Three patients (patients 5, 13, and 20) with systemic treatment of < 1 month, or no systemic treatment, are not depicted. The treatment course for patient 29 is depicted from 2016, when stage IV disease progressed after the initial diagnosis in 2012. *MET* mesenchymal-to-epithelial transition, *IHC* immunohistochemistry

observed a significant association between PD-L1 expression in *MET* exon 14-mutated tumors (80.0%) compared with *MET* wt^{IHCpositive} tumors (50.0%). In contrast to other studies, we detected fewer cases co-expressing PD-L1 and MET in patients without *MET* alterations [41]. Therefore, our data support that *MET* dysregulation is associated with PD-L1 expression, which was also described by previous studies where PD-L1 expression in 63% of tumors with *MET* exon 14 mutations was detected [42].

Four NSCLC patients in our cohort with *MET* exon 14 alterations and *MET* amplification had long-lasting response to immunotherapy with immune checkpoint inhibitors administered as second- or further-line treatment. Such therapy has shown to prolong PFS in *KRAS* mutant tumors and tumors with high tumor mutational burden (TMB), but not in patients with tumors harboring unique oncogenic alterations such as *EGFR* [47–51]. Partial or complete responses of more than 18 months were seen in six patients (46.2%) with *MET* exon 14 skipping mutations but without concurrent oncogenic mutations or *MET* amplification [52]. Such responses might as well have occurred through the presence of several co-mutations [53]. In contrast, 36 patients from the IMMUNOTARGET registry with *MET* dysregulation (23 with exon 14 mutation, 13 with amplification) had an objective response rate to immunotherapy of 16%, probably reflecting a population of patients undergoing immunotherapy at later lines [54]. In another study, *MET* amplification was not associated with greater benefit from nivolumab treatment [55, 56]. Studies on the co-mutational profiles of *MET*-altered tumors are inconclusive. However, several studies, including one large NGS-based study [11], reported that *MET* exon 14 skipping alterations are mutually exclusive with other oncogenic mutations, except for *MET* amplification and *MDM2* amplification [11, 12, 40]. In *MET*-amplified tumors, a negative correlation between the level of amplification and oncogenic co-mutations was shown with high-level *MET*-amplified tumors (MET/CEP7 ≥ 5) harboring concomitant drivers in 41% (11/27) of cases, and low-level *MET*-amplified tumors in 62% (32/52) of cases [57]. In this study, we demonstrate, in a subset of advanced-stage NSCLC, that *MET* amplification as well as *MET* exon 14 skipping alterations are not mutually exclusive events to *KRAS, BRAF,* and other oncogenic driver mutations. Twenty-five percent of *MET* exon 14-altered cases and 33% of *MET*-amplified cases harbored potentially targetable oncogenic co-mutations. In *KRAS*- and *EGFR*-mutated lung adenocarcinomas, *MET* amplification and mutations were detected [58]. In patients with *MET* exon 14-altered lung

cancer concurrent *MDM2* (35%), *CDK4* (21%) and *EGFR* amplifications (6.4%) were the most frequent concurrent genetic alterations [13]. Of interest, we detected that two *MET*-amplified tumors displayed amplification of the *HGF* gene, the ligand for the MET tyrosine kinase receptor, and this finding warrants further investigation.

MET TKIs improved the survival of patients harboring *MET* exon 14 skipping mutations as well as *MET* amplifications [34]; however, resistance to MET TKIs is observed in a broad number of patients [59]. Patients with *MET* exon 14-mutated tumors showed a response rate of 32% to the TKI crizotinib, 46% to tepotinib, and 68% to capmatinib [30, 31, 39] with unknown resistance mechanisms. In the course of treatment, many patients further develop acquired resistance to treatment with TKIs and therefore response to TKIs is usually transient [59, 60]. Co-occurring genomic alterations to *MET* alterations might explain resistance mechanisms. To this end, co-mutations in *KRAS* might reduce treatment efficacy with MET TKIs, since activation of the *RAS* pathway was reported to be associated with poorer outcomes in patients with *MET* exon 14 skipping alterations [38, 61]. However, one of the four patients with *KRAS* co-mutation and amplification (Fig. 2a, patient 8) received crizotinib as second-line therapy, reaching 18 weeks of PFS compared with a median PFS of 9 weeks in the other patients in our cohort with *MET* exon 14-mutated tumors.

Previous studies have shown that MET IHC is an inefficient screening tool for genomic changes in *MET* [16, 20–23]. In our study, 7% of tumors with genomic alterations in *MET* were MET IHC-negative, all either squamous cell or pleomorphic carcinomas. However, in the subset of genomic *MET*-altered adenocarcinomas, all *MET* exon 14-altered or -amplified tumors were also positive by IHC. These results suggest that MET IHC screening could be considered for adenocarcinomas in resource-limited settings, where upfront NGS-based molecular testing is not readily available. However, all positive cases require an NGS-based confirmation, since only 29.9% of MET IHC-positive cases in our study harbored a *MET* genomic alteration.

Currently, molecular analysis does not include testing for *MET* alterations as part of routine. Our study also underlines the importance of such analysis in the presence of other alterations. We believe that over the next years, when NGS becomes part of routine testing for patients with NSCLC, better knowledge of such co-alterations and clinical outcome will allow to improve treatment for these patients and possibly understanding of resistance mechanisms to MET-targeted therapies.

This study has several limitations. It was a single-center, retrospective study and does not present universal MET NGS, MET FISH, and PD-L1 assessment. In the group of MET IHC-negative tumors, NGS data were available for a minority of patients, and consequently, characterization of those tumors and comparison with MET overexpressing could not be reported. For the purpose of this study, we analyzed all tumor specimens for c-MET in order to understand molecular features of tumors exhibiting MET overexpression. Therefore, we have enriched our cohort with MET IHC-positive cases (50%). In the literature, MET overexpression was detected by IHC in 22–24% of NSCLC tumors [10, 16, 20, 21]. Additionally, IHC was not confirmed by another experienced pathologist and PD-L1 results were obtained by EIL3N assay, which is not the most used assay worldwide and other assays were shown to be superior due to staining intensity, scoring range, and pathologist preference [62]. By enriching our cohort with MET IHC-positive cases, we also report higher prevalence of *MET* alterations (14.7% with *MET* exon 14 skipping, 10.1% with *MET* amplification). In the literature, mutations within the juxtamembrane domain of *MET* encoded by exon 14 were reported in 3–4% of NSCLCs [10–12] and high-level *MET* amplification in 3–5% of lung adenocarcinoma [16–19].

Moreover, this was a single-center study with a limited number of patients. Due to the small sample size of patients with MET exon 14 alterations and MET amplification, it is difficult to draw conclusions by comparing the molecular profiles of the four patients who received immunotherapy and responded to this treatment, with patients with a similar molecular profile not receiving immunotherapy. Finally, the latest MET TKIs capmatinib and tepotinib have been demonstrated to be superior to crizotinib in patients with MET exon 14 alterations, and only one patient in our cohort received capmatinib, therefore we cannot reach any conclusions to relate response to such targeted treatments and molecular features.

5 Conclusion

We were able to show the presence of oncogenic co-mutations in tumors with *MET* exon 14 skipping alterations and *MET* amplification, and described the association of *MET* exon 14 skipping alterations with PD-L1 expression.

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Declarations

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Ethics approval This study was conducted according to the law and regulations of the local Ethics Commission under reference number KEK ZH-2021-00381. All patients included in the study consented to their participation.

Availability of data and material The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Authors' contributions Conceptualization: AF, LB, SH, ACF. Methodology: AF, ACF, MR. Investigation: AF, JR. Data curation: AF, MR. Validation: LB, ACF. Formal analysis: AF. Visualization: AF, SH. Resources: SH, MR, ER, ACF. Writing: AF, ACF. Review and editing: LB, SH, MR, JR, ER, CB, ACF. Supervision: ACF. Funding acquisition: SH, ACF.

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