Original Article

Frequency of *MET* exon 14 skipping mutations in non-small cell lung cancer according to technical approach in routine diagnosis: results from a real-life cohort of 2,369 patients

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Background: Mesenchymal epithelial transition receptor (*MET*) alterations, including *MET* exon 14 skipping mutation, are oncogenic in non-small cell lung cancer (NSCLC) and may confer sensitivity to targeted therapy. Given the rarity and the diversity of exon 14 skipping mutations, diagnosis may be challenging on small-biopsy specimens.

Methods: Between March 2014 and May 2018, tissue samples from patients with metastatic NSCLC were analysed for *MET* exon 14 skipping mutation as part of routine practice in the Pathology Department of the Hospices Civils de Lyon, France. Over the study period, Sanger sequencing and/or two different DNA-based next generation sequencing (NGS) assays were used.

Results: Genomic alterations of *MET* exon 14 were detected in 2.6% (62/2,369) samples of NSCLC analysed for *MET* exon 14 mutations. Patients were mainly women (38/62, 61%) without smoking history (22/39, 56%) and the median age was 75 years. *MET* exon 14 skipping mutations were diagnosed by NGS in 50 cases and by classical Sanger sequencing in 12 cases. The frequency of *MET* mutations was 15.4% when Sanger sequencing was performed at the request of the clinician and 4.1% when the DNA-based NGS assay coverage included the 3' and 5' parts of the *MET* exon 14 and performed systematically.

Conclusions: The frequency of genomic alterations is highly dependent on patient selection and the technical approach.

Keywords: Lung adenocarcinoma; *MET* exon 14; splice site variant; DNA-based next generation sequencing assays (DNA-based NGS assay)

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Introduction

Oncogenic alterations occurring in non-small cell lung cancer (NSCLC) are attractive because they may confer sensitivity to targeted therapies and help personalise medicine with the use of targeted therapy (1). Alterations of the gene coding for the mesenchymal epithelial transition receptor (*MET*), a tyrosine kinase receptor, have been reported in NSCLC, including copy number variations (polysomy and amplification) and mutational activating variants (2,3). According to the Cancer Genome Atlas, the latter occur in 7% of pulmonary adenocarcinoma (2) with different frequencies according to ethnicity (4,5). The most frequent mutations are *MET* exon 14 skipping mutations that activate MET through reduction of MET degradation and increased MET activity (6,7). Because of the highly diverse sequence composition of *MET* exon 14 skipping mutations, the frequency of these mutations is strongly dependent on the technique used to detect *MET* alterations. It varies from 3% of pulmonary adenocarcinoma using direct sequencing or hybrid capture new generation sequencing (7,8) to 4.3% using whole-exome sequencing (2).

The purpose of this retrospective study was to describe the frequency of *MET* exon 14 skipping mutations according to the different technical changes that have been used over recent years in a real-life context.

Methods

Patients and tumour samples

Between March 2014 and May 2018, tissue samples from patients with metastatic NSCLC were analysed for *MET* exon 14 skipping mutation as part of routine practice in the Pathology Department of the Hospices Civils de Lyon, France. A consent form was systematically obtained through the umbrella protocol of the biological specimen resource core facility of the Hospices Civils de Lyon. The present study was approved by the ethics committee of the Hospices Civils de Lyon. Clinical data, such as gender, age, smoking history, stage of the disease, and histopathological reports were retrospectively collected from all centres which had referred the cases for molecular diagnosis and ultimately managed the patients. Based on medical histopathological report and/or histopathological slides reviewed by two thoracic pathologists, lung carcinomas were classified according to the World Health Organization histopathological classification (1).

C-MET protein expression

Standard 4-µm formalin-fixed paraffin-embedded (FFPE) sections were subjected to immunohistochemical (IHC) analysis using a c-MET antibody (SP44, Ventana, Basel, Switzerland). Staining for c-MET was scored semi-quantitatively on a four-tier scale from 0 (absent) to 3 (strong membranous staining in >50% of tumour cells) as previously described (9). In case of a score of 2 (moderate expression) or 3 (high expression), fluorescent *in situ* hybridization (FISH) analysis was performed to confirm *MET* amplification.

MET amplification

MET gene copy number amplification was assessed by FISH using the Zytolight, MET/CEN7 dual color probe kit (Clinisciences, Nanterre, France). *MET* amplification was defined as a gene copy number ≥6 in at least 100 tumour cell nuclei or as the presence of clusters in >3 nuclei. Polysomy was defined as a ratio MET:CEN7 <2. In cases of exhausted samples, the analysis was performed on at least 25 cells.

MET gene exon 14 analysis

DNA was extracted from FFPE lung tumour tissue after laser microdissection (Leica, LMD 6000, Wetzlar, Germany; QIAmp DNA micro-kit, QIAGEN, Venlo, The Netherlands). In the Hospices Civils de Lyon, routine *MET* exon 14 genetic alterations were detected by three different methods over the duration of the study: (I) from 1 March 2014 to 30 April 2016 (period 1), Sanger sequencing of *MET* exon 14 and its splice sites (NM 001127500.2: c.2942-106 to c.3082+104) was performed only upon clinician request after investigation of other drivers by multiplex *KRAS/EGFR/BRAF* Sanger sequencing (*KRAS/EGFR/BRAF* negative + *MET* Sanger sequencing); (II) from 1 May 2016 to 31 August 2017 (period 2), next generation sequencing (NGS) was performed systematically for prospective samples using a custom ampliseq panel including the part of the *MET* exon 14 coding region (NM 001127500.2:c.2942-5 to c.3082+54) and Ion Personal Genome Machine technology (PGM Ion Torrent, ThermoFisher Scientific, Waltham, MA, USA) (period 2, NGS panel V1 only). Since this coverage is insufficient for *MET* splice site mutation detection (in particular at the 5' end) and in case wild-type *KRAS/EGFR/BRAF*, Sanger sequencing completed the analysis upon clinician request (period 2, NGS panel V1

Table 1 Clinical and histopathological characteristics

Characteristics	Total population, n=62
Gender, n [%]	
Female	38 [61]
Male	24 [39]
Median age (years), median [range]	75 [46–97]
Smoking history, n [%]	
Never	22 [35]
Current or former	17 [27]
Missing data	23 [37]
Type of sample, n [%]	
Endoscopic biopsies	38 [61]
Surgical samples	15 [24]
Percutaneous biopsies	7 [11]
Cell-block	2 [3]
Histological type of NSCLC, n [%]	
Adenocarcinoma	49 [79]
Squamous carcinoma	5 [8]
Sarcomatoid carcinoma	4 [6]
NSCLC-NOS	4 [6]
Clinical stage, n [%]	
I	2 [3]
II	3 [5]
III	8 [13]
IV	23 [37]
Missing data	26 [42]

NSCLC-NOS, non-small cell lung carcinoma-not otherwise specified.

negative + *MET* Sanger sequencing). For some previous samples, already analysed for the main oncogenic drivers before the beginning of the second period, only Sanger sequencing was performed upon clinician request (period 2, *KRAS/EGFR/BRAF* negative + *MET* Sanger sequencing); (III) from 1 September 2017 to 30 May 2018 (period 3), the custom ampliseq panel was modified to cover *MET* exon 14 splice sites (NM 001127500.2: c.2942-97 to c.2994 and c.3031 to c.3082+181; NGS V2). In case of unpublished *MET* exon 14 mutations, *MET* exon 14 skipping was confirmed by an RNA-approach technique currently available in our department. The interested reader can find details about the technical aspects of NGS and RNA assay in the Supplementary appendix online.

The *MET* exon 14 molecular analysis was performed after (period 1) or in parallel (period 2 and 3) of *EGFR/KARS/BRAF/HER2*, screened by sequencing and of ALK and ROS1, screened by immunohistochemistry.

Results

Patients and samples characteristics

A total of 2,369 samples from patients with NSCLC were analyzed for *MET* exon 14 skipping mutations from March 2014 until May 2018. Among these, 62 (2.6%) tumours harboured *MET* exon 14 genetic alterations. Patients were mainly women, (61% of cases) and the median age was 75 years (range, 46–97 years). Clinical data are summarized in *Table 1*.

Histopathological characteristics

Among the 62 samples harbouring a *MET* exon 14 mutation, 47 assays were performed on small samples (endoscopic/percutaneous biopsies or cell-block, 76%). Among these 47 assays, 38 (81%) were taken from bronchial biopsies, 7 (15%) from percutaneous biopsies and two were cytological samples (4%). In addition, 15/62 (24%) were taken from lung or pleural surgical biopsies. Overall, 49 (79%) tumours were adenocarcinomas, 5 (8%) were squamous carcinomas, 4 (6%) were sarcomatoid carcinomas, and 4 (6%) were classified as NSCLC-NOS (not otherwise specified; *Table 1*). MET IHC analysis was performed on 22 samples; 19 (86%) of these were positive (*Figure S1*). FISH analysis, performed on positive cases, found amplification (n=2; *Figure S2*) or polysomy (n=1) in 3/19 (16%) cases (*Table S1*).

Mutational characteristics

Considering all study periods, genomic alterations on the skipping site of *MET* exon 14 were detected in 2.6% of lung NSCLC. The description of the mutation type and its implication on the donor or acceptor site are described in *Figure 1*. Among these 62 mutations, 50 (81%) cases were diagnosed by NGS, while 12 (19%) mutations were identified by Sanger sequencing. During the second period one mutation in the part of the gene not covered by the

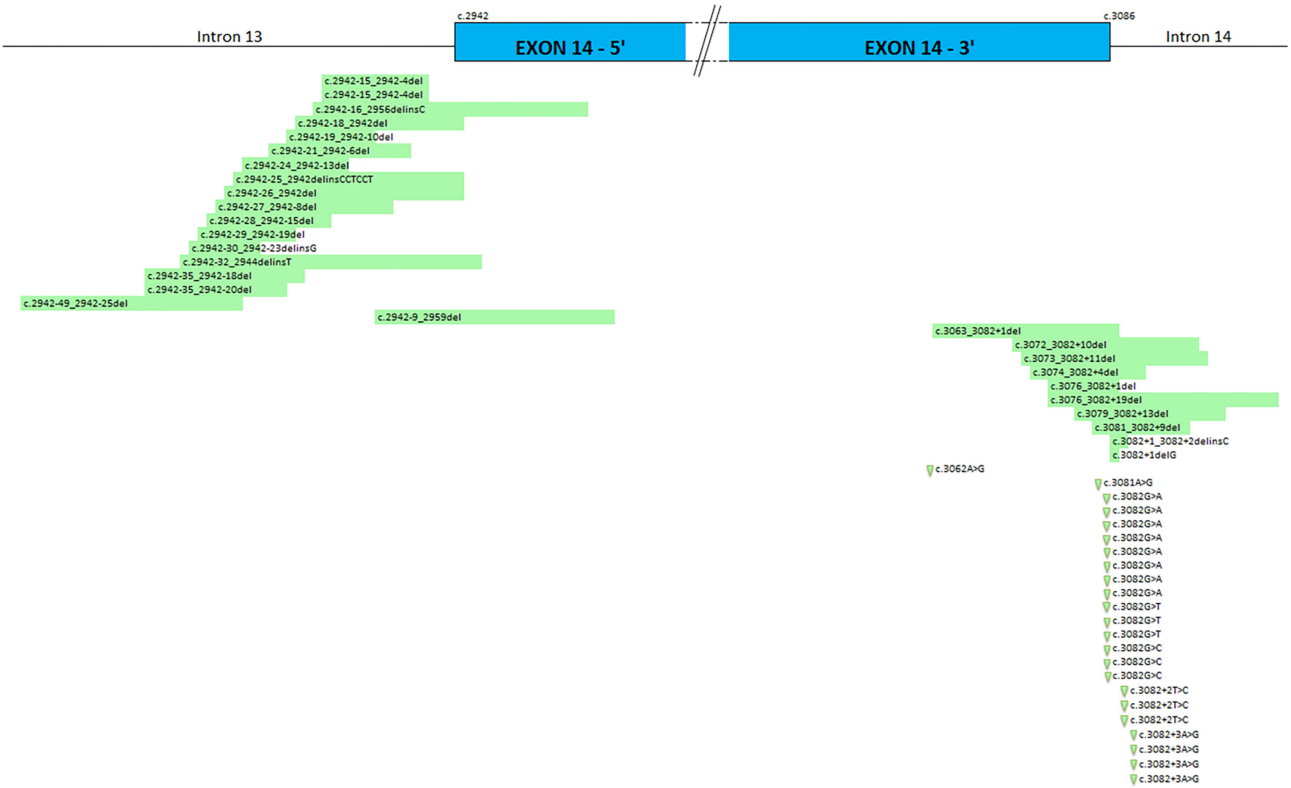

Figure 1 Mapping of *MET* exon 14 skipping mutations. The values indicate the position of the *MET* exon 14 skipping mutation on the *MET* exon 14 gene. *MET* substitutions are indicated by green triangles; *MET* deletions are indicated by green bars, the size of the bar is proportional to the size of the deletion. The corresponding position on the cDNA is indicated next to each alteration.

NGS panel V1was diagnosed by Sanger sequencing (*Table 2*). The frequency of *MET* exon 14 alterations varied depending on the selection of patients and technical approach used; the highest was found in patients who were known to have wild-type *KRAS/EGFR/BRAF* (15.4%). Among patients in whom only NGS was used, the frequency of *MET* exon 14 alterations was the greatest when the NGS panel covered the 3' and 5' of *MET* exon 14 (panel V2, 4.1%; *Table 3*). Co-occurring genomic mutations were present in three cases (5%) including one *KRAS* G12C mutation, one *NRAS* Q61L mutation and one *MET* H1112Y substitution.

Discussion

In this large cohort of patients with NSCLC, we show that the frequency of *MET* exon 14 skipping mutations differed over time in accordance with the technical approach used. Other than clinical correlates, we also report the co-occurrence of RAS and *MET* mutations.

The present study confirms some clinical and histopathological data. First, *MET* exon 14 skipping mutation were mostly observed in women and those who had never smoked, which is in line with previously reported features of patients with NSCLC harbouring oncogenic driver alteration (10); interestingly, the median age of the population was higher than that reported in case of *EGFR* or *ALK* alteration (4,11). This has a significant potential clinical relevance as elderly people are often not able to be treated by classical chemotherapy and targeted therapy could be a less toxic alternative. Furthermore, *MET* exon 14 skipping mutations were first described in sarcomatoid carcinoma (12-14), but herein they were mainly diagnosed in lung adenocarcinoma, as has widely been reported elsewhere (7,15,16). This underlines that the molecular screening of such mutations should not be limited to sarcomatoid carcinoma. The study also confirms that MET immunohistochemistry does not represent a valuable tool to screen patients for *MET* genomic alterations, including *MET* exon 14 skipping mutation as well as *MET* amplification. Furthermore, the results herein also confirm

Table 2 Detection of *MET* exon 14 skipping mutations on genomic DNA during the three periods of study

Period	NGS sequencing	Sanger sequencing	Total
Period 1 (n=30)			6/30, 20.0%
KRAS/*EGFR*/*BRAF* negative + *MET* Sanger sequencing	–	6	
Period 2 (n=1,507)			22/1,507, 1.5%
NGS panel V1 only (n=1,459)	16	–	16/1,459, 1.1%
NGS panel V1 negative + *MET* Sanger sequencing (n=31)	–	1	1/31, 3.2%
KRAS/*EGFR*/*BRAF* negative + *MET* Sanger sequencing (n=17)	–	5	5/17, 29.4%
Period 3 (n=832)			34/832, 4.1%
NGS panel V2	34	–	
Total	50	12	62/2,369, 2.6%

NGS panel V1 does not include the *MET* exon 14 skipping mutation in 5' while NGS panel V2 includes 3' and 5' MET exon14 skipping mutations. NSCLC, non-small cell lung carcinoma; NGS, nest generation sequencing.

Table 3 Detection of *MET* exon 14 skipping mutations on genomic DNA depending of the selection of the patients

Period of study	NGS panel V1 (n=16)	NGS panel V2 (n=34)	Sanger sequencing (n=12)	Total (n=62)
KRAS/*EGFR*/*BRAF* negative + *MET* Sanger sequencing (n=78)				12/78, 15.4%
Period 1 (n=30)	–	–	6	6/30, 20.0%
Period 2 (n=48)	–	–	6	6/48, 12.5%
NGS only (n=2,291)				50/2,291, 2.2%
Period 2 (panel V1; n=1,459)	16	–	–	16/1,459, 1.1%
Period 3 (panel V2, n=832)	–	34	–	34/832, 4.1%

NSCLC, non-small cell lung carcinoma; NGS, nest-generation sequencing.

that co-occurring mutations are observed in 5% of NSCLC specimens that harbour a *MET* exon 14 skipping alteration (5,12,17) which precludes the prescription of *MET* tyrosine kinase inhibitors for such patients. Ultimately, a key clinical point for routine practice is that screening for *MET* exon 14 skipping mutations should be systematic irrespective of the tumour status for other oncogenic driver alteration, and the clinical characteristics of patients.

The main purpose of this study was to analyse the impact of technical approach to detect *MET* exon 14 skipping mutations in routine practice. For instance, there were differences in the frequency of *MET* exon 14 skipping mutations according to the period of study and the sequencing design. When *MET* alterations were detected by Sanger sequencing in patients known to have wild-type *KRAS*/*EGFR*/*BRAF*, the frequency was very high which is explained mainly by the selection of the patients. To better evaluate this frequency, it would have been interesting to consider all patients who had received molecular analysis, but this was not possible to identify such individuals for the first study period. The lowest frequency of *MET* exon 14 skipping mutations detection was observed during the period when NGS was used systematically but the panel only covered part of the exon 14 and without further Sanger sequencing. In the most recent period, patients were not selected and the NGS panel covered the 3' and 5' of *MET* exon 14; the frequency of *MET* exons 14 skipping mutations is the same as that reported in other studies, including those using whole-exome sequencing (2) and a RNA-based assay (18).

Furthermore, three-quarters of the *MET* alterations were diagnosed on small samples in line with routine practice in the setting of advanced and metastatic disease and FFPE specimens. Recently, Davies *et al.* (18) described the higher sensitivity of the RNA-based NGS assay employing anchored multiplex PCR for target enrichment comparing

to a DNA-based NGS assay that employs an amplicon-mediated target enrichment, the latter being the approach used herein. Furthermore, RNA-based NGS assay allow the detection of *ALK*, *ROS1*, *RET*, *NTRK1*, and *NRG1* translocations in a single assay, that could be suitable for small samples. However, as expected, some samples herein that were positive using the DNA-based approach were uninformative when analysed by the RNA-based approach due to the poor quality or low quantity of RNA. In routine practice, we do not often have the possibility to work on frozen samples or on a large amount of tissue and the assay used should be adapted to these aspects. To perform biomarker analysis on all kinds of samples, including cytological samples, a DNA-based NGS assay that employs an amplicon-mediated target enrichment is a more suitable technique to detect the more frequent genomic alterations. The frequency of *MET* exon 14 skipping mutations observed in the most recent period of this study confirm that a DNA-based assay with a good coverage of *MET* exon 14 skipping mutations could detect these alterations with a same frequency as an RNA-based assay in an unselected population.

To conclude, the frequency of genomic alterations is highly dependent on patient selection and the technical approach. For routine practice, a compromise has to be made between sensitivity and feasibility, the DNA-based NGS assay used herein seems to be a practical alternative to RNA-based techniques for small FFPE specimens.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jtd.2020.04.21). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The present study was approved by the ethics committee of the Hospices Civils de Lyon (No. N° 19-17). A consent form was systematically obtained through the umbrella protocol of the biological specimen resource core facility of the Hospices Civils de Lyon.

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Supplementary

Sequence analysis

DNA was extracted from FFPE lung tumour tissue after laser microdissection ensuring a high (>60%) tumour content (Leica, LMD 6000, QIAmp DNA micro-kit, QIAGEN, Venlo, The Netherlands). DNA was quantified with a Qubit fluorometer (Thermofischer, Waltham, MA, USA). PCR and sequencing reactions were performed in duplicate using a 1 to 5 ng DNA input. Regions sequenced around *MET* exon 14 are specified in the main text. Samples were defined as wild-type only in the absence of variants with a minimal depth of 300×. Only variants present on both replicas were considered as mutations.

mRNA analysis

RNA was extracted from FFPE lung tumour tissue after macrodissection ensuring a least a 20% tumour content (RNeasy FFPE kit, QIAGEN). RNA was quantified with a Qubit fluorometer (Thermofischer) and 25 to 200 ng RNA were hybridized with a Nanostring (Seattle, WA, USA) custom panel comprising probes for MET exon 13/14 junction and for MET exon 13/15 junction. Count were recorded by a nCounter Digital Analyser (Nanostring). RNA quality and quantity were checked using four housekeeping genes: counts should >100 for *GUSB* and *POLR2A* and over 1,000 for *GAPDH* and *OAZ1*. Counts >100 for the exon 13/15 junction indicate a *MET* exon 14 skipping.

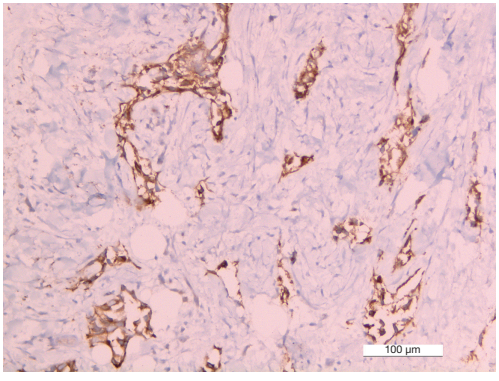

Figure S1 Immunohistochemical staining of MET protein in a pleural metastasis specimen of a lung adenocarcinoma. Membranous score 3 expression of MET protein is present on the tumour cells.

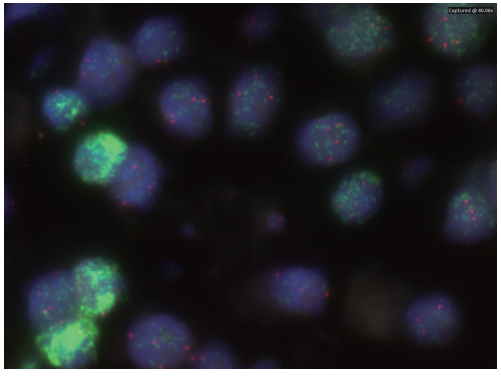

Figure S2 *MET* fluorescent *in situ* hybridization. *MET* amplification ≥3 nuclei with clusters of *MET* signals is shown. Green = *MET* probe; red = centromeric probe.

Table S1 Comparison between MET protein expression and *MET* CNV on 22 samples harbouring *MET* exon 14 skipping mutation

Patient No.	MET IHC	MET CNV (copy number; ratio)
1	3	No CNV (4.4; 0.8)
2	0	NP
3	2	NP
4	3	Amplification (11.3; 4.5)
5	3	Polysomy (7; 1)
6	0	NP
7	3	No CNV* (2.4; 0.96)
8	3	No CNV* (3.74; 1.43)
9	0	NP
10	2	No CNV* (2.5; 1.0)
11	3	No CNV (3.96; 1.01)
12	3	No CNV (4.6; 0.6)
13	2	No CNV* (3.6; 0.9)
14	3	No CNV (5.9; NA)
15	2	No CNV* (2.2; 0.8)
16	3	Amplification (6.5; 1)
17	2	No CNV* (2.5; 0.7)
18	2	No CNV* (2.79; 0.73)
19	2	No CNV* (3.37; 1.02)
20	3	No CNV (5.3; 2.29)
21	2	No CNV* (4.3; 1.3)
22	3	No CNV* (5.8; 1.3)

*, analysis performed on <50 tumour cells. MET IHC grades: 0 absence of MET expression, 2 moderate expression, 3 strong expression. NP, not performed; IHC, immunohistochemistry; CNV, copy number variation.