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Acquired *KRAS* mutation and loss of low-level *MET* amplification after durable response to crizotinib in a patient with lung adenocarcinoma

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ABSTRACT

Objectives: Resistance to tyrosine kinase inhibitor (TKI) therapy occurs inevitably in lung cancer patients with targetable genetic alterations. *MET* amplification has found to be an oncogenic driver in lung cancer with several reports showing response to MET TKI especially in cases with high-level amplification.
Materials and methods: We report the case of a patient with lung adenocarcinoma harbouring low-level *MET* amplification and strong MET expression who was treated with crizotinib.
Results: The patient developed a durable response to crizotinib. A *KRAS* mutation and loss of *MET* amplification was found in a new lesion at time of progression as a potential mechanism of acquired resistance.
Conclusion: *MET* amplification is a continuous biomarker with responses to MET TKI observed even in patients with low-level amplification. *KRAS* mutations may act as a resistance mechanism to MET inhibition in *MET* dependent lung cancer.

1. Introduction

Over the last years, a growing number of genetic alterations has been identified in non-small cell lung cancer (NSCLC) predicting response to targeted treatment. Beside mutations in *EGFR* and *BRAF* as well as rearrangements of *ROS1* and *ALK*, aberrations of *MET* have been subject to preclinical and clinical research.

Oncogenic dysregulation of the MET pathway may occur throug diverse molecular mechanisms, most commonly *MET* exon 14 skipping mutations, *MET* amplifications or overexpression but also *MET* translocation [1,2]. *MET* amplification and overexpression hereby represent continuous biomarkers. So far no cut-offs could be defined that reliably predict response to TKI treatment [3,4]. Moreover, little is known about acquired resistance mechanisms to targeted therapy of MET-positive NSCLC.

2. Case report

A 61-year-old female former smoker (30 pack years) was diagnosed with an adenocarcinoma in the central left lung in November 2012 with mediastinal lymph node involvement (UICC stage IIIA). Pneumonectomy with systemic lymphadenectomy was performed in February 2013 after neoadjuvant chemoradiotherapy (4 cycles of carboplatin and paclitaxel plus radiation ad 45 Gy). The initial molecular analyses revealed no mutations in *KRAS* (exon 2 and 3), *PIK3CA* (Exon 9 and 20) *BRAF* (Exon 11 and 15) and *EGFR* (Exon 18, 19 and 21). *ALK* fluorescence in-situ hybridization (FISH) detected no *ALK* translocation. *MET* FISH was performed as described by Schildhaus et al. and showed ≥ 4 *MET* signals in 62% of cells and an average *MET* gene copy number (GCN) of 4.22, fulfilling the criteria of low-level *MET* amplification (Table 1) [5].

In February 2014, a CT scan showed new lymphadenopathy of right cervical and supraclavicular lymph nodes. A biopsy of the cervical lymph node was analysed by next generation sequencing using a panel of 14 genes including *KRAS*, *EGFR* and *PIK3CA* as well as by FISH for *ALK*, *ROS1* and *RET*. *MET* FISH showed 53% of cells with ≥ 4 *MET* signals and a GCN of 3.6, confirming the low-level *MET* amplification. Again, no other mutations were found throughout molecular analyses,

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Table 1
Summary of molecular alterations detected in the baseline, the pre-treatment and the post-treatment biopsy samples.

	Baseline biopsy	Pre-treatment biopsy	Post-treatment biopsy
Spot of biopsy	Central left lung	Right cervical lymphnode	Right middle lobe of the lung
***MET* GCN**	4.22	3.62	2.32
***MET/CEN7* ratio**	1.47	1.19	1.25
% of cells with ≥ 4 *MET* signals	62	53	10
MET IHC score/% of cells	Not done	3+/95	Not done
NGS results	*PTEN* p.G209Efs*12, *TP53* p.V272M	*PTEN* p.G209Efs*12, *TP53* p.V272M	*KRAS* p.G12V

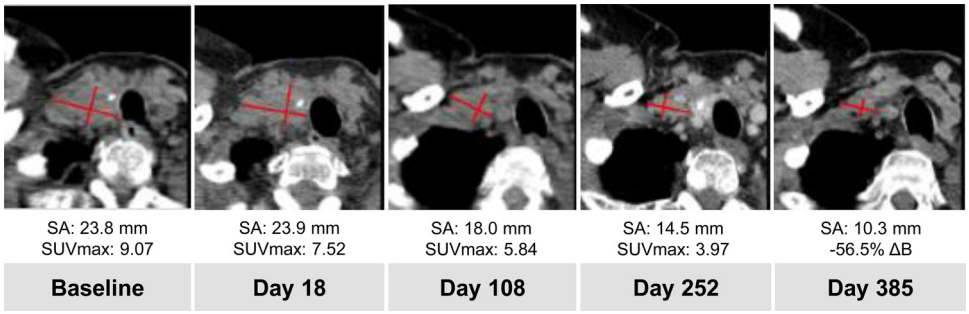

Fig. 1. CT scans at baseline and at treatment follow-up. Numbers indicate the diameter of the short axis (SA) and maximum standardized uptake value (SUVmax) of the single hottest lesion. ΔB: change in size of the short axis compared to baseline.

including *KRAS* and *MET* sequencing as well as *ALK* and *ROS1* FISH (Table 1). Additionally, immunohistochemistry revealed a strong expression (3+) of MET in 95% of cells (Table 1). In view of these results and after obtaining written informed consent we decided to initiate therapy with the MET/ALK/ROS1 inhibitor crizotinib in May 2014. Because of the simultaneous intake of sertraline, bearing the potential to induce QTc prolongation, the patient received crizotinib at a dose of 200 mg BID. A baseline PET/CT scan showed isolated FDG uptake in supraclavicular and cervical lymph nodes, with a maximum standard uptake value (SUVmax) of 9.07 in the single hottest lesion. Follow-up PET/CT scans showed a slow but constant reduction of SUVmax of the single hottest lesion to 3.97 after 8 months (252 days) of treatment (Fig. 1). CT scans confirmed the response in the right supraclavicular lymph node with a maximum reduction of the short axis of 56,8% (Fig. 1).

Crizotinib treatment was discontinued in February 2016 after 20,7 months of treatment due to progression of the right cervical and the right supraclavicular lymph node metastases. In March 2017 radiotherapy of these lymph nodes was performed. Subsequent CT scans showed response to radiotherapy and the patient was free of any local or systemic therapy until February 2018. Then, a PET/CT revealed a new suspect lesion of the right middle lobe (not shown). A biopsy was performed by bronchoscopy which showed recurrence of lung adenocarcinoma. Again, the sample was analysed using next-generation sequencing and ddPCR analysis showing a *KRAS* mutation in Exon 2 (p.G12 V) (Table 1). *MET* FISH revealed the loss of *MET* amplification (10% of cells with ≥ 4 *MET* signals, GCN 2.32).

3. Discussion

MET amplification and expression may be used as predictive biomarkers for response to MET inhibition in NSCLC [4,6]. However, *M*both are continuous biomarkers and no definitive cut-offs predictive for response to MET inhibition could be established so far. Variables used to define different levels of amplification include the *MET*-to-chromosome-7-centromere ratio (MET/CEP7), the mean *MET* gene copy number per cell (GCN) or the number off cells showing ≥4 or ≥5 *MET* signals [5,7]. Variations in the amplification thresholds used in different studies make it difficult to compare results concerning the frequency of *MET* aberrations as well as the response to targeted therapy. Since the first report of successful crizotinib treatment in a patient with high-level *MET* amplified NSCLC, several case reports have been published demonstrating response to crizotinib in patients with lung adeno- and squamous-cell carcinomas harbouring high-level *MET* amplifications [6,8,9]. Moreover, several studies currently investigate different MET TKIs in patients with MET -dependent lung cancer. In the phase I trial of crizotinib, *MET* amplification was initially defined by *MET/CEP7* ratio (low ≥1,8 to ≤2,2; medium > 2,2 to > 5; high ≥5). Preliminary results showed an ORR of 16.7% in the intermediate group and an ORR of 50% in the high-amplified group. Notably, no responses were seen in the low-amplified group [4]. The phase I trial of capmatinib recruited patients based on the *MET/CEP7* ratio or MET immunohistochemistry. Retrospective analyses revealed best response rates in patients with a *MET* GCN of ≥6, that was therefore defined as high-level. No significant response signal was seen in the other patients [3]. The validity of MET expression as a biomarker for response to MET inhibition is even less well characterized.

Our case illustrates the problem with the use of continuous biomarkers for response prediction. Although most patients with low-level *MET* amplification may not respond to therapy with MET TKIs, a small subset does, most notably those with high MET expression. Our case also shows, that the level of MET expression by IHC, does not necessarily correlate with the level of *MET* amplification by FISH. Defining a strict amplification cut-off would exclude this subset from a potentially effective treatment option. Further investigation is needed to better select patients with different levels of *MET* amplification or expression who do may benefit from targeted therapy.

The low-level *MET* amplification in our patient initially presented mutually exclusive from other known driver genes – including *KRAS*. After treatment with crizotinib, the biopsy at disease progression showed loss of *MET* amplification but revealed a *KRAS* mutation as a potential mechanism of resistance. By repeated molecular analyses with a uniform NGS panel as well as by ddPCR analyses, we confirmed that the tumor samples obtained before treatment with crizotinib harboured no *KRAS* mutation. However, we were not able to detect the *TP53* and *PTEN* mutations that were found in the pre-treatment samples. This argues in favour for the selection of treatment-desensitized tumour clones caused by the high level of spatial and temporal clonal heterogeneity of the disease. Nevertheless, since progression occurred in the middle lobe and the previous samples originated from the primary

tumor and a cervical lymph node, progression due to a secondary carcinoma may not be ruled out. However, our findings are in line with published data showing that enhanced *KRAS* activation through mutations or amplification may contribute to resistance to MET inhibition [10–12].

In a recent study, Noonan et al. analysed patients with different levels of *MET* amplification for so called oncogene overlap. They found that in the low-level group co-occurring mutations in *EGFR*, *KRAS*, *ALK*, *ERBB2*, *BRAF*, *NRAS*, *ROS1* or *RET* could be detected in 62% and 52% of patients respectively, suggesting that in these patients, *MET* amplification may rather be a coincident event than a true driver [13]. Selecting patients with low-level *MET* amplified and high MET expressing, driver mutation-negative NSCLC for targeted therapy could possibly enhance response rates to MET TKI in this subset of patients.

Conflict of interest statement

Richard Riedel

RR has received honoraria from Boehringer-Ingelheim and Novartis and Travel funding from Boehringer-Ingelheim, Loxo Oncology, Novartis and Lilly. His institution received research funding from Pfizer, Bristol-Myers Squibb, Novartis, MSD, Janssen Pharmaceuticals

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Carina Heydt

CH has not declared any conflict of interest with regard to the submitted work.

Carsten Kobe

CK has not declared any conflict of interest with regard to the submitted work.

Anne Bunck

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Stephan Schäfer

SS has not declared any conflict of interest with regard to the submitted work.

Rieke N. Fischer

RF has received honoraria from BMS, MSD, Roche, Boehringer-Ingelheim, Novartis, Astra Zeneca. She received Travel Funding from Mediolanum Bioscience.

Her institution received research funding from Pfizer, Bristol-Myers Squibb, Novartis, MSD, Janssen Pharmaceuticals

Matthias Scheffler

MS has been a consultant for BMS, Boehringer-Ingelheim, Takeda and Roche and has received Travel funding from Boehringer-Ingelheim and Mediolanum.

His institution received research funding from Pfizer, Bristol-Myers Squibb, Novartis, MSD, Janssen Pharmaceuticals

Diana S. Y. Abdulla

DA has received honoraria from BMS, Boehringer-Ingelheim, MSD, Novartis, Roche, Healthcare Consulting Cologne, Abbvie and Travel funding from AstraZeneca, BMS, Boehringer-Ingelheim, MSD, Novartis, Roche, Abbvie, Loxo Oncology Her institution received research funding from Pfizer, Bristol-Myers Squibb, Novartis, MSD, Janssen Pharmaceuticals

Lucia Nogová

LN has received honoraria from Pfizer, Celgene, Novartis, Roche, Boehringer Ingelheim, Janssen Pharmaceuticals, Bristol-Myers Squibb and has been a consultant for Novartis, Boehringer Ingelheim, Bristol-Myers Squibb, Roche, Janssen Pharmaceuticals, Pfizer. Her institution received research funding from Pfizer, Bristol-Myers Squibb, Novartis, MSD, Janssen Pharmaceuticals

Sophia Koleczko

Her institution received research funding from Pfizer, Bristol-Myers Squibb, Novartis, MSD, Janssen Pharmaceuticals

Sabine Merkelbach-Bruse

SMB has received honoraria from Astra Zeneca, BMS, Roche has been a consultant for Novartis, BMS, Roche, Pfizer

Reinhard Büttner

RB has been a consultant for Pfizer and Novartis regarding MET inhibitors and is a co-founder and CSO of Targos Molecular Pathology, Kassel germany.

Jürgen Wolf

JW has received honoraria from Abbvie, AstraZeneca, BMS, Boehringer-Ingelheim, Chugai, Ignyta, Lilly, MSD, Novartis, Pfizer, Roche and has been a consultant for Abbvie, AstraZeneca, BMS, Boehringer-Ingelheim, Chugai, Ignyta, Lilly, MSD, Novartis, Pfizer, Roche. He received Travel funding from Abbvie, AstraZeneca, BMS, Boehringer-Ingelheim, Chugai, Ignyta, Lilly, MSD, Novartis, Pfizer, Roche. His institution received research funding from Pfizer, Bristol-Myers Squibb, Novartis, MSD, Janssen Pharmaceuticals

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