CANCER BIOLOGY & THERAPY
2019, VOL. 20, NO. 6, 837–842
https://doi.org/10.1080/15384047.2019.1566049

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RESEARCH PAPER

Case report: HER2 amplification as a resistance mechanism to crizotinib in NSCLC with MET exon 14 skipping

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ABSTRACT

Patients with non-small cell lung cancer (NSCLC) harboring MET exon 14 skipping can benefit from crizotinib treatment. Currently, the main resistance mechanisms to crizotinib are MET D1228N and Y1230C mutations. We reported a case of a Chinese NSCLC patient with MET exon 14 skipping detected by targeted next-generation sequencing (NGS) achieved clinical and imaging remission after crizotinib treatment. Then, amplification of multiple genes such as erb-b2 receptor tyrosine kinase 2 (HER2) was detected when disease progressed, indicating novel resistance mechanisms to crizotinib. Ultimately the patient died from cancer-related factors. This was the first NSCLC case with MET exon 14 skipping which reported the HER2 gene amplification at the time of progression during crizotinib treatment, indicating that bypass mechanisms contribute to the development of acquired resistance to MET inhibitors.

ARTICLE HISTORY
Received 28 September 2018
Revised 10 December 2018
Accepted 25 December 2018

KEYWORDS
NSCLC; MET 14 skipping; crizotinib; resistance; HER2 amplification; NGS; targeted therapy

Background

As the research on the NSCLC genome and targeted therapy moves along, more and more molecular targets for anti-tumor therapy have been applied into clinical practice,[1] including epidermal growth factor receptor (EGFR) gene,[2-4] anaplastic lymphoma kinase (ALK) gene,[5-7] and ROS proto-oncogene 1, receptor tyrosine kinase (ROS1) gene,[8] etc. MET proto-oncogene, receptor tyrosine kinase gene (MET) is another important driver gene in NSCLC.[5,9,10] Activation of MET includes mutation, amplification, and protein overexpression, which is a potential target for NSCLC treatment, indicating its association with prognosis.[11-13] Clinical evidence has demonstrated that MET is not only a driver proto-oncogene in lung cancer, but also one cause of acquired resistance to EGFR-targeted therapy.[14]

The MET exon 14-encoded portion of the juxtamembrane domain includes a binding site for Y1003 and c-Cbl E3 ubiquitin ligase. When MET exon 14 skipping mutations occur, the binding site for Y1003 and c-Cbl E3 ubiquitin ligase will be deleted, subsequently causing a decrease in receptor ubiquitination, a block in MET protein degradation, and continuously activated MET as a driver proto-oncogene.[11] It was reported by previous studies that the incidence of MET exon 14 skipping mutation in pancreatic cancer was approximately 3%,[15] much higher than the incidence of 0.9% in China shown by some domestic research.[16] There are various types of MET exon 14 skipping mutations, mainly DNA mutations at 5' splice site and 3' splice site of MET exon 14.[17] MET exon 14 skipping has been found in lung adenocarcinoma, lung squamous carcinoma, and pulmonary sarcomatoid carcinoma, for which clinical research indicated crizotinib treatment is effective.[18] The currently reported resistance mechanisms to crizotinib in patients with MET exon 14 skipping are MET D1228N and Y1230C mutations.[19,20] Currently known resistance mechanisms to kinase inhibitors can almost be categorized into the following types: second mutations, gene amplification, activation of bypass or downstream signaling pathways, histology and phenotypic transformation, etc.[21] Using targeted NGS of cancer-related genes, we detected the response of MET 14 exon skipping in the tumor tissue of a patient with lung adenocarcinoma to crizotinib treatment. After treatment progress, we did not detect point mutations of the MET gene, but found other acquired resistance mechanisms such as HER2 gene amplification.

Pathological report

A 65-year-old woman without a prior history of smoking was hospitalized for cough and chest pain. In Feb. 2015, the patient was diagnosed with stage Ib (pT2aN0M0) invasive lung adenocarcinoma (Figure 1) with alveolar adenocarcinoma as the main type (ALK IHC, negative; EGFR ARMS-PCR, negative). The patient received adjuvant chemotherapy (gemcitabine + carboplatin) after operation with DFS 8 months. In Oct. 2015, tumor recurrence and metastasis was detected by the follow-up CT scan, and the patient had the chemotherapy (first-line pemetrexed + carboplatin + bevacizumab, pemetrexed maintenance chemotherapy + bevacizumab) with PFS 14 months. In Jan. 2017, the progression of pulmonary lesions was detected by the follow-up CT scan, and the second-line chemotherapy (paclitaxel liposome + carboplatin) was given.

In Jun. 2017, the patient progressed, and tumor biopsy (via bronchofiberscope) and plasma were taken for mutation profiling using targeted NGS for 422 cancer-relevant genes [Supplemental Materials, Table 1]. According to the instructions of KAPA Library Quantification Kit, fragmented DNA or ctDNA (>20 ng) were obtained for sequencing library construction and Hiseq 4000 NGS platforms (Illumina) were used for

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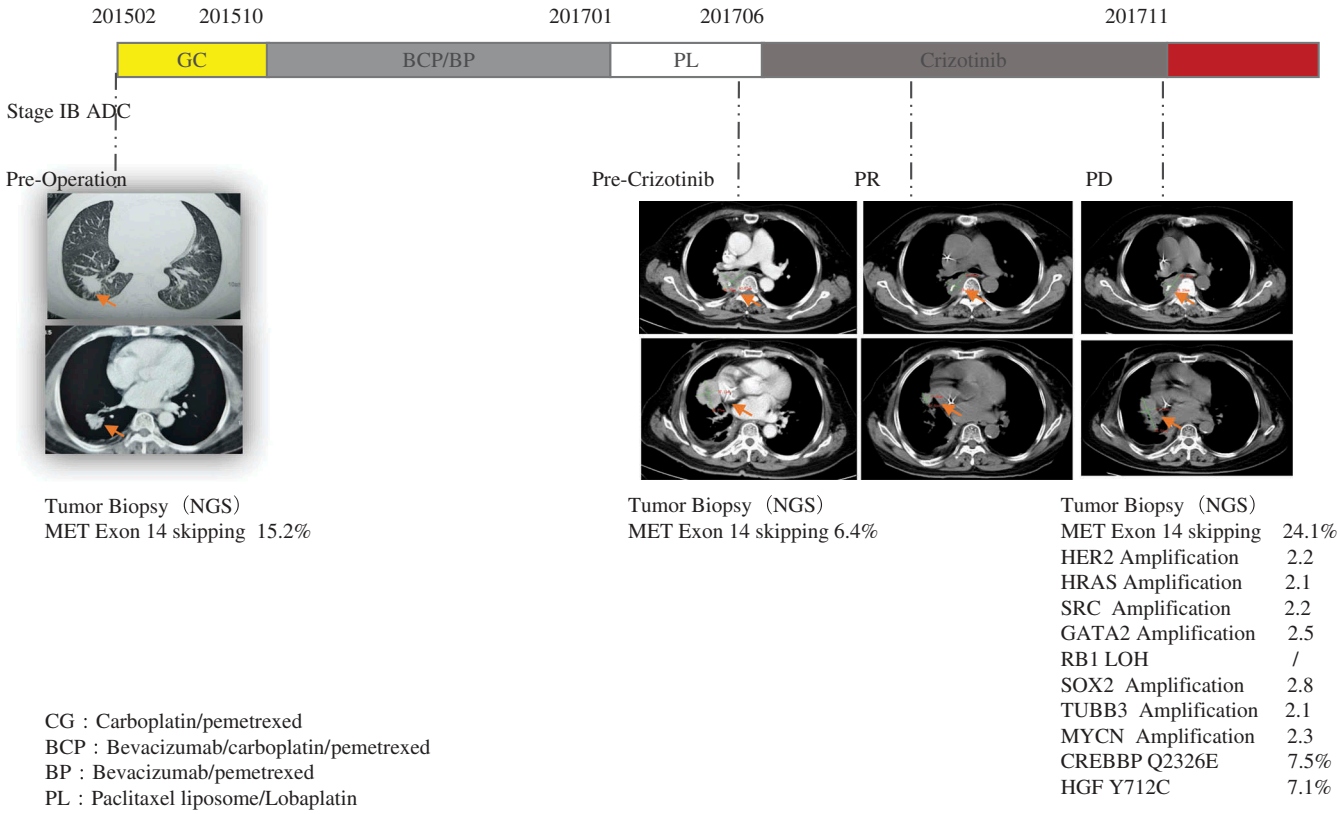

Figure 1. The patient can undergo a CT scan and have a tumor biopsy for NGS to ensure the efficacy of crizotinib and tumor progression during crizotinib treatment. MET: *MET proto-oncogene, receptor tyrosine kinase*; HER2: *erb-b2 receptor tyrosine kinase 2*; HRAS: *HRas proto-oncogene, GTPase*; SRC: *SRC proto-oncogene, non-receptor tyrosine kinase*; GATA2: *GATA binding protein 2*; RB1: *RB transcriptional corepressor 1*; SOX2: *SRY-box 2*; TUBB3: *tubulin beta 3 class III*; MYCN: *MYCN proto-oncogene, bHLH transcription factor*; CREBBP: *CREB binding protein*; HGF: *hepatocyte growth factor.*

high-throughput sequencing. MET exon 14c.2942-17_2942-6del12 skipping mutation was detected in both tissue and plasma of the patient (tissue abundance 6.4%; plasma abundance 0.5%) (Figure 2) and other tumor-specific mutations were not detected. From Jun. 2017, the patient has undergone crizotinib treatment, and the tumor regressed after 42 days indicated by tumor imaging, showing partial response (PR).

In Nov. 2017, as the tumor progressed, tumor biopsy (via bronchofiberscope) and plasma were taken again for NGS. MET exon 14c.2942-17_2942-6del12 skipping mutation was detected with plasma abundance 1.7% and tissue abundance 24.1%. In the biopsy, we also detected that almost 2.2-fold amplification of the ERBB2 gene, almost 2.1-fold amplification of the HRAS gene, almost 2.2-fold amplification of the SRC gene, almost 2.5-fold amplification of the GATA2 gene, a single copy number loss of the RB1 gene, almost 2.8-fold amplification of the SOX2 gene, almost 2.1-fold amplification of the TUBB3 gene, almost 2.3-fold amplification of the MYCN gene, Q2326E mutation of the CREBBP gene with abundance 7.5%, and Y712C mutation of HGF gene with abundance 7.1%. IHC was used to measure HER2 protein expression in tumor biopsy, emphasizing the fact that HER2 over-expressed (Figure 3).

In Dec. 2017, a retrospective NGS analysis of the patient's paraffin-embedded tissue after operation was performed and it detected MET exon 14c.2942-17_2942-6del12 skipping mutation (MAF 15.2%).

Discussion

We observed a case of alveolar adenocarcinoma in a patient with MET exon 14 skipping. The patient had response to crizotinib with a PFS of nearly 5 months. Liu et al. reported the incidence of MET exon 14 skipping is 0.9% among the Chinese patients with NSCLC, of whom 83% are with lung adenocarcinoma, 0.8% with lung squamous carcinoma, and 0.8% with adenosquamous carcinoma of the lung.[16] Currently, the histological type of patients with lung adenocarcinoma harboring MET exon 14 skipping has not been reported yet.

Profile 1001 analyzed the efficacy of crizotinib in treating NSCLC with MET exon 14 skipping. This research obtained an OR of 44% and found crizotinib was generally well tolerated. The median duration of therapy in Profile 1001 was 5.3 months, which was consistent with the PFS of nearly 5 months in this report.

Several case reports have explored the resistance mechanisms to crizotinib in patients with MET exon 14 skipping mutations.[19,20,22] Those patients at first received first-line or second-line chemotherapy and local radiotherapy. When the disease progressed, they received crizotinib therapy and achieved partial response (PR) until the disease progressed again. Then they accepted gene detection again. A patients was de tedetected new MET D1228N mutation and the pre-existing MET exon 14 skipping mutation, D1010H; in another case, the level of pre-existing MET exon 14 skipping

Table 1. The list of 422 genes panel.

ABCB1	ABCB4	ABCC2	ADH1A	ADH1B	ADH1C	AIP	AKT1	AKT2	AKT3	ALDH2	ALK
AMER1	APC	AR	ARAF	ARID1A	ARID1B	ARID2	ARID5B	ASCL4	ASXL1	ATF1	ATIC
ATM	ATR	ATRX	AURKA	AURKB	AXIN2	AXL	BAI3	BAK1	BAP1	BARD1	BCL2
BCL2L11	BCR	BIRC3	BLM	BMPR1A	BRAF	BRCA1	BRCA2	BRD4	BRIP1	BTG2	BTK
BUB1B	c11orf30	CASP8	CBL	CBLB	CC2D2B	CCND1	CCNE1	CD274	CD74	CDA	CDC73
CDH1	CDK10	CDK12	CDK4	CDK6	CDK8	CDKN1A	CDKN1B	CDKN1C	CDKN2A	CDKN2B	CDKN2C
CEBPA	CEBPB	CEBPD	CEP57	CHD4	CHEK1	CHEK2	CLEC2D	CREBBP	CRKL	CSF1R	CTCF
CTLA4	CTNNB1	CUL3	CUX1	CXCR4	CYLD	CYP19A1	CYP2A13	CYP2A6	CYP2A7	CYP2B6	CYP2C19
CYP2C9*3	CYP2D6	CYP3A4	CYP3A5	DAXX	DDR2	DENND1A	DHFR	DHFRL1	DICER1	DNMT3A	DPYD
DUSP2	EGFR	EML4	EP300	EPAS1	EPCAM	EPHA2	EPHA3	EPHA5	EPHB2	ERBB2	ERBB2IP
ERBB3	ERBB4	ERCC1	ERCC2	ERCC3	ERCC4	ERCC5	ESR1	ETV1	ETV4	EWSR1	EXT1
EXT2	EZH2	FANCA	FANCC	FANCD2	FANCE	FANCF	FANCG	FANCL	FANCM	FAT1	FBXW7
FGF19	FGFR1	FGFR2	FGFR3	FGFR4	FH	FLCN	FLT1	FLT3	FLT4	FOXA1	FOXP1
FRG1	GATA1	GATA2	GATA3	GATA4	GATA6	GNA11	GNA15	GNAQ	GNAS	GRIN2A	GRM3
GRM8	GSTM1	GSTM4	GSTM5	GSTP1	GSTT1	HDAC2	HDAC9	HGF	HLA-A	HNF1A	HNF1B
HRAS	HSD3B1	IDH1	IDH2	IGF1R	IGF2	IKBKE	IKZF1	IL7R	INPP4B	IRF2	JAK1
JAK2	JAK3	JARID2	JUN	KDM5A	KDM6A	KDR	KEAP1	KIF1B	KIF5B	KIT	KITLG
KLLN	KMT2A	KMT2B	KMT2C	KMT2D	KRAS	LHCGR	LMO1	LRP1B	LYN	LZTR1	MAP2K1
MAP2K2	MAP2K4	MAP3K1	MAP3K4	MAP4K3	MAX	MCL1	MDM2	MDM4	MECOM	MED12	MEF2B
MEN1	MET	MGMT	MITF	MLH1	MLH3	MLLT1	MLLT3	MLLT4	MPL	MRE11A	MSH2
MSH6	MTHFR	MTOR	MUTYH	MYC	MYCL	MYCN	MYD88	MYH9	NAT1	NAT2	NBN
NCOR1	NF1	NF2	NFE2L2	NFKBIA	NKX2-1	NKX2-2	NKX2-4	NOTCH1	NOTCH2	NOTCH3	NPM1
NQO1	NRAS	NRG1	NSD1	NTRK1	NTRK3	PAK3	PALB2	PALLD	PARK2	PARP1	PARP2
PAX5	PBRM1	PDCD1	PDCD1LG2	PDE11A	PDGFRA	PDGFRB	PDK1	PGR	PHOX2B	PIK3C3	PIK3CA
PIK3R1	PIK3R2	PKHD1	PLAG1	PLK1	PMS1	PMS2	POLD1	POLD3	POLE	POLH	POT1
PPP2R1A	PRDM1	PRF1	PRKACA	PRKACG	PRKAR1A	PRKCI	PRKDC	PRSS1	PRSS3	PTCH1	PTEN
PTK2	PTPN11	PTPN13	PTPRD	QKI	RAC1	RAC3	RAD50	RAD51	RAD51C	RAD51D	RAF1
RARA	RARG	RASGEF1A	RB1	RECQL4	RELN	RET	RHOA	RICTOR	RNF43	ROS1	RPTOR
RRM1	RUNX1	RUNX1T1	RUNX3	SBDS	SDC4	SDHA	SDHB	SDHC	SDHD	SEPT9	SETBP1
SETD2	SF3B1	SGK1	SLC34A2	SLC7A8	SMAD2	SMAD3	SMAD4	SMAD7	SMARCA4	SMARCB1	SMO
SOS1	SOX1	SOX14	SOX2	SOX21	SOX3	SPOP	SPRY4	SRC	SRY	STAG2	STAT3
STK11	STMN1	STT3A	SUFU	TEK	TEKT4	TERC	TERT	TET2	TGFBR2	THADA	TMEM127
TMPRSS2	TNFAIP3	TNFRSF11A	TNFRSF14	TNFRSF19	TNFSF11	TOP1	TOP2A	TP53	TP63	TPMT	TSC1
TSC2	TSHR	TTF1	TUBB	TUBB2A	TUBB2B	TUBB3	TUBB4A	TUBB4B	TUBB6	TYMS	U2AF1
UGT1A1	VEGFA	VHL	WAS	WISP3	WRN	WT1	XPA	XPC	XRCC1	YAP1	ZNF2
ZNF217	ZNF703										

mutation D1010H has decreased to 10.9%, while that of MET Y1230C mutation has increased to 3.5%; in another case, there emerged new MET exon 19 D1228N/H and Y1230H mutations on the basis of the pre-existing MET exon 14 skipping mutation, which meant the co-occurrence of three mutations. The above cases indicated that D1228 and Y1230 mutations might be the main reason why patients with MET exon 14 skipping mutations resisted to crizotinib. MET Y1230 and D1228 mutations were certificated to be the mechanisms of acquired resistance to MET inhibitors in vivo. When the patient in our report had partial relief after crizotinib treatment, we conducted a biopsy and ctDNA detection. HER2 gene amplification was detected in the biopsy. HER2, also known as ERBB2, is a transmembrane glycoprotein with receptor tyrosine kinase (RTK) activity and is a member of the epidermal growth factor receptor (EGFR) family. HER2 gene amplification and protein overexpression, without the need of ligand activation, can directly induce the formation of HER2 homodimers or heterodimers, activate receptor tyrosine kinase (RTK) and downstream signaling pathways, and promote proliferation and metastasis of tumor cells. HER2 gene amplification can elevate HER2 protein expression and participate in tumor initiation and progression and affect prognosis through promoting proliferation, invasion and metastasis of tumor cells, and may reduce sensitivity of tumors to EGFR-TKIs, serving as one of the resistance mechanisms of first-generation EGFR-TKIs.[23] Crizotinib inhibited continuous MET activation caused by MET exon 14 skipping, whereas elevated HER2 protein expression also promoted the proliferation of tumor cells, suggesting maybe bypass activation can also be a resistance mechanism to crizotinib in the treatment of NSCLC with MET exon 14 skipping.

When the patient in our report had partial relief after crizotinib treatment, a biopsy was performed, in which we detected SOX2 gene amplification and a single copy number loss of the RB1 gene. SOX2 is a stem cell transcription factor which plays a critical role in embryogenesis. It belongs to a set of genes (Oct4, SOX2, NANOG) which can reprogram human somatic cells to pluripotent stem cells.[24,25] SOX2 is overexpressed in various histologic types of lung cancers, such as small-cell lung cancer (SCLC), lung squamous carcinoma and lung adenocarcinoma.[26] The spectrum of genomic variants in small-cell lung cancer has already identified SOX2 as a potential target for therapeutic intervention.[27] RB1 gene plays a regulatory role in the G1 phase of the cell cycle, and therefore RB1 gene deletions will lead to loss of G1 control. The gene has a critical role in SCLC transformation.[28] Molecular sequencing confirmed that 100% of SCLC cells transformed from resistant EGFR mutant NSCLC had RB1 gene deletions. This gene change didn't exist in NSCLC before SCLC transformation or the remaining NSCLC after SCLC transformation, indicating RB1 gene deletions played a key role in SCLC transformation.[29] What's more, SOX2 gene amplification and a single copy number loss of the RB1 gene detected in the biopsy suggested there might exist transformation to SCLC after crizotinib resistance. In 2013, NCCN Guidelines for NSCLC pointed out that one of the resistance mechanisms to EGFR-TKIs is transformation

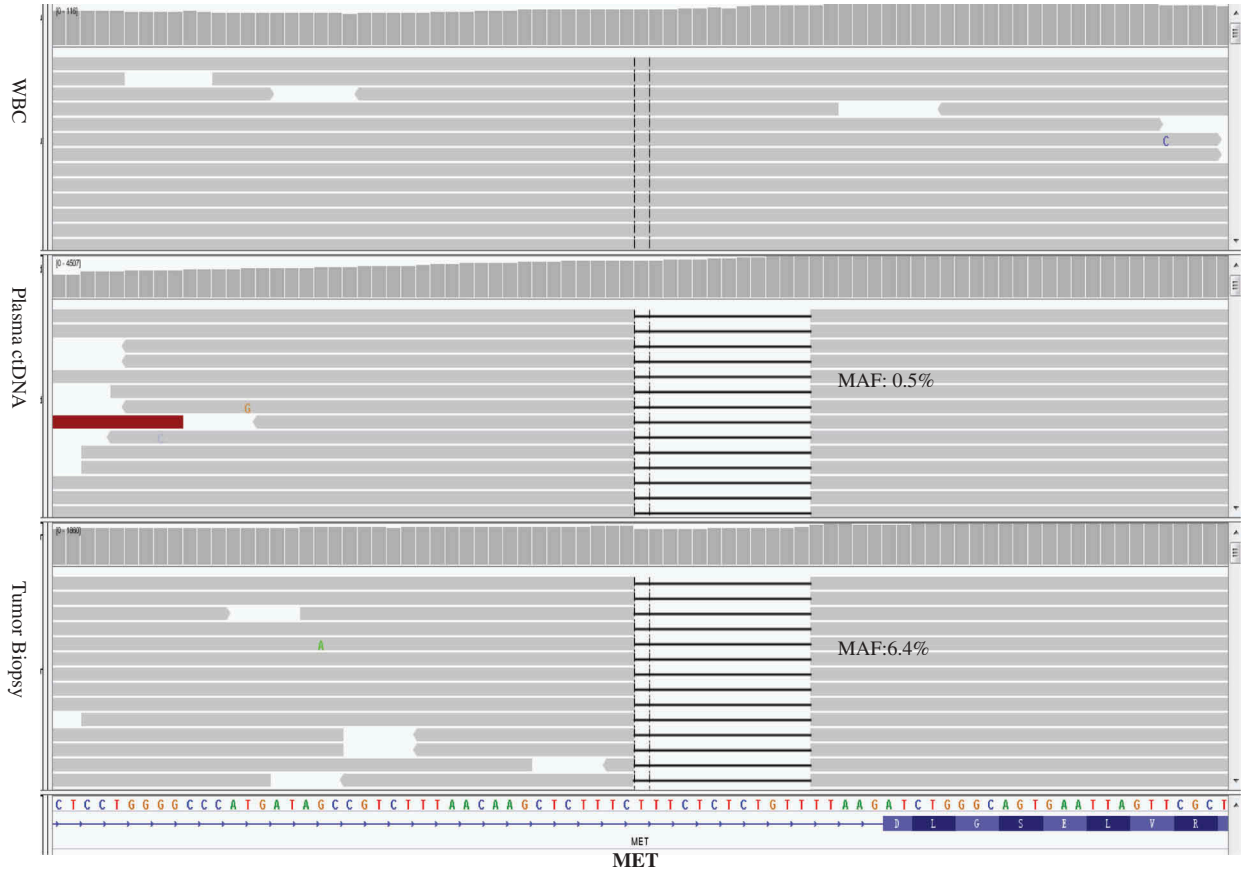

Figure 2. MET exon 14 skipping mutation identified in the pre-crizotinib tumor biopsy and plasma ctDNA.

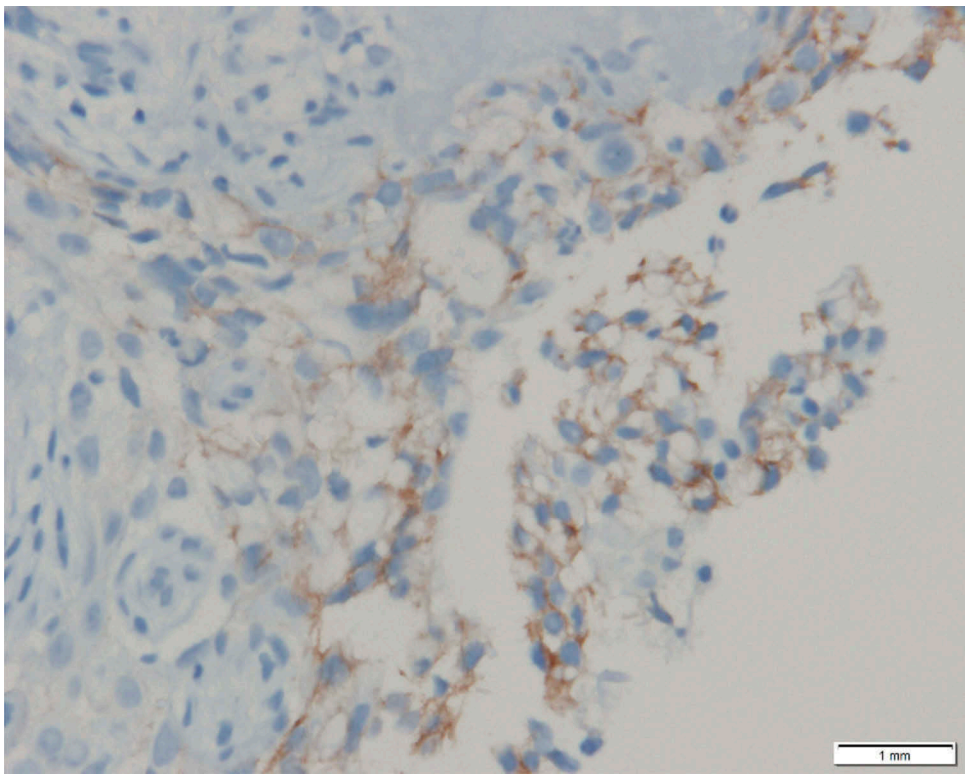

Figure 3. HER2 over-expression by IHC at 400× magnification.

from NSCLC to SCLC, accounting for approximately 3%–15%.[25] The case in our report indicated that SCLC transformation might be one of the resistance mechanisms in crizotinib treatment of MET exon 14 skipping, which still needs more evidence.

The resistance machanisms is wide-ranging for cancer's ability, second-site mutations in the target gene and bypass tract signaling both can develop in response to target drugs. For NSCLC with MET exon 14 skipping, although second-site mutations in MET gene had been reported and demonstrated to be mechanisms of resistance to MET inhibitors, to our knowledge, this is the first clinical report of Her2 amplification arising in a patient with MET exon 14 skipping. The bypass tract signaling was emphasized to be resistance mechanisms to MET inhibitors and the range of resistance mechanisms observed may provide further insight into clinical decision.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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