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Lung adenocarcinoma with sarcomatoid transformation after tyrosine kinase inhibitor treatment and chemotherapy

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ABSTRACT

Objectives: Lung cancers have various acquired resistance mechanisms that lead to treatment failure and disease progression, including secondary epidermal growth factor receptor (*EGFR*) exon 20 T790 M mutations, EGFR downstream or bypass pathway activation, and histologic transformation from adenocarcinoma to small cell carcinoma, squamous cell carcinoma, or sarcomatoid carcinoma.

Materials and Methods: This study compared the pathological and immunohistochemical characteristics before and after sarcomatoid transformation. Six advanced cases of lung adenocarcinoma that developed sarcomatoid transformation after treatment were collected.

Results: Five cases had classic EGFR mutations and one had a ROS1 rearrangement. The interval from initial diagnosis to sarcomatoid transformation ranged from 9 to 88 mo (median of 31.5 mo). The median survival after sarcomatoid transformation was 2.5 mo (1–16 mo). Before sarcomatoid transformation, all cases demonstrated typical adenocarcinoma features, including acinar, micropapillary, or solid/cribriform patterns, negative or weak focal vimentin staining, and strong E-cadherin expression. Histologic features of sarcomatoid transformation included giant cell features (6/6), loose cellular cohesion (6/6), strong staining for vimentin (6/6), decreased or lost E-cadherin expression (5/6), and high PD-L1 expression (5/6; one case demonstrated high PD-L1 staining at initial diagnosis). High MET expression and MET copy number gain (two samples with high polysomy and three with true amplification) were observed in five cases with EGFR mutation treated with tyrosine kinase inhibitors (TKI). One case exhibited MET amplification prior to the start of TKI treatment.

Conclusion: Sarcomatoid transformation is a type of lung cancer histologic evolution with a poor prognosis and a high proportion of cases with aberrant MET activation and PD-L1 expression.

1. Introduction

Lung cancer is the leading cause of cancer-related death worldwide [1]. In Taiwan, epidermal growth factor receptor (*EGFR*) mutations are the most common driver mutations and can be found in 55.7% of patients with lung adenocarcinoma [2]. In addition to the discovery of several important driver mutations, a number of agents targeting mutations in genes including *EGFR*, anaplastic lymphoma kinase (*ALK*), *ROS1*, *MET*, and *RET*, have been developed, with some demonstrating impressive clinical responses [3–14]. First- and second-generation EGFR tyrosine kinase inhibitors (EGFR-TKIs), including gefitinib, erlotinib, and afatinib, are currently being used as first-line therapies for patients with advanced *EGFR* mutation-positive lung adenocarcinoma. However, tumor cells can develop acquired resistance to target therapies after an average of 12–15 months [15]. Acquired resistance mechanisms include secondary acquired *EGFR* T790 M mutations, activation of bypass pathways, and histological transformation such as small-cell or squamous-cell transformation [15–17]. Patients with acquired *EGFR*-T790M-positive advanced non-small-cell lung carcinoma (NSCLC) can be treated with osimertinib, a third-generation EGFR-TKI that demonstrates improved efficacy compared with the conventional chemotherapy consisting of platinum therapy plus pemetrexed [18].

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Similar to first and second generation TKIs, tumor cells can become resistant to osimertinib by the acquisition of the *EGFR* C797S mutation, loss of the T790 M mutation, activation of bypass or downstream pathways, or histological transformation [19].

Activation of EGFR-independent bypass pathways or histologic transformation from adenocarcinoma to small-cell carcinoma or squamous-cell carcinoma can also make tumor cells resistant to EGFR TKIs [20–30]. EGFR-independent bypass pathways include *MET* amplification, human epidermal growth factor receptor 2 (*HER2*) amplification, fibroblast growth factor receptor 1 (*FGFR1*) amplification, insulin-like growth factor 1 receptor (IGF1R) activation, and AXL activation [19–21,25–27]. The tyrosine kinase receptor MET (c-Met) belongs to the hepatocyte growth factor (HGF) receptor family and is encoded by the *MET* gene. c-MET is essential for embryonic development, organogenesis, and wound healing [22]. *MET* gene amplification is another important resistance mechanism that leads to treatment failure of EGFR inhibitors [15,17,19–21]. Other *MET* mutations, including exon 14 deletion and *MET* rearrangements, have been identified in NSCLCs [10–12].

Phenotypic changes can be observed in adenocarcinomas that have acquired resistance to EGFR TKIs [23,27]. Conventional adenocarcinoma may undergo histologic evolution into small-cell carcinoma, squamous-cell carcinoma, or epithelial-to-mesenchymal transition (EMT) [23–31]. EMT has been observed in clinical cases as well as in tumor cell lines [23,24]. However, most EMT studies have been based on tumor cell lines, with EMT being defined as increased vimentin expression or decreased E-cadherin expression [23,24]. Clinical cases with sarcomatoid transformation confirmed by pathological examination are rarely reported [23]. In our daily practice, NSCLCs with sarcomatoid transformation is far less frequent than those with small-cell or squamous-cell carcinoma transformation. In the current study, we report six cases of lung adenocarcinoma with sarcomatoid transformation after treatment and describe their histologic and immunohistochemical features. As c-MET overexpression was incidentally found in two cases, we also evaluated c-MET expression and MET gene status before and after sarcomatoid transformation in all six cases.

2. Materials and methods

2.1. Case selection

Six cases initially diagnosed as lung adenocarcinoma with sarcomatoid transformation identified in subsequent biopsies between January 2017 and May 2019 in the Department of Pathology at National Taiwan University Hospital (NTUH) were reviewed. Clinical and demographic data were collected from patient medical records, including sex, age, smoking history, tumor site, tumor size, tumor stage, *EGFR/ROS1* mutations, treatment history, and clinical outcomes. EGFR mutation analysis was performed using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) in our institute which could also assess *KRAS*, *BRAF*, and *HER2* mutations [2]. In this study, *KRAS*, *BRAF*, and *HER2* mutations were not detected in any of the six patients. Tumor staging was performed according to the eighth edition of the American Joint Committee on Cancer guidelines [32]. The pathological features of specimens acquired before and after sarcomatoid transformation were evaluated by two pathologists (M.S.H. and Y.H.L.). The study (201901022RINA) was approved by the Research Ethics Committee of NTUH.

2.2. Immunohistochemistry (IHC)

Tissue sections (4 μm) were deparaffinized and rehydrated. Immunohistochemistry was performed using a Ventana BenchMark XT Autostainer (Ventana, Tucson, AZ, USA). The slides were allowed to react with antibody specific for c-MET (SP44, 1:50, Ventana), CK (AE1/AE3/PCK26, ready-to-use, Ventana), CK7 (SP52, ready-to-use, Ventana), TTF-1 (SP141, ready-to-use, Ventana), vimentin (V9, ready-to-use, Ventana), E-cadherin (36, ready-to-use, Ventana), ALK (D5F3) CDx Assay (Ventana), PD-L1 (SP263) Assay (Ventana), and ROS1 (D4D6, 1:250; Cell Signaling Technology, Beverly, MA, USA). The staining intensity of c-MET was categorized as 0, no staining; 1+, weak cytoplasmic staining; 2+, moderate cytoplasmic staining; and 3+, strong cytoplasmic staining. MET-positive immunostaining was defined as 2+ or 3+ staining intensity in ≥ 50% of tumor cells according the criteria used in the METLung trial (MetMab criteria) [33]. PD-L1 expression was evaluated using the Tumor Proportion Score (TPS), and tumor cells that demonstrated any perceptible membrane staining were included. The staining results were scored.

2.3. Detection of MET gene amplification by fluorescence in situ hybridization (FISH)

The commercial ZytoLight SPEC *MET*/CEN 7 Dual Color Probe (Zytovision, Germany) was used to assess *MET* gene amplification using a previously described method [33]. Briefly, 4-μm thick paraffin-embedded tissue sections were deparaffinized in xylene (three times, 10 min each), followed by two 5-min washes in 100% ethanol. Sections were then treated with FISH Pretreatment Reagent (Abbott Molecular, Des Plaines, IL, USA) at 80 °C for 30–50 min, after which the sections were treated with protease mixed with protease buffer. Sections were hybridized with *MET*/CEN 7 FISH probes as per the manufacturer's instructions. Results were analyzed using a fluorescence microscope (Zeiss AXIO Imager.D2) and Axio-Vision 4.5 software. For each case, 50 non-overlapping nuclei of tumor cells were selected and evaluated. *MET* gene amplification was considered positive when more than 10% of the evaluated cells contained a tight gene cluster of ≥15 copies, a *MET*/CEN7 ratio ≥ 2.0 (truly amplified), or ≥ 5.0 copies of the *MET* gene/cell (high polysomy) [33–35].

3. Results

3.1. Clinical features of lung adenocarcinoma with sarcomatoid transformation after treatment

The clinical features of the six cases of lung adenocarcinoma that developed sarcomatoid transformation after treatment are summarized in Table 1. The study cases included one male and five female patients. The patient ages ranged from 36 to 76 yr, with a mean age of 52.7 yr. Except for the patient in Case 1, none of the patients had a history of being smokers. Tumor size ranged from 2.5 to 9 cm in maximum diameter, with a mean diameter of 5.7 cm. The initial clinical stage for all six patients was stage IV. At the time of initial diagnosis, each of the patients had malignant pleural effusion, with five having distant metastases. In contrast to Case 1, which had a *ROS1* rearrangement, the other five cases were found to have classic *EGFR* mutations. Case 1 received only chemotherapy, while the other five patients received EGFR-TKIs followed by chemotherapy due to disease progression. Cases 3 and 6 had acquired *EGFR* T790 M mutations, and both patients received osimertinib. The interval from the initial diagnosis of lung adenocarcinoma to pathologically confirmed sarcomatoid transformation ranged from 9 to 88 mo, with a median interval of 31.5 mo. All patients passed away due to disease progression during follow-up, with a median overall survival time since diagnosis of 39.5 mo (12–89 mo) and a median survival since the time of sarcomatoid transformation of 2.5 mo (1–16 mo).

3.2. Pathological features of lung adenocarcinoma before and after sarcomatoid transformation

The histologic and immunohistochemical features of the lung adenocarcinomas before and after sarcomatoid transformation for the six patients included in the study are summarized in Table 2. Most of the

Table 1
Clinical information of lung ADC cases with sarcomatoid transformation.

Case	Sex	Age	Smoking	Site	Tumor size (cm)	TNM / Stage	Metastatic sites found at initial diagnosis/ during treatment	Treatment before sarcomatoid transformation/Clinical response after sarcomatoid transformation	Interval between initial diagnosis and sarcomatoid transformation (mo)	Survival time: overall/after sarcomatoid transformation (mo)
1	M	36	+	RLL	7.0	cT4N2M1b, stage IVA	bilateral lungs, pleura, brain/ liver, penis	Chemotherapy (cisplatin/pemetrexed/ bevacizumab)/primary tumor stable but progression of brain, lung, and liver metastases under chemotherapy	78	81/3
2	F	41	−	LLL	5.0	cT4N0M1c, stage IVB	bilateral lungs, pleura, bone/brain, heart	*TKI (gefitinib, erlotinib, afatinib) + chemotherapy (cisplatin/ pemetrexed) /primary tumor stable but progression of brain, lung, and heart metastases under chemotherapy	88	89/1
3	F	53	−	RLL	2.5	cT4N3M1c, stage IVB	bilateral lungs, pleura, brain, bone, liver/adrenal gland, left eye (retina)	TKI (erlotinib) + chemotherapy (cisplatin/pemetrexed) + TKI (osimertinib)/progression of lung, brain, liver, adrenal gland, metastases under osimertinib	35	36/1
4	F	56	−	LLL	4.1	cT3N0M1c, stage IVB	lung (ipsilateral), pleura, brain, bone/liver, retroperitoneum, peritoneum	*TKI (gefitinib, afatinib) + chemotherapy (cisplatin/ pemetrexed/ bevacizumab) + immunotherapy (pembrolizumab)/progression of brain, bone, liver, retroperitoneum, peritoneal metastases under chemotherapy	9	25/16
5	F	54	−	RLL	6.4	cT3N0M1c stage IVB	lung (ipsilateral), pleura, bone/ retroperitoneum, abdominal wall	*TKI (gefitinib, erlotinib, afatinib) + chemotherapy (cisplatin/ pemetrexed)/progression of intraabdominal and abdominal wall metastases under chemotherapy	10	12/2
6	F	76	−	RLL	9.0	cT4N2M1a, stage IVA	lung (ipsilateral), pleura/brain	*TKI (gefitinib, erlotinib) + TKI (osimertinib) + chemotherapy (gemcitabine/pemetrexed)/progression of brain metastases under chemotherapy	39	43/4

Abbreviations: ADC adenocarcinoma; RLL right lower lobe; LLL left lower lobe; TKI tyrosine kinase inhibitor; TNM tumor node, and metastases.

* The switch of TKI was due to side effects including watery diarrhea or hepatotoxicity. TKI was replaced by chemotherapy due to disease progression and newly developed metastases.

Table 2
Pathological features of lung ADC before and after sarcomatoid transformation.

Case	Biopsy /Site	Pathological Findings	Immunohistochemistry						Mutation Analysis	
			TTF-1	CK	CK7	vimentin	E-cadherin	PD-L1	EGFR	Other
1	1st biopsy (brain)	ADC (solid/cribriform)	(+)	(+)	(+)	(−)	(+)	1%	wild type	ROS1 fusion
	2nd biopsy (penis)	sarcomatoid transformation (giant cells)	(-)	(+), focal	(+), focal	(+)	(-)	100%	wild type	ROS1 fusion
2	1st biopsy (lung)	ADC (acinar/micropapillary)	(+)	(+)	(+)	(−)	(+)	25%	exon 19 del	N/A
	2nd biopsy (atrium)	sarcomatoid transformation (giant cells)	(+)	(+)	(+), focal	(+)	(-)	50%	exon 19 del	N/A
3	1st biopsy (lung)	ADC (acinar/micropapillary)	(+)	(+)	(+)	(−)	N/A	N/A	exon 19 del	N/A
	2nd biopsy (lung)	ADC (solid with occasional lumina)	(+)	(+)	(+)	(+), focal < 10%	(+)	0%	exon 19 del + exon 20 T790M	N/A
	3rd biopsy (lung)	sarcomatoid transformation (giant cells/solid nests with occasional lumina)	(-)	(+)	(+)	(+)	(+)	100%	exon 19 del + exon 20 T790M	N/A
4	1st biopsy (lung)	ADC (micropapillary)	(+)	(+)	(+)	(−)	(+)	95%	exon 18 G719 + exon 19 del	N/A
	2nd biopsy (supraclavicular LN)	sarcomatoid transformation (giant cells)	(+), weak	(+)	(+), focal	(+)	weak, 5%	90%	exon 18 G719 + exon 19 del	N/A
5	1st biopsy (lung)	ADC (micropapillary)	(+)	(+)	(+)	(+), focal < 10%	(+)	5%	exon 21 L858R	N/A
	2nd biopsy (abdominal wall)	sarcomatoid transformation (giant cells)	(+), weak	(+)	(−)	(+)	(−)	90%	exon 21 L858R	N/A
6	1st biopsy (effusion)	ADC (micropapillary)	(+)	(+)	(+)	(+), focal < 10%	(+)	< 5%	exon 21 L858R	N/A
	2nd biopsy (lung)	ADC (small nests/ micropapillary)	(+)	N/A	(+)	N/A	N/A	N/A	exon 21 L858R + exon 20 T790M	N/A
	3rd biopsy (lung)	sarcomatoid transformation (giant cells/solid nests)	(+)	(+), focal	(+), focal	(+)	weak, 90%	< 5%	exon 21 L858R + exon 20 T790M	N/A

Abbreviations: ADC, adenocarcinoma; N/A, not applicable.

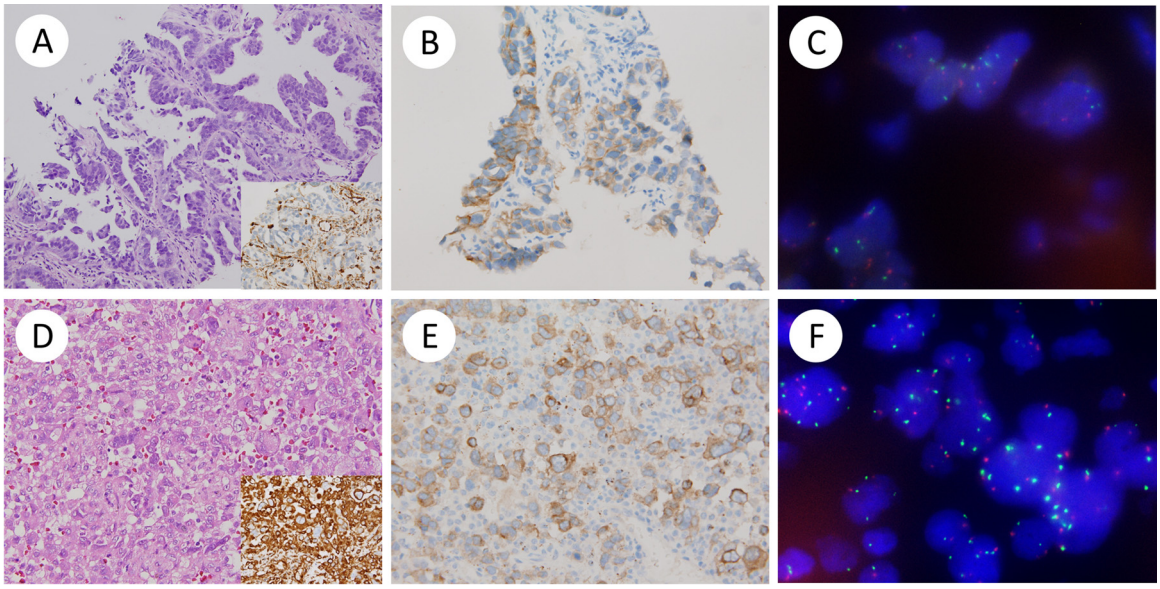

Fig. 1. Case 2 at initial diagnosis. (A) Adenocarcinoma cells arranged in a micropapillary-predominant pattern, negative for vimentin (inset), moderate staining (2+) for c-MET (B), but no *MET* amplification according to *MET* fluorescence *in situ* hybridization (FISH) (C). (D) After treatment and disease progression, re-biopsy showed sarcomatoid transformation features including the presence of giant cells, loose cellular cohesiveness, strong staining for vimentin (inset), moderate staining (2+) for c-MET (E), and *MET* gene gain (high polysomy) according to *MET* FISH (F). Original magnification: A, 200×; B, D, and E, 400×.

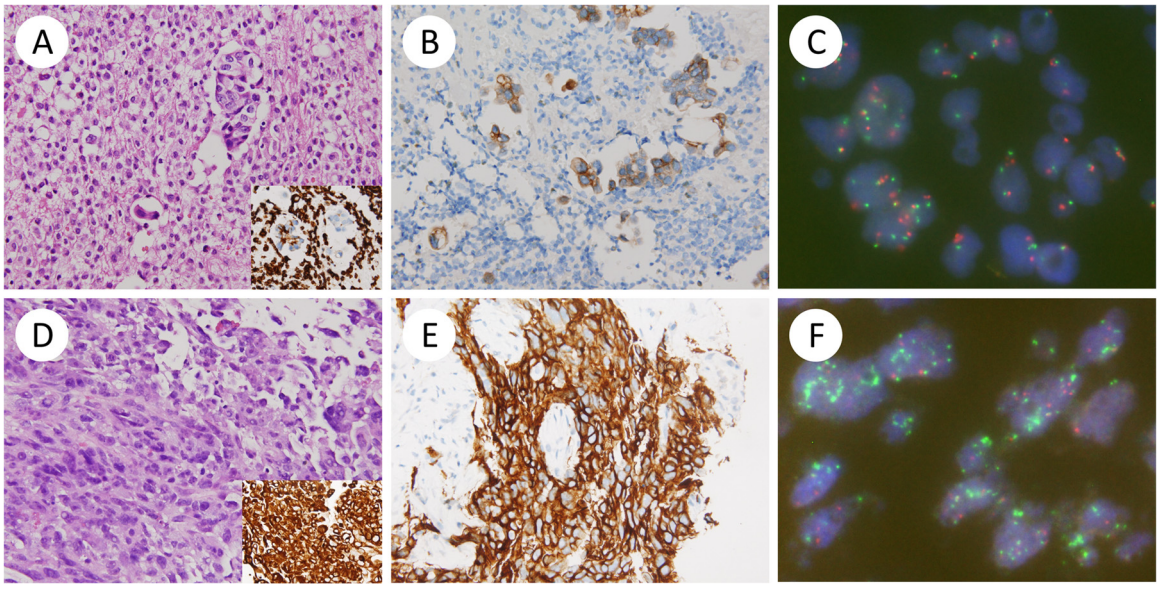

Fig. 2. Case 6 at initial diagnosis. (A) Adenocarcinoma cells arranged in micropapillary or small solid nests in pleural effusion with negative or weak focal staining for vimentin (inset), and moderate staining (2+) for c-MET (B). (C) No *MET* amplification according to MET fluorescence *in situ* hybridization (FISH), but *MET* gene copy number heterogeneity was present with some cells having high polysomy. (D) After treatment and disease progression, re-biopsy showed sarcomatoid transformation features including giant cells, loose intercellular cohesiveness, strong staining for vimentin (inset), strong staining (3+) for c-MET (E), and *MET* amplification according to *MET* FISH (F). Original magnification: A, B, D, and E, 400×.

specimens evaluated were biopsies, since all of the patients had advanced clinical stage disease at the time of first diagnosis. Pathologically, Case 1 with a *ROS1* rearrangement had mixed histopathology with both solid and cribriform patterns (Supplementary file). The other five cases with classic *EGFR* mutations displayed acinar or micropapillary patterns. After sarcomatoid transformation, the tumor cells became more undifferentiated and showed features of giant cell carcinoma such as loose intercellular cohesiveness, eosinophilic cytoplasm, marked nuclear pleomorphism, tumor giant cells, or marked tumor necrosis (Figs. 1–3 and Supplementary file). Undifferentiated sarcomatoid cells were the exclusive cell type (4/6, 66.7%) or the predominant cell type (2/6, 33.3%) in the re-biopsy specimens.

Prior to sarcomatoid transformation, immunohistochemistry showed that all cases were strongly positive for CK, CK7, TTF-1, and E-cadherin. Two cases showed weak focal vimentin staining (< 10% of tumor cells) prior to sarcomatoid transformation. After sarcomatoid transformation, all cases showed strong and diffuse staining for vimentin. All cases other than Case 3 demonstrated an absence (three cases) or marked decrease (two cases) in E-cadherin expression. CK expression was preserved in all cases but decreased in intensity in two cases; TTF-1 expression was preserved in four cases. The PD-L1 assay (TPS) revealed that PD-L1 expression levels markedly increased after sarcomatoid transformation in four cases. PD-L1 expression increased from 0 to 5% pre-transformation to 90–100% after transformation in

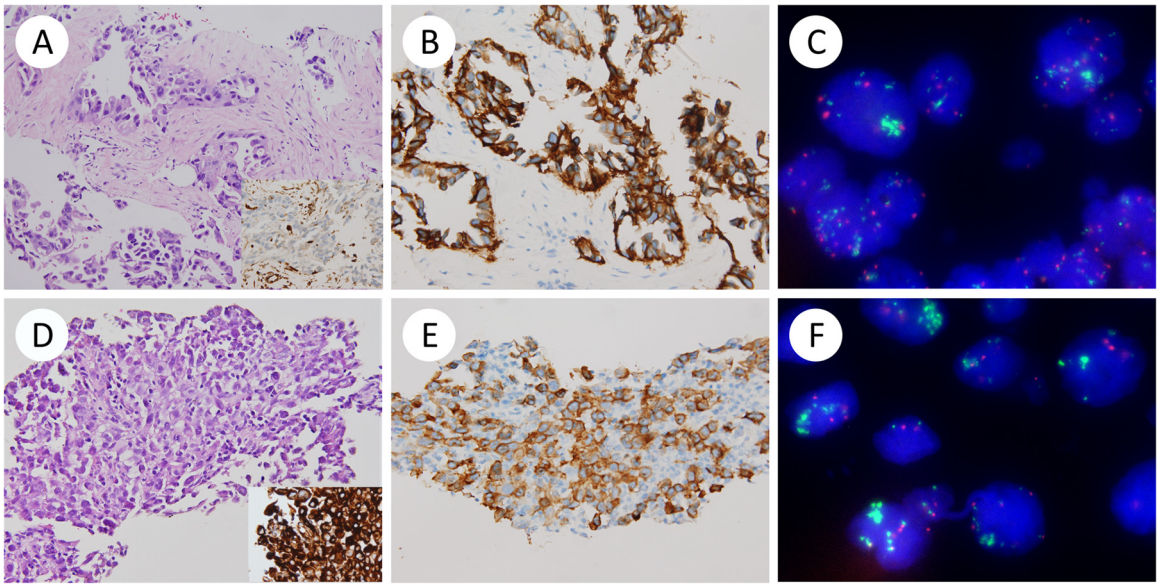

Fig. 3. Case 4 at initial diagnosis. (A) Adenocarcinoma cells arranged in a micropapillary pattern and negative staining for vimentin (inset) with strong staining (3+) for c-MET (B), and *MET* amplification according to *MET* fluorescence *in situ* hybridization (FISH) (C). (D) After treatment and disease progression, re-biopsy showed sarcomatoid transformation features including giant cells, loose intercellular cohesiveness, strong staining for vimentin (inset), strong staining (3+) for c-MET (E), and *MET* amplification according to *MET* FISH (F). Original magnification: A, 200×; B, D, and E, 400×.

three cases and went from 25% to 50% in one case. PD-L1 expression before and after sarcomatoid transformation in the remaining two cases was nearly unchanged, with high PD-L1 expression (≥ 90%) in Case 4 and low PD-L1 expression (< 5%) in Case 6.

3.3. c-MET immunohistochemistry and status of the MET gene

Results of the c-MET immunohistochemistry and *MET* gene FISH analyses are summarized in Table 3. Case 1, which had the *ROS1* rearrangement, had negative staining for c-MET and no *MET* amplification. For the other five cases with classic *EGFR* mutations, one exhibited *MET* amplification at the time of initial diagnosis (Fig. 3), while the other four cases developed increased *MET* gene copy numbers after treatment with TKI and chemotherapy, two having high polysomy (Fig. 1) and two having true amplification (Fig. 2 and Supplementary file). The initial biopsy specimens of Cases 2, 5, and 6 showed intratumor heterogeneity with high polysomy of the *MET* gene in a minority of the tumor cells. Using the criteria from the METLung trial [33], a 3+ c-MET IHC score correlated well with *MET* gene gain (amplification or high polysomy) detected by FISH.

4. Discussion

Oncogene-driven NSCLC that is initially responsive to TKIs can acquire resistance through various mechanisms within 1–2 years of starting therapy [17,23]. A second point mutation in *EGFR* is the most common mechanism leading to drug resistance. Fortunately, this form of resistance may be overcome by treating patients with third generation TKIs. Currently, detection of the *EGFR* T790 M mutation is crucial in lung cancer treatment. Activation of EGFR bypass pathways can lead to acquired resistance to TKIs. However, it is more difficult to detect bypass pathway activation, which usually requires high-quality specimens and methods such as FISH or next generation sequencing. Unlike secondary mutations or bypass pathway activation, histologic evolution is a unique resistance mechanism that can be readily observed by pathological examination. Small-cell transformation is the most common type of histologic evolution, and it is found in 3–10% of lung cancer patients with acquired resistance [17,23,29,36,37]. Small-cell lung

Table 3
c-MET IHC and FISH results of lung ADC before and after sarcomatoid transformation.

Case	Biopsy Findings	c-MET IHC			FISH		
		Intensity†	Proportion (%)	MetMab Criteria	Amplification	High Polysomy	Average MET Gene Copy Number/Cell
1	1st biopsy: ADC	0	0	(-)	(-)	(-)	2.4
	2nd biopsy: sarcomatoid transformation	(1+)	5	(-)	(-)	(-)	3.2
2	1st biopsy: ADC	(2+)	70	(+)	(-)	(-)	4.6#
	2nd biopsy: sarcomatoid transformation	(2+)	75	(+)	(-)	(+)	6.3
3	2nd biopsy: ADC	(1+)	25	(-)	(-)	(-)	2.8
	3rd biopsy: sarcomatoid transformation	(3+)	95	(+)	(-)	(+)	9.6
4	1st biopsy: ADC	(3+)	95	(+)	(+)	(-)	8.4
	2nd biopsy: sarcomatoid transformation	(3+)	95	(+)	(+)	(-)	> 15
5	1st biopsy: ADC	(1+)	30	(-)	(-)	(-)	4.5#
	2nd biopsy: sarcomatoid transformation	(3+)	90	(+)	(+)	(-)	> 15
6	1st biopsy: ADC	(2+)	75	(+)	(-)	(-)	2.6#
	3rd biopsy: sarcomatoid transformation	(3+)	100	(+)	(+)	(-)	> 15

Abbreviations: ADC, adenocarcinoma; FISH, fluorescence *in situ* hybridization; IHC, immunohistochemistry.
† (0), no staining (1+), weak cytoplasmic staining; (2+), moderate cytoplasmic staining; and (3+), strong cytoplasmic staining.
# These cases showed MET gene copy number heterogeneity with some cells having high polysomy.

carcinoma (SCLC) transformed samples not only retain their original *EGFR* mutations but are also associated with a high frequency of *TP53*, *Rb1*, and *PIK3CA* mutations [17,31,36,37]. Studies have demonstrated that *TP53* and *Rb1* mutations may be found at the initial diagnosis of NSCLC, while activation of the phosphoinositide 3-kinase (PI3K) pathway, an EGFR downstream pathway, is a later genetic event that occurs after SCLC transformation [31,36,37]. Squamous cell carcinoma transformation is another form of histologic evolution that confers drug resistance to NSCLC and has been observed in both clinical cases and cell line studies [28,38].

EMT histologic evolution can also lead to drug resistance [23,24]. However, most reports of EMT-based drug resistance have been reported in cancer cell line studies, with the diagnosis of EMT based on the expression of vimentin and the loss of E-cadherin expression in tumor cells, rather than on histologic examination [23,24]. Clinical cases with sarcomatoid transformation that can be readily observed by pathologists are rare [23]. Sequist et al. reported that a resistant lung adenocarcinoma cell line developed a spindle cell morphology and vimentin expression consistent with EMT [23]. They also reported three lung cancer patients with phenotypic sarcomatoid changes at the time of TKI resistance with no other resistance mechanisms being identified [23]. Chung et al. reported that a lung cancer patient whose tumor acquired resistance to TKI demonstrated EMT with no other known resistance mechanisms detected, such as *EGFR* T790 M mutation or *MET* amplification [39]. EMT is considered to be an intrinsic resistance mechanism to EGFR inhibitors [23,27,39]. The incidence rate of EMT varies from 1% to 8% in different studies [23,25–27]. The incidence rate of sarcomatoid change at the time of TKI resistance in biopsy specimens is estimated to be 5% (5/100) in our institute during this study period since there are an average of 40 re-biopsy cases per year. Studies focusing on NSCLC with EMT or sarcomatoid transformation after treatment are rare and, to the best of our knowledge, the current study is the largest case series focusing on changes in clinicopathological features before and after sarcomatoid transformation. Our findings are consistent with those of previous groups in that sarcomatoid transformation can be a mechanism of resistance leading to treatment failure.

According to the 2015 World Health Organization (WHO) classification of lung tumors, pulmonary sarcomatoid carcinoma (PSC) is a specific pathological category in lung cancer classification with different subtypes including pleomorphic carcinoma, spindle cell carcinoma, giant cell carcinoma, carcinosarcoma, and pulmonary blastoma [40]. Each subtype has its own characteristic histologic features, and diagnosis is usually dependent on surgically resected specimens. PSC is uncommon and comprises less than 1% of all NSCLC, with most cases arising in male patients, especially smokers [40,41]. Immunohistochemically, PSC typically demonstrates decreased or absent expression of CK, CK7, and TTF-1 in the sarcomatoid component [40,41]. Several genetic studies have shown that PSC is more commonly associated with *TP53* and *KRAS* mutations, with only a minority of cases reported to have classic EGFR mutations [42,43].

Recently, *MET* amplification and *MET* exon 14 skipping mutations were found to be important in the molecular pathogenesis of PSC. *MET* exon 14 skipping mutations are found in 12–22% and *MET* amplification in 13.6–25.6% of PSC [44–47]. In addition, PD-L1 overexpression is observed in over half of PSC [48–50]. Patients with PSC containing either aberrant MET activation or PD-L1 overexpression may benefit from MET inhibitors or immunotherapy.

In this study, all six cases with sarcomatoid transformation demonstrated features similar to giant cell carcinoma of PSC, and no spindle cell or true sarcomatous components were seen. Due to the advanced clinical stage, all specimens in this study were biopsies or small resected tissues, which could not be classified into specific PSC subtypes.

PSC and lung adenocarcinomas with sarcomatoid transformation after treatment have similar and dissimilar features in pathological and genetic profiles. Similar to PSC, the expression of PD-L1 was high in five of the six (83%) cases with sarcomatoid transformation when a TPS of 50% was used as the cut-off point. In contrast to PSC, the *EGFR* mutation rate in adenocarcinomas with sarcomatoid transformation was high because they retained their founder *EGFR* mutations after histologic evolution. Except for Case 1, *MET* gene gains were detected in five cases, with the gains being identified at initial diagnosis in one case and after receiving TKIs and undergoing sarcomatoid transformation in four cases. These findings suggest that MET pathway activation may play an important role in the pathogenesis of both *de novo* PSC and adenocarcinoma with sarcomatoid transformation that occurs after treatment. Additional studies are needed to determine whether adenocarcinoma with *MET* gene gain prior to TKI treatment is prone to sarcomatoid transformation after treatment.

In the current study, we demonstrated that sarcomatoid transformation was frequently associated with changes in the number of *MET* genes (amplification or high polysomy), as well as with high c-MET expression. MET is considered to be one of the bypass pathways leading to EGFR-TKI resistance. Similarly, small-cell transformation is usually associated with activation of the PI3K pathway, which is one of the downstream pathways of EGFR [23,29,31]. The current findings suggest that histologic evolution may be regarded as a phenotypic reflection of underlying genetic changes that occur when tumor cells develop acquired resistance. The prognosis for patients in our study with sarcomatoid transformation was poor, with a median survival of 2.5 mo from the time of sarcomatoid transformation. Similarly, *de novo* PSC is notorious for its poor prognosis in advanced cases and poor response to traditional chemotherapy. PSC is also well known to have unusual distant metastatic sites such as the retroperitoneum and gastrointestinal tract [40]. A pattern of unusual sites of metastatic disease is also present in patients with sarcomatoid transformation, as metastases were found in the penis, heart, eye (retina), retroperitoneum, and abdominal wall. Clinically, the development of unusual metastatic sites may be a useful clue suggestive of sarcomatoid transformation, and re-biopsy should be considered for pathological confirmation. Several MET inhibitors, such as crizotinib, cabozantinib, capmatinib, and glesatinib have been found to have anti-tumor efficacy in cell lines or xenograft models of *MET* exon 14 skipping mutants and *MET* amplification [51]. While a randomized phase III trial showed no benefit in using a MET inhibitor in the treatment of advanced NSCLC with MET overexpression [33], the utility of MET inhibitors in PSC or NSCLC with sarcomatoid transformation is unknown. Further clinical studies are needed to identify useful biomarkers for selecting lung cancer patients who may benefit from treatment with MET inhibitors.

Studies have used different criteria to evaluate c-MET IHC and FISH results, and the correlation between MET IHC and FISH is not as good as ALK IHC in *ALK*-rearranged lung cancer. In this study, patients with a c-MET IHC 3+ score typically demonstrated diffuse and complete circumferential membranous staining and strong cytoplasmic staining. Those with a c-MET IHC 2+ score presented a mixture of complete and incomplete circumferential membranous staining and moderate cytoplasmic staining. Patients with a c-MET IHC 1+ score presented incomplete circumferential membranous staining and weak cytoplasmic staining in less than 50% of the tumor cells. Using the criteria from the METLung phase III trial, our study revealed that c-MET IHC correlated well with *MET* FISH, except for the first biopsy specimens of Cases 2 and 6, which showed a 2+ level of c-MET IHC staining in 70% of tumor cells (Case 2) and 75% of tumor cells (Case 6), but average *MET* gene copy numbers of 4.6 and 2.6, respectively. It is worth noting that *MET* gene copy number heterogeneity was observed in these two cases, with some cells having high polysomy but the average signals per cell being less than five. According to the results of the METLung phase III trial, the concordance rate between IHC and FISH is higher in IHC 3+ cases compared with those with IHC 2+ scores [33]. Since there are other mechanisms that may lead to MET overexpression, such as *MET* exon 14 skipping mutations, it is reasonable to select patients with high MET

IHC expression, rather than using FISH. However, the METLung phase III trial showed that the use of the MET inhibitor onartuzumab plus erlotinib failed to improve clinical outcomes in patients with NSCLC selected by MET IHC as well as those with *MET* amplification confirmed by FISH [33]. Determining the appropriate criteria for MET IHC/FISH or other biomarkers for patients with NSCLC will require further studies.

In conclusion, we reported six cases of lung adenocarcinoma with sarcomatoid transformation that occurred after treatment, with a median interval of 31.5 mo after initial diagnosis. The histologic changes included features of giant cell carcinoma of PSC, loose intercellular cohesiveness, strong expression of vimentin, and decreased or lost E-cadherin staining. High MET expression and *MET* copy number gain (two high polysomy and three true amplification) were observed in five cases with *EGFR* mutation treated with EGFR-TKIs. One case was found to have *MET* amplification prior to the start of TKI treatment. PD-L1 overexpression (TPS ≥ 50%) was observed in the majority of cases (5/6). Overall, sarcomatoid transformation is another type of lung cancer histologic evolution that has a poor prognosis, unusual distant metastatic sites, and a high proportion of cases with aberrant MET activation and PD-L1 expression.

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Declaration of Competing Interest

None of the authors have potential conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.08.029.

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