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Molecular characterization of pulmonary sarcomatoid carcinoma: analysis of 33 cases

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Several targetable genetic alterations have been found in lung cancer, predominantly in adenocarcinomas, which have led to important therapeutic advancements with the advent of targeted therapy. In contrast, the molecular features and presence of targetable genetic abnormalities in pulmonary sarcomatoid carcinomas are largely unknown. Thirty-three cases of pulmonary sarcomatoid carcinoma were tested for approximately 2800 mutations in 50 oncogenes and tumor-suppressor genes, including *EGFR*, *KRAS*, *NRAS*, *TP53*, *BRAF*, *ERBB2*, *JAK3*, *AKT1*, *ATM*, *MET*, *KIT*, and *PIK3CA*. ALK immunostaining was performed, and *ALK* FISH was performed on cases with any degree of staining. Twenty-four of the 33 cases (72%) had at least one genetic abnormality: 19 cases (58%) had *TP53* mutations; 10 cases (30%) had *KRAS* mutations; *AKT1*, *JAK3*, *BRAF*, *NRAS*, and *PIK3CA* mutations were observed in 1 case each (3%). Six of the 19 cases (32%) with a mutation in *TP53* had simultaneous mutations in *KRAS* (18%). The cases with alterations in *JAK3*, *BRAF*, and *NRAS* also had mutations in *TP53*. The case showing a mutation in *PIK3CA* had a mutation in *KRAS*. No *EGFR* mutations were observed. One case had *ALK* gene rearrangement. *ALK* rearrangement was observed in a single case of sarcomatoid carcinoma (3%), which has currently available targeted therapy. Four tumors had mutations in genes with experimental molecular-based therapy, including *BRAF*, *NRAS*, *PIK3CA*, and *AKT1*. Testing for targetable mutations should be considered for patients with pulmonary sarcomatoid carcinoma, as a subset may benefit from currently approved drugs or clinical trials of novel therapeutic options available for other types of lung cancer.
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Pulmonary sarcomatoid carcinoma is a rare subtype of lung carcinoma, which is aggressive and poorly differentiated.[1–7] The poorly differentiated nature is reflected by the presence of at least 10% tumor giant cells or spindle-shaped cells, hence the designation as 'sarcomatoid'. The sarcomatoid areas may be observed as a form of progression of other types of lung carcinoma, or the entire tumor may be composed of sarcomatoid morphology. Areas of differentiated sarcoma may be observed, mostly commonly resembling rhabdomyosarcoma, chondrosarcoma, or osteosarcoma, and some examples show primitive/immature mesenchymal stroma.[1,3,8] In the current 2015 World Health Organization classification, sarcomatoid carcinomas are divided into five histological subtypes depending on the observed morphology: pleomorphic carcinoma, spindle cell carcinoma, giant cell carcinoma, carcinosarcoma, and pulmonary blastoma.[8] These histological subtypes are useful to pathologists in the recognition of the morphological spectrum of sarcomatoid carcinoma, but they do not appear to have clinical or therapeutic significance. Similar to other types of lung carcinoma, sarcomatoid carcinoma tends to affect older patients with a history of tobacco smoking, and a male predominance has been observed.[2,3,5,6,9,10] Treatment is the same as for other lung carcinoma with non-small cell morphology, but sarcomatoid carcinoma tends to have a poor prognosis even in early-stage disease.[8]

Substantial advancements in the treatment of lung cancer have occurred during the past decade, predominantly in adenocarcinomas. This accomplishment is largely due to the discovery of several targetable genetic alterations, leading to more effective therapy as well as a better understanding of cellular and molecular mechanisms of lung adenocarcinoma pathogenesis. Some of these molecular abnormalities have proven to be successful biomarkers and/or drug targets.[11–14] Currently, tumors with *ALK* rearrangement and *EGFR* mutations have molecular targeted therapies approved by the US

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Food and Drug Administration. Crizotinib and ceritinib are approved for use in *ALK* rearranged tumors,[15–17] while erlotinib, gefitinib, and afatinib are approved for tumors with *EGFR* mutations.[18–21] In the US population, these therapies are applicable to a minority of patients with lung carcinoma, as *ALK* rearrangement is present in $<5\%$ of lung adenocarcinomas and *EGFR* mutations are present in 15–25% of lung adenocarcinomas. *KRAS* mutations are present in a substantial number of lung adenocarcinomas in the US population (roughly 25–30%), but there are currently no approved targeted therapies for these mutations. Many additional rare mutations, predominantly within tyrosine kinase signaling complexes, have been identified in lung adenocarcioma, including *BRAF*, *NRAS*, *PIK3CA*, *AKT1*, *HER2*, *MET*, *KIF5B-RET*, *PTEN*, *ROS1*, and *MEK1*. This has resulted in several ongoing clinical trials, which are testing the newest potential therapies targeted at many of these genes.[11–14]

Although the molecular characteristics of the more common subtypes of lung carcinoma have been extensively studied, the molecular features and presence of targetable genetic abnormalities in pulmonary sarcomatoid carcinoma are largely unknown. The currently available chemotherapy for sarcomatoid carcinoma is highly ineffective,[4–7] and thus targeted therapy is a very attractive therapeutic option to improve outcome. A recent study reported frequent *MET* exon 14 skipping mutations in pulmonary sarcomatoid carcinomas, which may be targetable, in addition to mutations in other genes without currently available targeted therapy, including *TP53* and *KRAS*.[22,23] The aim of this study is to survey for potentially targetable genetic abnormalities in 33 cases of pulmonary sarcomatoid carcinoma, with the diagnosis previously confirmed based on morphology and immunohistochemistry.[24]

Materials and methods

The study was approved by the Mayo Clinic Institutional Review Board. Mayo Clinic surgical pathology archives were searched for cases of pulmonary sarcomatoid carcinoma from 1994 to 2011. All cases were re-reviewed by two pathologists (JMB and SBT), with histological and immunohistochemical confirmation of the diagnosis as previously described.[24] Cases with ambiguous morphology or immunophenotype were reviewed by a third pathologist (ESY), with consensus diagnosis. Cases felt to represent an alternative diagnosis were excluded, as were cases with insufficient remaining tissue for molecular testing, yielding a final study group of 34 cases.

DNA was extracted from formalin-fixed paraffin-embedded tumor samples and applied to the Ion AmpliSeq Cancer Hotspot Panel v2 to test by next-generation sequencing for approximately 2800 individual mutations in 50 oncogenes and tumor-suppressor genes, including *EGFR*, *KRAS*, *NRAS*, *TP53, BRAF*, *ERBB2*, *JAK3*, *AKT1*, *ATM*, *MET*, *KIT*, and *PIK3CA*, as previously described.[25] Mutational analysis was attempted in 34 cases, but 1 case was excluded owing to technical failure.

ALK immunohistochemistry was performed on formalin-fixed paraffin-embedded tumor sections from 34 cases (clone D5F3, rabbit monoclonal anti-human ALK, Cell Signaling Technology, Danvers, MA, USA). Fluorescent *in situ* hybridization using a break-apart probe for *ALK* rearrangement was performed on cases with any degree of ALK immunoreactivity.

Fluorescent *in situ* hybridization testing was also performed using a break-apart probe for *ROS1* rearrangement. This was performed on cases that did not have *KRAS* mutations or *ALK* rearrangements, as these genetic abnormalities are generally mutually exclusive of *ROS1* rearrangement. One case did not have sufficient tissue remaining for this analysis, so results were obtained on 21 cases.

Results

Complete patient characteristics, clinical information, and immunohistochemical profile of the majority of the study cases have been previously reported.[24] In brief, 34 patients were 20 men and 14 women, with mean age of 69 years (range, 46–93). Most patients (32, 94%) had a history of cigarette smoking (average 36 pack years; range, 5–150). Two were never smokers. Per current World Health Organization criteria, cases were composed of 23 pleomorphic carcinomas, 8 spindle cell carcinomas, 2 carcinosarcomas, and 1 giant cell carcinoma. Follow-up ranged from 1 to 112 months (mean 25.5 months). At last follow-up, 25 of the 34 patients were deceased (73.5%). Twenty patients (61%) had died of lung cancer, and the cause of death in the other 5 patients could not be confirmed. Nine patients were alive at last follow-up, 2 of which had persistent disease, and the remaining 7 (20.6%) were alive without evidence of disease.

A summary of mutational analysis results are presented in Tables 1 and 2 and Figures 1 and 2. No obvious genetic differences were noted between the different histological subtypes of sarcomatoid carcinoma. Twenty-four of the 33 cases (72%) showed at least one detectable genetic abnormality, and 9 cases (28%) had none of the tested mutations. Of the patients with no detectable mutations, 1 was a never smoker and 8 were former or current smokers. The most commonly observed mutations were in *TP53* exons 5–8, which were present in 19 patients (58%), all of which had a history of cigarette smoking. The next most commonly observed abnormalities were in *KRAS*, which was mutated in 10 cases (30%, Figure 3). Nine of the 10 *KRAS* mutations occurred at codon 12 (four G12V; two G12C; two G12D; and one G12A). The remaining *KRAS* mutation involved Q61H. Eight of the nine

Table 1 Summary of molecular alterations identified in 33 cases of pulmonary sarcomatoid carcinoma

No. of cases (%)	*No available targeted therapy*			*Experimental or FDA-approved molecular-based therapy*				
	TP53	*KRAS*	*JAK3*[a]	*BRAF*	*NRAS*	*PIK3CA*	*AKT1*	*ALK*
10 (30%)	x							
3 (9%)		x						
6 (18%)	x	x						
1 (3%)	x		x					
1 (3%)	x			x				
1 (3%)	x				x			
1 (3%)		x				x		
1 (3%)							x	
1 (3%)								x
8 (25%)								

[a]Variant of unknown significance.

Table 2 Summary of specific mutations observed

No available targeted therapy			*Experimental or FDA-approved molecular-based therapy*				*Total*
TP53	*KRAS*	*JAK3*	*BRAF*	*NRAS*	*PIK3CA*	*AKT1*	
C176Y							
L348F, E349							
G245D							
R213L							
P142H, C141W							
E285K							
Q104							
G279E							
L194F							
V157F							10/33 (30%)
	G12C						
	G12D						
	G12V						3/33 (9%)
V157F	G12A						
R196	G12C						
R337L	Q61H						
R273L	G12D						
C242F	G12V						
R175H	G12V						6/33 (18%)
I162F		V722I					1/33 (3%)
G105C, R175L			D594N				1/33 (3%)
T155P, R156P				A146T			1/33 (3%)
	G12V				E542K		1/33 (3%)
						E17K	1/33 (3%)

patients with *KRAS* mutations were smokers, while one never smoker had a *KRAS* G12D mutation. *AKT1*, *BRAF*, *NRAS*, and *PIK3CA* mutations were observed in one case each (3%). Of note, the *BRAF* mutation detected was a non-V600E mutation (D594N). One case had a *JAK3* V722I substitution, which is a variant of unknown significance and likely represents a polymorphism. No *EGFR* mutations were observed.

Ten of the 33 cases (30%) showed more than one mutation in the tested genes. Six of the 19 cases (32%) with a mutation in *TP53* had simultaneous mutations in *KRAS* (18%, Figure 4). The cases with mutations in *BRAF* and *NRAS* also had mutations in *TP53*, and the case with the *JAK3* variant of unknown significance had a mutation in *TP53*. The case showing a mutation in *PIK3CA* also had a mutation in *KRAS*.

One of the 34 cases (3%) was strongly positive for ALK immunohistochemistry, while all the other cases were completely negative. *ALK* gene rearrangement was subsequently confirmed by fluorescent *in situ* hybridization in the immunoreactive tumor (Figure 5), with *ALK* rearrangement present in 54% of tumor nuclei. This case showed no additional detected mutations and occurred in a 58-year-old Chinese woman with an 8 pack-year smoking history. She is currently crizotinib naive, as her resected tumor was stage IB and thus adjuvant therapy was not

initiated. Her tumor showed approximately 40% spindle cell morphology, while the remaining tumor cells showed solid epithelioid growth pattern with focal signet ring cells. *ROS1* fluorescent *in situ* hybridization was successful in 21 of the 22 cases that lacked *ALK* rearrangement and *KRAS* mutations, and all were negative for *ROS1* rearrangement.

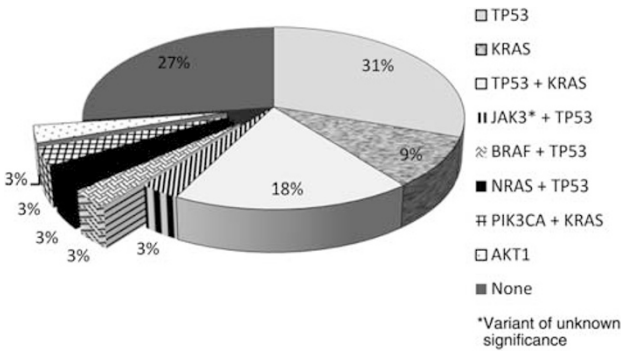

Figure 1 Summary of the mutations detected in 33 cases of pulmonary sarcomatoid carcinoma.

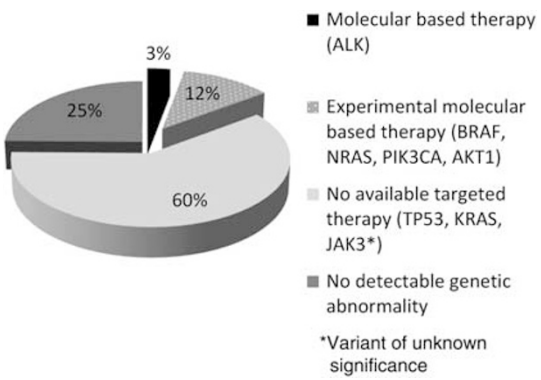

Figure 2 Summary of the targeted therapeutic options for the mutations detected in pulmonary sarcomatoid carcinoma.

Discussion

Lung cancer has been the most common cause of cancer-related deaths in the worldwide for decades[26] and still has a very poor prognosis in most cases, as many patients present with advanced stage disease. The advent of targeted therapy has revolutionized tumor testing and treatment strategies for lung adenocarcinoma, based on intense research into the molecular abnormalities driving this disease. However, translating the recent success observed in the treatment of some patients with lung adenocarcinomas to other histological subtypes of lung cancer has been a struggle, as other common types (squamous cell carcinoma and small cell carcinoma) have different molecular alterations.[11–14] The mutational profile of pulmonary sarcomatoid carcinoma is difficult to establish, given its relative rarity compared with these other more common histological types. However,

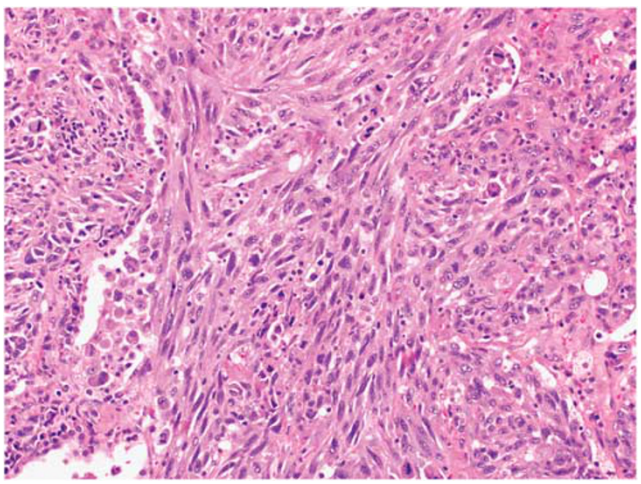

Figure 4 An example of sarcomatoid carcinoma showing both *KRAS* and *TP53* mutations. The tumor consisted exclusively of malignant spindled and pleomorphic cells.

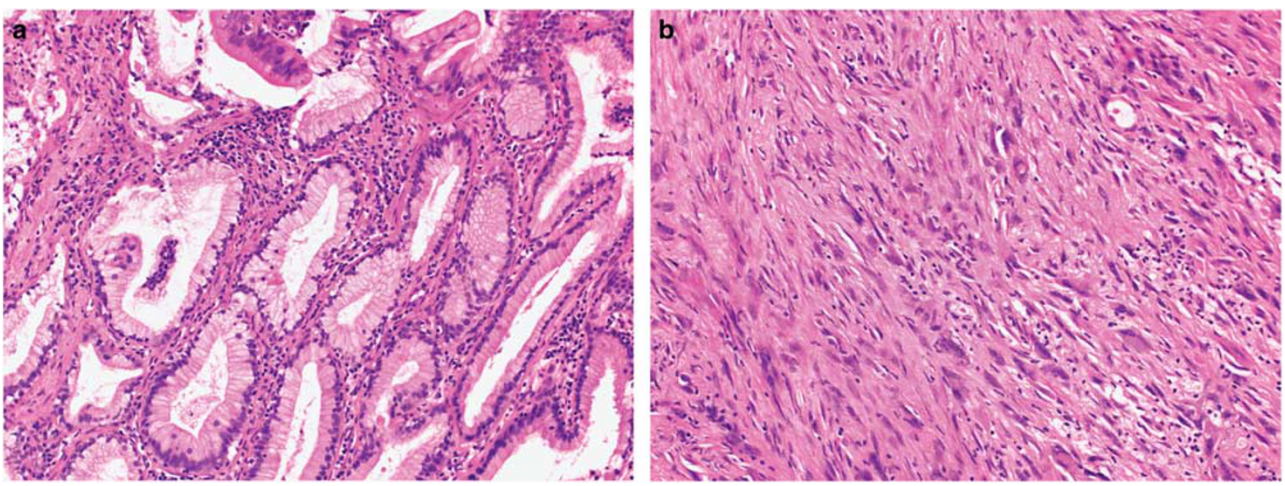

Figure 3 An example of sarcomatoid carcinoma showing *KRAS* mutation in isolation. The tumor had areas of mucinous adenocarcinoma (**a**) that transitioned to high-grade spindle cell and giant cell sarcomatoid regions (**b**).

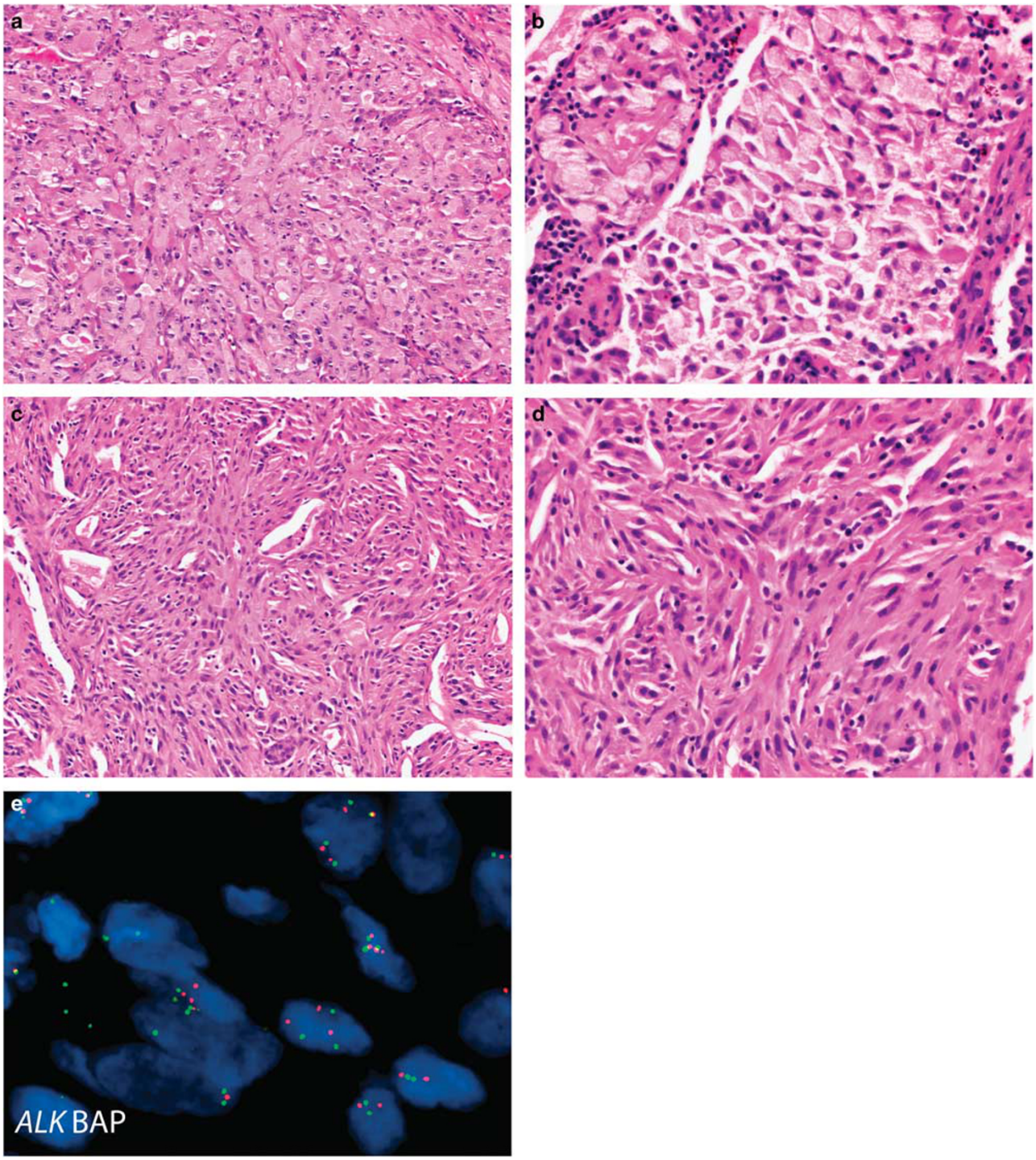

Figure 5 An example of sarcomatoid carcinoma showing *ALK* mutation alone. The tumor had areas of solid growth (**a**) as well as foci of signet ring-cell formation (**b**), which transitioned to areas of high-grade spindle cell morphology (**c** and **d**). *ALK* rearrangement was confirmed by FISH (**e**, break apart of red and green signals).

molecular analysis of sarcomatoid carcinoma represents an important step toward better understanding of the molecular underpinnings and potential treatment options for this aggressive tumor type.

Our results demonstrate that mutations in the tumor-suppressor gene *TP53* are very common in pulmonary sarcomatoid carcinoma, present in 72% of cases. This is not surprising, given that *TP53* mutations are common in various types of high-grade human malignancies, including other types of lung carcinoma, where it has been found in 45–90% of tumors.[27] *KRAS* mutations were frequent as well, present in 30% of cases, which is very similar to the rate observed in pulmonary adenocarcinomas

occurring in the US population, which are usually associated with cigarette smoking.[12] Mutations in *KRAS* and *TP53* have been observed in other studies of pulmonary sarcomatoid carcinoma,[22] a mutation profile closely resembling that of smoking-related lung adenocaricnoma. The genetic similarity between smoking-related adenocarcinoma and sarcomatoid carcinoma is consistent with histopathological observations that many sarcomatoid carcinomas represent a form of high-grade tumor transition from an underlying adenocarcinoma. Furthermore, *KRAS* mutations were often observed in conjunction with *TP53* mutations in sarcomatoid carcinoma, which are invariably high grade, as opposed to the low-grade mucinous morphology that is classically associated with *KRAS* mutations alone.[28] Some of our *KRAS*-mutated tumors showed areas of typical mucinous adenocarcinoma, with abrupt transition to high-grade sarcomatoid areas; these relatively 'dedifferentiated' areas may correspond to the accumulation of additional genetic hits associated with tumor progression, including mutations in *TP53*. However, as the mutations in these morphologically distinct areas of the tumor were not investigated separately, this cannot be confirmed from our data.

Although both *TP53* and *KRAS* mutations are difficult targets for therapeutic intervention, there have been several clinical trials[29–31] for *KRAS* mutant adenocarcinomas, as well as preclinical model studies,[32] with the purpose of optimizing the treatment for this subgroup. Numerous studies have shown various therapeutic approaches for cancers harboring *TP53* mutations,[33–35] including clinical trials specific for lung cancer employing therapies designed to inhibit immune blockade by the tumor (anti-CTLA 4 and anti-PD-1/PD-L1).[36]

Four tumors in our study had mutations in genes with experimental molecular-based therapy for lung carcinoma, namely *BRAF*, *NRAS*, *PIK3CA*, and *AKT1*. Lung cancer response to the *BRAF* inhibitors dabrafenib and vemurafenib have been observed in cases reports,[37,38] and it is also under investigation in clinical trials.[12] However, the *BRAF* mutation observed in our study was a non-V600 mutation, which is inactivating and would not be amenable to BRAF inhibition therapy. Limited data have shown that MEK inhibitors may be effective in patients with inactivating *BRAF* mutations.[39] *NRAS* mutations have been found in approximately 1% of lung carcinoma,[40] and preclinical models have demonstrated sensitivity to metformin and trametinib combination therapy.[41] *PIK3CA* mutations are detected in 2% of lung carcinomas,[42] and preclinical data suggest a high sensitivity to PI3K inhibitors.[43,44] *AKT1* mutations have been observed in 1% of lung adenocarcinomas,[12,45] and targeting AKT1 using microRNAs have shown *in vitro* and *in vivo* suppression of lung tumorigenesis.[46]

One case of *ALK* rearranged sarcomatoid carcinoma was identified (3% of total cases), which to our knowledge is quite a rare phenomenon.[47,48] Thus it seems that rare cases of sarcomatoid carcinoma show mutations in this gene, for which Food and Drug Administration-approved targeted therapies could potentially be used. Presently, *ALK*-rearranged lung adenocarcinomas have two Food and Drug Administration-approved targeted therapies, crizotinib and ceritinib, and a number of next-generation ALK tyrosine kinase inhibitors are in clinical development. Crizotinib is a multitargeted tyrosine kinase inhibitor with activity against MET, ALK, and ROS1, that is an effective treatment for many patients with *ALK*-rearranged lung cancer.[15,49–52] However, virtually all patients develop resistance to crizotinib therapy, usually within 1 year.[53–59] The higher potency ALK tyrosine kinase inhibitor ceritinib has been studied in both crizotinib-naive and crizotinib-resistant patients and has received Food and Drug Administration approval for crizotinib-resistant or -intolerant *ALK*-rearranged lung carcinoma.[17] Alectinib is another highly potent and selective ALK inhibitor that has been granted breakthrough-therapy designation by the Food and Drug Administration for ALK-positive advanced lung carcinomas that have progressed on crizotinib therapy.[60–63]

Given that both *KRAS* and *ALK* mutations were observed in our sample of sarcomatoid carcinomas, which are genetic abnormalities typically associated with pulmonary adenocarcinoma, it is interesting that no *EGFR* mutations were observed. *EGFR* mutations are present in a significant number of pulmonary adenocarcinomas in the United States (15–20%). This may indicate that adenocarcinomas with *EGFR* mutations are less likely to undergo high-grade sarcomatoid transformation than tumors with *KRAS* or *ALK* mutations, as may be predicted by their often low-grade nature, regions of lepidic growth, and more indolent course compared with *EGFR*-negative tumors. It may be that smoking status is also related, as the vast majority of our tumors occurred in smokers, while *EGFR*-mutated tumors occur in a higher percentage of non-smoking patients. A prior genetic study of sarcomatoid carcinoma performed on Chinese patients did show *EGFR* mutations present in sarcomatoid carcinoma,[64] so these mutations can occur in this tumor type, but may be much more uncommon in the United States than in Asian countries where these mutations are known to be more prevalent.

A recent next-generation sequencing study of pulmonary sarcomatoid carcinoma showed *MET* exon 14 skipping mutations in 22% of cases.[22] We did not identify any *MET* mutations using the cancer hotspot panel in the current study. To further explore this, a manual review of the raw sequencing data was performed specifically looking for *MET* alterations, but we did not identify any point mutations/indels within the intron/exon boundary at the 5′ end of exon 14. The 3′ end (including exon/intron boundary) of exon 14 was not interrogated with our hotspot panel. The discordant results

between the current study and the previously published study may be explained by the different next-generation sequencing methodologies (ie, deep sequencing of targeted regions *vs* wide-scale genomic sequencing) and is a potential limitation of our study method.

In summary, our next-generation sequencing-based analysis of common mutations revealed that pulmonary sarcomatoid carcinomas have a genetic phenotype very similar to that of high-grade lung adenocarcinoma from smokers, characterized by frequent *TP53* and *KRAS* gene mutations present in the same tumor. We identified one sarcomatoid tumor (3%) with an *ALK* translocation, which could have benefited from targeted therapy. Although sarcomatoid carcinomas are a rare pulmonary neoplasm, our results suggest that they should be evaluated for potential targetable mutations in the same manner as adenocarcinomas, to improve therapeutic options for this aggressive form of lung cancer.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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