ORIGINAL ARTICLE

Pulmonary Sarcomatoid Carcinomas Commonly Harbor Either Potentially Targetable Genomic Alterations or High Tumor Mutational Burden as Observed by Comprehensive Genomic Profiling

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ABSTRACT

Introduction: Pulmonary sarcomatoid carcinoma (PSC) is a high-grade NSCLC characterized by poor prognosis and resistance to chemotherapy. Development of targeted therapeutic strategies for PSC has been hampered because of limited and inconsistent molecular characterization.

Methods: Hybrid capture–based comprehensive genomic profiling was performed on DNA from formalin-fixed paraffin-embedded sections of 15,867 NSCLCs, including 125 PSCs (0.8%). Tumor mutational burden (TMB) was calculated from 1.11 megabases (Mb) of sequenced DNA.

Results: The median age of the patients with PSC was 67 years (range 32–87), 58% were male, and 78% had stage IV disease. Tumor protein p53 gene (*TP53*) genomic alterations (GAs) were identified in 74% of cases, which had genomics distinct from *TP53* wild-type cases, and 62% featured a GA in *KRAS* (34%) or one of seven genes currently recommended for testing in the National Comprehensive Cancer Network NSCLC guidelines, including the following: hepatocyte growth factor receptor gene (*MET*) (13.6%), *EGFR* (8.8%), *BRAF* (7.2%), erb-b2 receptor tyrosine kinase 2 gene (*HER2*) (1.6%), and ret proto-oncogene (*RET*) (0.8%). *MET* exon 14 alterations were enriched in PSC (12%) compared with non-PSC NSCLCs

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Drs. Schrock and Li equally contributed to this work.

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(~3%) ($p < 0.0001$) and were more prevalent in PSC cases with an adenocarcinoma component. The fraction of PSC with a high TMB (>20 mutations per Mb) was notably higher than in non-PSC NSCLC (20% versus 14%, $p = 0.056$). Of nine patients with PSC treated with targeted or immunotherapies, three had partial responses and three had stable disease.

Conclusion: Potentially targetable GAs in National Comprehensive Cancer Network NSCLC genes (30%) or intermediate or high TMB (43%, >10 mutations per Mb) were identified in most of the PSC cases. Thus, the use of comprehensive genomic profiling in clinical care may provide important treatment options for a historically poorly characterized and difficult to treat disease.

Keywords: Lung sarcomatoid; Genomic profiling; Tumor mutational burden; BRAF; MET exon 14; Immunotherapy

Introduction

Pulmonary sarcomatoid carcinoma (PSC), also referred to as sarcomatoid carcinoma of the lung, is a rare subtype of aggressive NSCLC that accounts for approximately 0.4% of lung malignancies.[1] Classification by the WHO includes five PSC histological subtypes: pleomorphic carcinoma, spindle cell carcinoma, giant cell carcinoma, carcinosarcoma, and pulmonary blastoma.[2,3] However, diagnosis of PSC is often challenging, particularly when only small biopsy specimens are available.[4,5] PSCs are typically defined by the presence of an epithelial component and a sarcoma-like component, and they have been thought to be associated with epithelial-mesenchymal transition.[2,4,6] Patients with PSC have an extremely poor prognosis relative to other stage-matched NSCLCs, largely because of lack of response to conventional therapies and extremely quick recurrence after surgical resection.[1,5,7–9]

The efficacy of current treatment options for disseminated PSC is limited on account of resistance to chemotherapy and low responsiveness to radiotherapy, as well as owing to a lack of genomic understanding necessary to develop targeted therapeutic strategies. Most mutational studies are based on small numbers of specimens and a limited scope of analysis focusing only on those genes most frequently mutated in NSCLCs, and they have yielded inconsistent results.[10–14] However, recent studies have identified targetable hepatocyte growth factor receptor gene (*MET*) exon 14 skipping and *KRAS* alterations in a significant fraction of cases.[12,15,16] Isolated reports of responses to targeted therapy have been presented,[15,17,18] but PSC remains a tumor type with little demonstrated efficacy of any targeted therapy in the clinic. Herein, we present results of comprehensive genomic profiling (CGP) of the largest series to date of patients with PSC, including patients with diverse genomic alterations (GAs) experiencing clinical benefit to targeted therapies and immunotherapy. Importantly, this study is the most in-depth genomic analysis of sarcomatoid carcinoma or carcinosarcoma originating from any anatomic site.

Methods

A series of 15,867 NSCLCs were assayed prospectively with a validated CGP platform (August 2012–July 2016). DNA was extracted from 40 μM formalin-fixed paraffin-embedded sections, and CGP was performed on hybridization-captured, adaptor ligation–based libraries to a mean coverage depth of $\times$650 for 236 or 315 cancer-related genes plus select introns from 19 or 28 genes frequently rearranged in cancer.[19] All classes of GAs were identified, including base substitutions, insertions/deletions, copy number alterations, and rearrangements. Tumor mutational burden (TMB) was calculated by using a novel algorithm as the number of somatic base substitution or indel alterations per megabase (Mb) of the coding region target territory of the test (currently 1.11 Mb) after filtering to remove known somatic and deleterious mutations and extrapolating that value to the exome or genome as a whole (Frampton et al., manuscript in preparation). A subspecialty board–certified thoracic pathologist independently reviewed all PSC cases to confirm the diagnosis; however, definitive subclassification was largely not feasible given limited available tissue. Tumors classified as pulmonary blastomas were not included in this study. Ordinal and categorical relationships were examined by using the Mann-Whitney *U* test and Pearson's chi-square test with Yates' continuity correction, respectively. Approval for this study, including a waiver of informed consent and a Health Insurance Portability and Accountability Act waiver of authorization, was obtained from the Western Institutional Review Board (protocol no. 20152817).

Results

Genomic profiles for 125 PSCs, representing 0.8% of NSCLC cases, were analyzed. The cohort of 125 patients with PSC was 58% male, with a median age of 67 years (range 32–87). Clinical disease stage was available for a subset of cases, and 78% of patients (64 of 82) were stage IV, 11% (nine of 82) were stage III, 9% (seven of 82) were stage II, and 2% (two of 82) were stage IB. Smoking status was not routinely available for most patients with PSC.

CGP identified a median of five GAs per tumor, and at least one GA was identified in all but one case (99%). The most frequently altered genes were tumor protein p53 gene (*TP53*) (73.6%), cyclin-dependent kinase inhibitor 2A gene (*CDKN2A*) (37.6%), *KRAS* (34.4%), cyclin-dependent kinase inhibitor 2B gene (*CDKN2B*) (23.2%), and neurofibromin 1 gene (*NF1*) (17.6%) (Fig. 1*A*). Distinct genomic

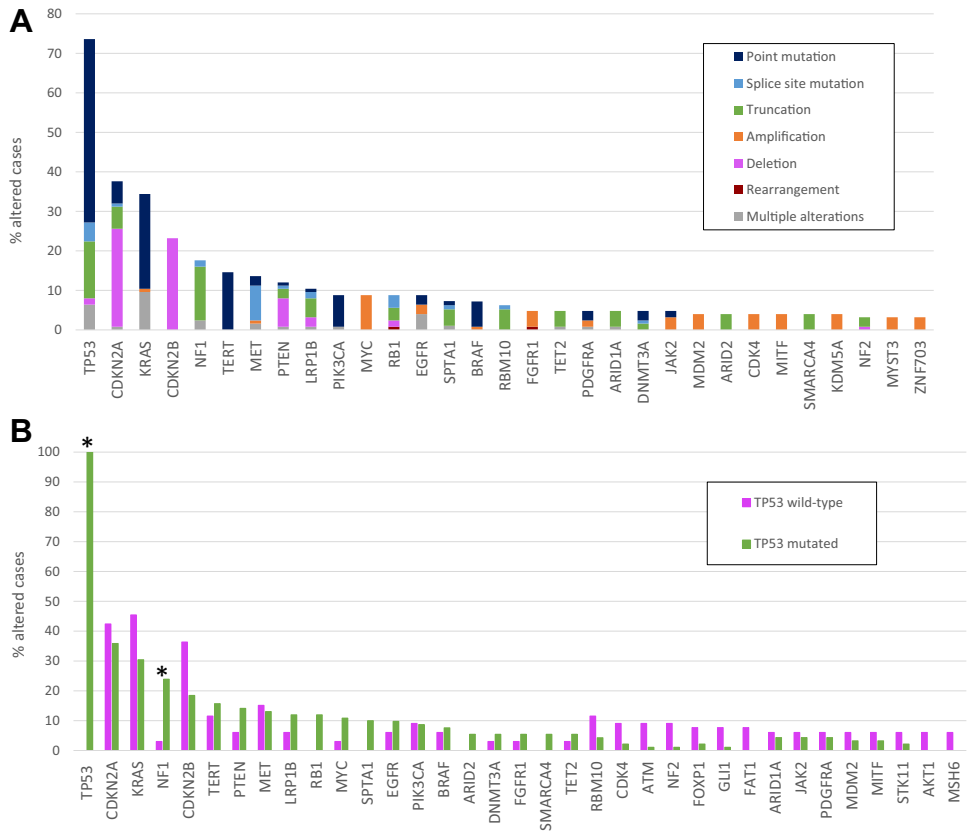

Figure 1. (*A*) Frequency of genomic alterations in pulmonary sarcomatoid carcinoma for genes altered in more than 3% of cases in this series. (*B*) Frequency of genomic alterations in tumor protein p53 gene (*TP53*) wild-type (n = 33) and *TP53*-mutated (n = 92) pulmonary sarcomatoid carcinoma for genes altered in more than 5% of cases in either subset. Asterisk indicates genes for which the difference in frequency of alteration between the two subsets was statistically significant ($p < 0.05$). *CDKN2A*, cyclin-dependent kinase inhibitor 2A gene; *CDKN2B*, cyclin-dependent kinase inhibitor 2B gene; *NF1*, neurofibromin 1 gene; *TERT*, telomerase reverse transcriptase gene; *MET*, hepatocyte growth factor receptor gene; *PTEN*, phosphatase and tensin homolog gene; *LRP1B*, LDL receptor related protein 1B; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha gene; *MYC*, v-myc avian myelocytomatosis viral oncogene homolog gene; *RB1*, retinoblastoma 1 gene; *SPTA1*, spectrin alpha, erythrocytic 1 gene; *RBM10*, RNA binding motif protein 10 gene; *FGFR1*, fibroblast growth factor receptor 1 gene; *TET2*, tet methylcytosine dioxygenase 2 gene; *PDGFRA*, platelet derived growth factor receptor alpha gene; *ARID1A*, AT-rich interaction domain 1 gene; *DNMT3A*, DNA methyltransferase 3 alpha gene; *CDK4*, cyclin-dependent kinase 4 gene; *MITF*, melanaogenesis associated transcription factor gene; *SMARCA4*, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 gene; *KDM5A*, lysine demethylase 5A gene; *NF2*, neurofibromin 2 gene; *MYST3*, lysine acetyltransferase 6A gene; *ZNF703*, zinc finger protein gene; *ATM*, ATM serine/threonine kinase gene; *FOXP1*, forkhead box P1 gene; *GLI1*, GLI family zinc finger 1 gene; *FAT1*, FAT atypical cadherin 1 gene; *STK11*, serine/threonine kinase 11 gene; *AKT1*, AKT/serine threonine kinase 1 gene; *MSH6*, mutS homolog 6 gene.

profiles were observed among *TP53* wild-type (*TP53*-WT) (n = 33) and *TP53*-mutated (*TP53*-mut) (n = 92) cases (Fig. 1*B*). *NF1* alterations were significantly more common in *TP53*-mut versus in *TP53*-WT cases (23.9% versus 3.0%, $p = 0.017$), and neurofibromin 2 gene (*NF2*) alterations were more common in *TP53*-WT than in *TP53*-mut cases (9.1% versus 1.1%, $p = 0.096$), whereas retinoblastoma 1 gene (*RB1*) alterations were found only in *TP53*-mut cases (12.0% versus 0%, $p = 0.085$). *KRAS* alterations were more common in *TP53*-WT versus in *TP53*-mut cases (45.5% versus 30.4%), but the difference was not statistically significant ($p = 0.179$).

A significant proportion of PSC cases (30%) also harbored GAs in genes recommended for testing in the NSCLC National Comprehensive Cancer Network (NCCN) guidelines, including *MET* (17 of 125 [13.6%]), *EGFR* (11 of 125 [8.8%]), *BRAF* (nine of 125 [7.3%]), erb-b2 receptor tyrosine kinase 2 gene (*HER2*) (two of 125 [1.6%]), and ret proto-oncogene (*RET*) (one of 12 [0.8%]) (Fig. 2). However, no anaplastic receptor tyrosine kinase gene (*ALK*), *ROS1*, *RET*, or neurotrophic tyrosine kinase receptor, type 1 gene (*NTRK*) fusion genes were identified. *MET* alterations were most frequent exon 14 skipping mutations (15 cases, including one with concurrent *MET* amplification), *MET* amplification alone (one case), and H1094L (one case). *EGFR* alterations included L858R mutation (four cases, including one with concurrent *EGFR* amplification and

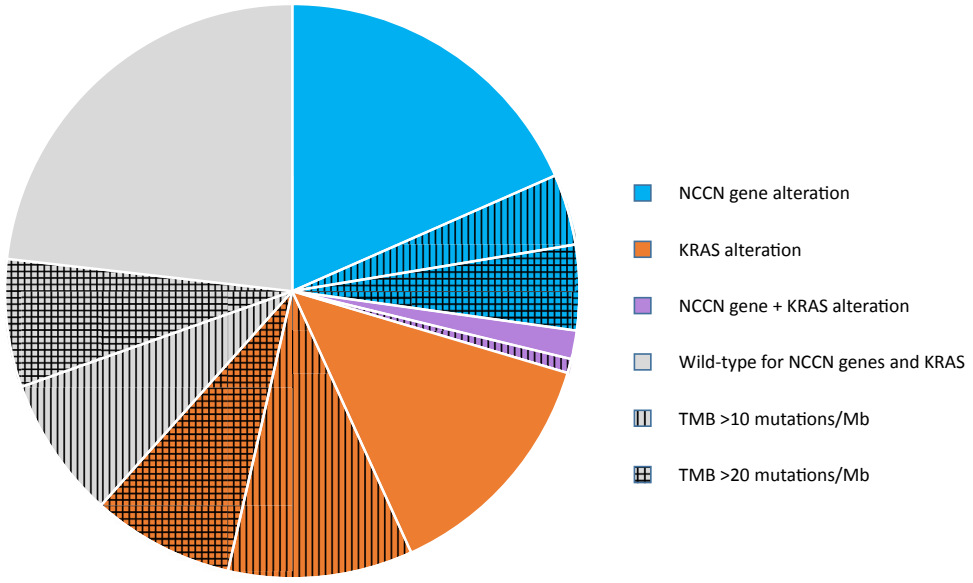

Figure 2. Frequency of alterations in genes recommended for testing in the National Comprehensive Cancer Network (NCCN) NSCLC guidelines, *KRAS* alterations, and overlap with a tumor mutational burden (TMB) of more than 10 mutations per megabase (Mb) or more than 20 mutations per Mb.

one with K860I), *EGFR* amplification alone (three cases), exon 20 insertion (two cases, both with concurrent amplification), exon 19 deletion (one case with concurrent amplification), and I759T mutation (one case). *BRAF* alterations included V600E (three cases); G469A/V (three cases); and amplification, G466E, and D594N (one case each). *HER2* (*ERBB2*) alterations included amplification and a C-terminal splice site mutation. The single *RET* alteration was an amplification. In most cases, the NCCN gene was the sole driver detected.

The median TMB in this series of PSCs was 8.1 mutations per Mb (mean 13.6, range 0–165.2), 20% of cases had a TMB greater than 20 mutations per Mb, and 43% of cases had a TMB greater than 10 mutations per Mb (see Fig. 2). Of 88 cases that were wild type for the seven NCCN NSCLC genes (*EGFR*, *HER2*, *MET*, *ALK*, *ROS1*, *RET*, and *BRAF*), the median TMB was 9.9 mutations per Mb, in contrast to 6.3 mutations per Mb in the 37 cases with NCCN NSCLC gene alterations. For *KRAS*-altered cases, median TMB was 10.8 mutations per Mb (see Fig. 2). *TP53*-WT tumors had a significantly lower TMB (median 5.0) compared with *TP53*-mut tumors (median 10.1) ($p < 0.001$). Overall, 77% of cases had an intermediate or high TMB (>10 mutations per Mb) and/or an alteration in an NCCN NSCLC gene or *KRAS* (see Fig. 2).

An adenocarcinoma component was identified in 34% of PSCs in this series (42 of 125). The genomic profile of the 42 cases with an adenocarcinoma component was very similar overall to that of cases without an adenocarcinoma component (Fig. 3). Notably, *MET* alterations were enriched in cases with an adenocarcinoma component (nine of 42 [21.4%]) than in those without an adenocarcinoma component (eight of 83 [9.6%]), and *MET* exon 14 skipping alterations were further enriched in cases with an adenocarcinoma component (19.0% versus 8.4%); however, neither difference was significant ($p = 0.124$ and $p = 0.152$, respectively). TMB in cases with an adenocarcinoma component was lower than in cases without an adenocarcinoma component (median 6.8 versus 9.9), but this difference was also not significant ($p = 0.176$).

Clinical outcomes were available for 13 PSC cases through discussion with the treating physicians in the context of appropriate consent. Ten of thirteen patients received targeted or immunotherapy, and of the nine of those patients who could be assessed, three had partial responses and three had stable disease (Table 1).

For example, stage IIIB PSC was diagnosed in a 54-year-old African American female current smoker in 2015, when she presented with obstructive pneumonia. She had a 6.4-cm primary lesion in the left lower lobe that was associated with subcarinal left hilar lymphadenopathy and a subpleural left upper lung nodule. Pathologic review of the resections revealed a necrotic high-grade tumor. Immunohistochemistry (IHC) revealed diffuse cellular localization of vimentin and pankeratin, and the epithelial membrane antigen and cytokeratin 7 were weakly expressed (Supplementary Fig. 1).

Because of the patient's poor performance status and severe obstructive symptoms, she was initially treated with palliative chest radiation, followed by systemic chemotherapy consisting of three cycles of carboplatin and gemcitabine. Her restaging scan 4 months after diagnosis demonstrated disease progression with new

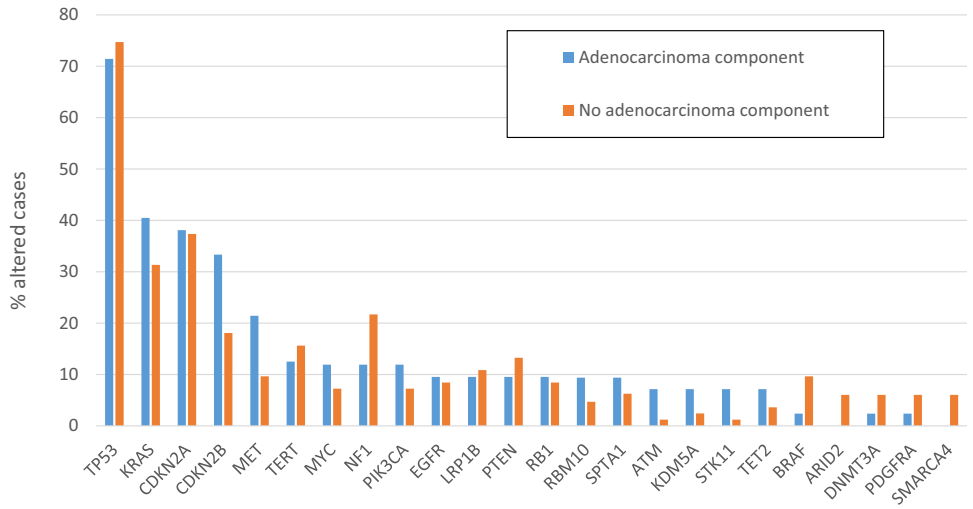

Figure 3. Frequency of genomic alterations in pulmonary sarcomatoid carcinoma with an adenocarcinoma component compared with in pulmonary sarcomatoid carcinoma without an adenocarcinoma component. Cases were reviewed and classified by a subspecialty board-certified thoracic pathologist. Graph includes genes altered in more than 5% of cases in either subset. The difference in frequency of alteration between the two subsets was not statistically significant ($p < 0.05$) for any of the genes shown. *TP53*, tumor protein p53 gene; *CDKN2A*, cyclin-dependent kinase inhibitor 2A gene; *CDKN2B*, cyclin-dependent kinase inhibitor 2B gene; *MET*, hepatocyte growth factor receptor gene; *TERT*, telomerase reverse transcriptase gene; *MYC*, v-myc avian myelocytomatosis viral oncogene homolog gene; *NF1*, neurofibromin 1 gene; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha gene; *LRP1B*, LDL receptor related protein 1B; *PTEN*, phosphatase and tensin homolog gene; *RB1*, retinoblastoma 1 gene; *SPTA1*, spectrin alpha, erythrocytic 1 gene; *ATM*, ATM serine/threonine kinase gene; *KDM5A*, lysine demethylase 5A gene; *STK11*, serine/threonine kinase 11 gene; *TET2*, tet methylcytosine dioxygenase 2 gene; *ARID2*, AT-rich interaction domain 2 gene; *DNMT3A*, DNA methyltransferase 3 alpha gene; *PDGFRA*, platelet derived growth factor receptor alpha gene; *SMARCA4*, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 gene.

peritoneal, pancreatic, and bone metastases (Fig. 4*A*). CGP was performed to explore targeted therapeutic options and identified multiple alterations, including BRAF V600E and a *TP53* mutation (see Table 1), prompting treatment with vemurafenib (960 mg twice a day). Eight weeks after initiation of the treatment, computed tomography scan showed an excellent partial response, including a significant reduction of pancreatic and peritoneal metastatic lesions that continued at 7 months into treatment (Fig. 4*B*). Treatment was complicated by acute pericarditis due to previous radiotherapy and a rash that was managed with clindamycin gel and tetracycline. The patient continued to receive vemurafenib therapy for 10 months, until she experienced disease progression.

Case 2 is that of a 46-year-old male former heavy smoker, in whom stage IIIB pleomorphic NSCLC was diagnosed in June 2015, when he presented with severe left upper back pain and was found to have a very large, necrotic, and highly invasive mass of the left upper lung with associated chest wall, mediastinal, and vertebral invasion. The results of positron emission tomography/computed tomography and brain magnetic resonance imaging were negative for distant metastatic disease. Pathologic review noted a poorly differentiated tumor with sarcomatoid features with strong cytokeratin and calretinin positivity. The patient was treated initially with concurrent chemoradiation (cisplatin/etoposide for two cycles with 54 Gy of radiotherapy), with a response in the primary tumor but rapid progression with a new adrenal metastasis. He began receiving chemotherapy (gemcitabine/docetaxel), and CGP and immune biomarker studies were pursued. He had a minor initial response to second-line chemotherapy, with progression noted by December of 2015 in a worsening adrenal lesion.

Results of CGP of a lung biopsy revealed a high TMB (31 mutations per Mb) and no targetable alterations in any of the seven NCCN NSCLC genes (*EGFR*, *HER2*, *ALK*, *ROS1*, *RET*, *MET*, and *BRAF*). The results of tests of the tumor were also negative for *KRAS* mutation, but the tumor did harbor a *TP53* mutation (see Table 1). Programmed death ligand 1 (PD-L1) staining was positive, with a tumor proportion score of 80% (Dako PharmDx 22c3 antibody, Dako, Carpenteria, CA). Given the lack of targeted options but high TMB and PD-L1 expression, pembrolizumab therapy was initiated; however, 1 week into treatment the patient presented with hemorrhagic brain metastases and had additional complications requiring two episodes of cardiac resuscitation. Despite

Table 1. PSC Cases with Available Clinical Outcomes

Case	GAs	Sex	Age	Tumor Stage[a]	TMB	Smoking Status	Prior Chemotherapy	Targeted or Immunotherapy	Treatment Duration	Best Response to Targeted or Immunotherapy
1	*BRAF* V600E, *FGFR1* amplification, *TP53* E171*, *MYST3* amplification, *U2AF1* S34F, *ZNF703* amplification	F	54	3B	8.1	Current	Yes	Vemurafenib	10 mo	PR
2	CCND3 amplification, *FLT1* R781Q, *VEGFA* amplification, *CDK6* amplification, *MYCL1* amplification, *NOTCH3* R2031fs*54, *TP53* R249S, *CREBBP* E1724*, *CREBBP* K1176fs*3, *JUN* amplification, *PBRM1* A137fs*37	M	46	3B	31	Former heavy smoker	Yes	Pembrolizumab	10 mo, ongoing	PR
3[b]	*MET* exon 14 splice site (2888-5_2890TTAAGATC>A), *MET* exon 14 splice site (3028+2T>G), *MET* H1094Y, *NF1* R1362*, *TSC1* splice site 2041+1G>C, *TP53* Y220C	M	61	4	2.5	Never	Yes	Crizotinib	5 mo	PR
4	*MET* exon 14 splice site (3027_3028+2delAGGTA), *CDKN2A/B* loss, *TP53* G245S, *SPTA1* R575H	F	71	4	7.2	Never	No	Crizotinib; nivolumab	4.5 mo; 6 mo, ongoing	Stable disease[c]; Stable disease
5	*MET* amplification, *MET* splice site 3028+3A>T (exon 14), PD-L1/PD-L2 amplification, *JAK2* amplification, CDK6 amplification, *CDKN2A* p16 E69* and p14 G83V, *TP53* K164fs*15, *MLL2* R2105H, *PIK3CG* amplification, *SPTA1* L1395fs*24	F	71	4	15	Current	Yes	Nivolumab; crizotinib	3 mo; <1 mo	PD[e]; not evaluable on account of toxicity
6	*BRAF* V600E, *SETD2* W1341_Q1343>*	M	75	4	11	Never	Yes	Vemurafenib	6 wk	PD
7	*BRAF* G469V, *KRAS* amplification, *KRAS* G12C, *CCND2* amplification, *CDK4* amplification, *MDM2* amplification, FGF23/6 amplification, *KDM5A* amplification, *MSH6* L1060fs*19	M	86	3B	17	Former heavy smoker	Yes	Dabrafenib	2 wk	Not evaluable; further therapy declined
8	*EGFR* E746_A750del, *EGFR* amplification, *CDKN2A/B* loss, *TP53* K164N, *GRIN2A* V1018M	F	63	Unk	3.6	Never	Unk	Afatinib	5 mo	Stable disease[d]
9	*EGFR* amplification, *EGFR* H773_V774insNPH, *TP53* L330P, *BCL6* I449V, *PIK3C2B* P717L, *RB1* E817fs*2	M	56	Unk	3.6	Unk	Yes	Cetuximab + afatinib	3 mo	PD
10	*EGFR* L858R, *EGFR* K860I, *PD-L1/PD-L2* amplification, *JAK2* amplification, *PTEN* Q245*, *NTRK1* amplification, *TERT* promoter -124C>T, *TP53* splice site 919+1G>T, *CHEK2* S422fs*15, *NOTCH2* P6fs*27	F	68	2B	7.2	Unk	Unk	Erlotinib	5 mo	Stable disease[c]

[a]At time of comprehensive genomic profiling.
[b]Previously published case report (Lee et al.[18]).
[c]Tumor shrinkage but less than 30% reduction.
[d]Dose reduced to 30 mg because of toxicity.
[e]Mixed response noted.

GA, genomic alteration detected by comprehensive genomic profiling; TMB, tumor mutational burden (mutations per megabase); *FGFR1*, fibroblast growth factor receptor 1 gene; *TP53*, tumor protein p53 gene; *MYST3*, lysine acetyltransferase 6A gene; *U2AF1*, U2 small nuclear RNA auxiliary factor 1 gene; *ZNF703*, zinc finger protein gene; PR, partial response; *CCND3*, cyclin D3 gene; *FLT1*, fms related tyrosine kinase 1 gene; *VEGFA*, vascular endothelial growth factor A gene; *CDK6*, cyclin-dependent kinase 6 gene; *MYCL1*, v-myc avian myelocytomatosis viral oncogene lung carcinoma derived homolog gene; *NOTCH3*, notch 3 gene; *CREBBP*, CREB binding protein gene; *JUN*, Jun proto-oncogene, AP-1 transcription factor subunit gene; *PBRM1*, polybromo M gene; *MET*, hepatocyte growth factor receptor gene; *NF1*, neurofibromin 1 gene; *TSC1*, tuberous sclerosis 1 gene; PD, progressive disease; *CDKN2A*, cyclin-dependent kinase inhibitor 2A gene; *CDKN2B*, cyclin-dependent kinase inhibitor 2B gene; *SPTA1*, spectrin alpha, erythrocytic 1 gene; *JAK2*, Janus kinase 2 gene; *MLL2*, lysine methyltransferase 2D gene; *PIK3CG*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit gamma gene; *SETD2*, SET domain containing gene; *CCND2*, cyclin D2 gene; *CDK4*, cyclin-dependent kinase 4 gene; *MDM2*, MDM2 proto-oncogene gene; *KDM5A*, lysine demethylase 5A gene; *MSH6*, mutS homolog 6 gene; *GRIN2A*, glutamate ionotropic receptor NMDA type subunit 2A gene; UNK, unknown; *BCL6*, B-cell CLL/lymphoma 6 gene; *PIK3C2B*, phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type beta gene; *RB1*, retinoblastoma 1 gene; *PD-L1*, programmed death ligand 1 gene; *PD-L2*, programmed death ligand gene; *PTEN*, phosphatase and tensin homolog gene; *NTRK1*, neurotrophic tyrosine kinase receptor, type 1 gene; *TERT*, telomerase reverse transcriptase gene; *CHEK2*, checkpoint kinase 2 gene; *NOTCH2*, notch 2 gene.

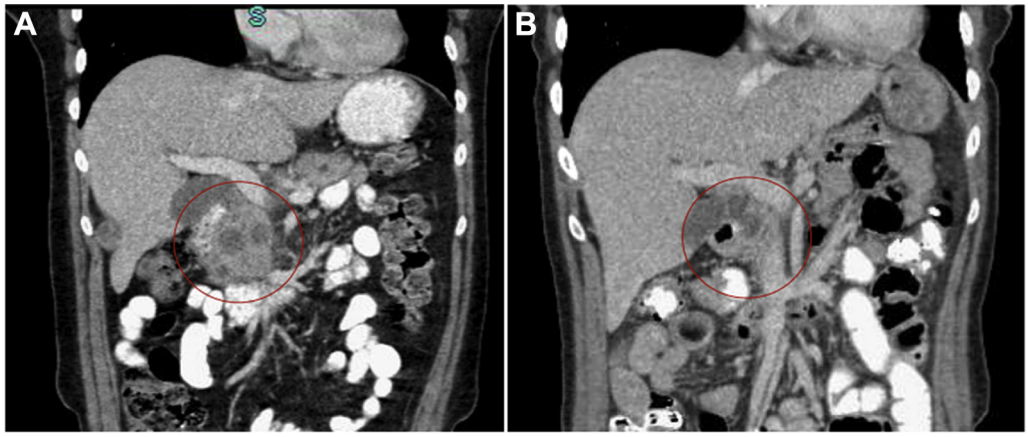

Figure 4. Case 1. (*A*) Computed tomography scan of the patient with *BRAF* V600E–positive pulmonary sarcomatoid carcinoma before initiation of vemurafenib reveals a large peritoneal mass. (*B*) After 7 months of vemurafenib therapy, there was significant improvement.

this, his condition soon stabilized and he completed a course of whole brain radiation. He resumed pembrolizumab therapy and enjoys an excellent clinical and radiographic response both in the central nervous system and systemically (ongoing for 10 months) (Fig. 5). Although treatment was complicated by an episode of hyperthyroidism requiring a short course of steroids as well as methimazole, he has continued to

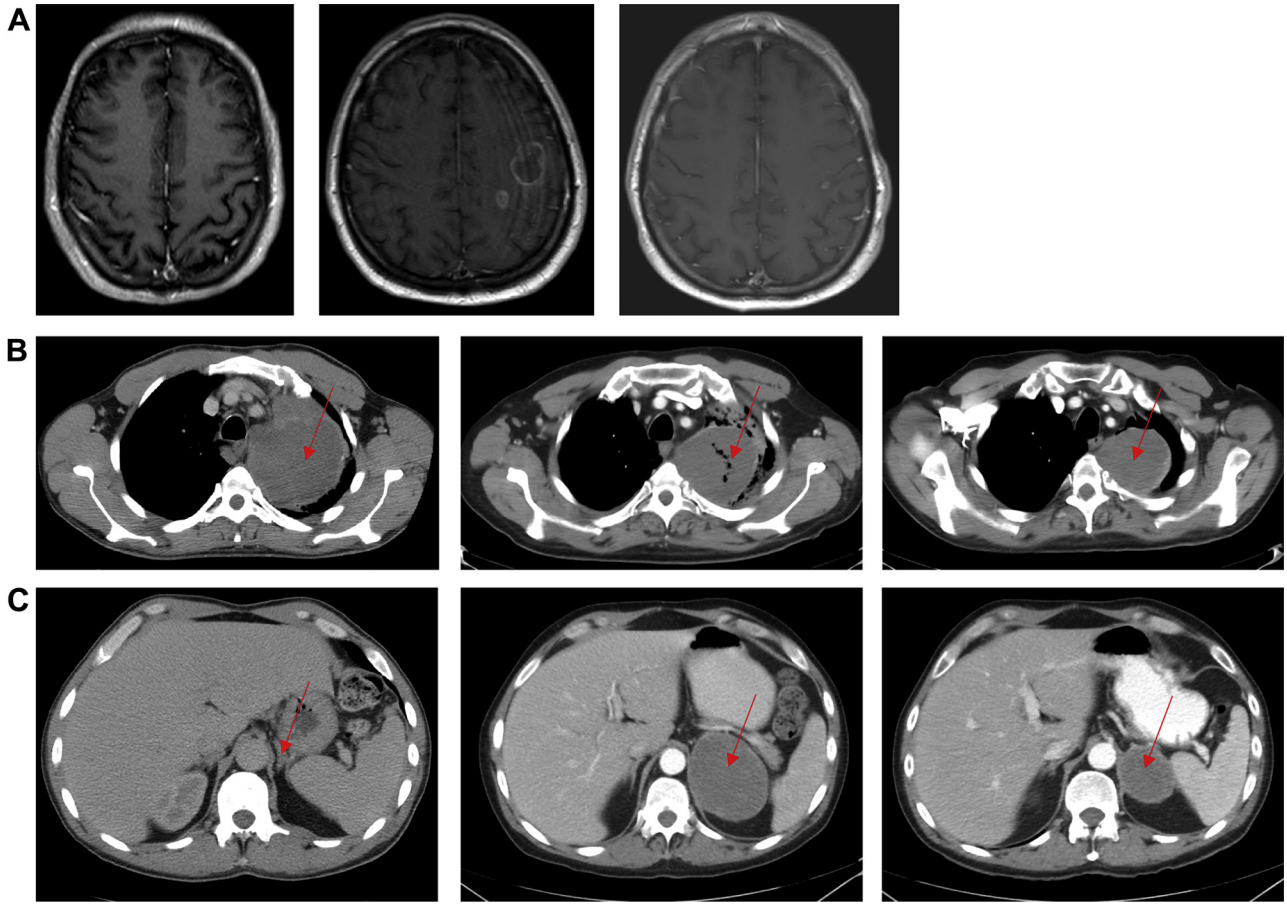

Figure 5. Case 2. Computed tomography (CT) scans of the patient with high tumor mutational burden and programmed death ligand 1–positive pulmonary sarcomatoid carcinoma at diagnosis (*left*), before initiation of pembrolizumab (*center*), and after 8 months of pembrolizumab therapy (*right*). (*A* and *B*) CT scans of brain (*A*) and lung (*B*) lesions, which also received radiation. At diagnosis the primary lung lesion measured 9.3 × 10.6 × 12 cm (*B* [*left panel*]). (*C*) Abdominal CT scans showing the adrenal mass, which was reduced by 35% (from 89 mm to 57 mm in the largest dimension) during the course of pembrolizumab treatment (*center panel* to *right panel*).

receive pembrolizumab without further interruptions and was able to return to work in August 2016.

Discussion

The exceedingly poor prognosis of PSC and its resistance to cytotoxic chemotherapy define a high need for effective systemic therapy; however, the delineation of the utility of molecularly targeted therapies for PSC has been hampered by a poor understanding of the underlying genomic drivers. Moreover, it has remained unclear whether identification of oncogenic alterations will effectively predict response to matched targeted therapy in PSC. These challenges of management are shared across sarcomatoid carcinomas arising in other organs, such as sarcomatoid renal cell carcinoma (sRCC) and ovarian carcinosarcoma.[20,21]

We performed CGP on 125 PSC cases, and *TP53*, *CDKN2A*, *KRAS*, *CDKN2B*, and *NF1* were the most frequently altered genes (>15% of cases). These results are consistent with previous studies of sRCC and sarcomatoid mesothelioma, in which *TP53* and *CDKN2A/B* were among the most frequently altered genes.[21–23] However, the frequency of *TP53* mutation is a defining feature in our PSC series (74%), enriched compared with a frequency of 46% in the lung adenocarcinoma data set of The Cancer Genome Atlas,[24] with a distinct genomic profile in *TP53*-WT tumors. We did not find a high rate of AT-rich interaction domain 1 gene (*ARID1A*) (5%) or BRCA1, DNA repair associated gene (*BRCA1*)/BRCA2, DNA repair associated gene (*BRCA2*) alterations (1%–2%), as have been reported in some other sarcomatoid tumors studied with limited molecular testing.[21,25] Given that this is the largest cohort of sarcomatoid carcinomas studied with CGP, the possibility of conserved classes of sRCC and PSC is intriguing and may indicate common pathways for the development of sarcomatoid carcinoma. This disease is thought to arise from a dedifferentiation of carcinoma to acquire a sarcoma-like morphologic pattern, and it carries a significantly worse prognosis relative to matched epithelial-only tumors. The cosegregation of *NF1* and *RB1* alterations with *TP53*-mut PSC and *NF2* with *TP53*-WT PSC, which is consistent with findings in sRCC,[22] may indicate distinct pathways for the evolution of sarcomatoid carcinoma. If the latter is true, further investigation is warranted in sarcomatoid carcinomas of other organs, as well as in a larger series of PSCs, with an eye to understanding implications for response to targeted therapy and even rapid clinical intervention, given the often quick declines of these patients.

We identified GAs in genes listed in the NCCN NSCLC guidelines in 30% of PSC cases (*MET* [13.6%], *EGFR* [8.8%], *BRAF* [7.2%], *HER2* [1.6%], and [*RET* 0.8%]); *KRAS* alterations were also identified in 34% of cases. These alterations included *MET* exon 14 skipping mutations (12%), which were enriched (19%) in cases with an adenocarcinoma component, and activating *EGFR* point mutations or indels (5.6%). *MET* amplification co-occurred with exon 14 skipping in 6.7% of cases (1 of 15), which is slightly lower than the frequency reported in prior studies,[16] although the sample size here is relatively small. Activating *BRAF* kinase domain mutations, including V600E, were found in six cases (4.3%) and kinase-impaired *BRAF* mutations, which have been shown to up-regulate RAF/MEK signaling through feedback activation, were observed in two cases.[26] In comparison, a recent report on nonsquamous NSCLCs using the same CGP platform reported alterations in NCCN genes in approximately 40% of cases, including *EGFR* (20%), *HER2* (6.0%), *BRAF* (5.7%), *MET* (5.6%), *ALK* (4.1%), *RET* (2.4%), and *ROS1* (1.5%); *KRAS* mutation was also reported in 32% cases in this study and thus appears to be comparable across NSCLCs, including PSC.[27] These results suggest that although overall potentially targetable alterations are somewhat less frequent in PSC than in other nonsquamous NSCLCs, some oncogenes, including *MET* and *BRAF*, may actually be altered more frequently in PSC.

Prior smaller studies of PSC have reported varying *EGFR* mutation frequencies of 0% to 28%, with consistent enrichment in Asian compared with white cohorts.[10–15,28] Prior studies have also reported lack of *ALK* rearrangements.[15] *MET* exon 14 mutations were previously identified in 8% to 22% of PSC cases,[15,16] whereas other studies have reported infrequent *MET* mutation[12,14,28]; however, the methodologies used in these latter studies may not have been capable of identifying the diverse alterations such as those shown to result in *MET* exon 14 skipping.[16] In this report we identified *KRAS* mutations in 34% of PSC cases, compared with reported frequencies of 3% to 39%.[11,14,12,13,28] Prior studies have reported *BRAF* mutation in only 0% to 3% of PSCs; however, several of these studies were small and utilized polymerase chain reaction–based sequencing limited to detection of specific mutations.[14,12,13,28] Overall, difficulty in accurately diagnosing PSC by using small biopsy specimens, varying histological makeup, differences in ethnicity, and inconsistent sequencing methodologies may contribute to variable alteration frequency between studies.[4]

In the *BRAF* V600E-mutated PSC case reported herein, vemurafenib treatment resulted in an excellent and durable response to therapy. Our results clearly indicate that *BRAF* V600E is an oncogenic driver in this case, and they demonstrate the utility of CGP in developing a targeted therapeutic strategy for individual patients with PSC. Results from a phase 2 basket trial of vemurafenib revealed that response rate varies significantly across different *BRAF* V600 mutation–positive

cancers.[29] Although such basket trials allow us to identify common nonmelanoma cancers that could benefit from BRAF-targeted therapy, it is difficult to design this type of trial for rare cancers or for cancers with low alteration frequency of the target. Therefore, reporting of individual outcomes to matched targeted therapy is critical to inform clinical practice.

Although PSC and smoking-related lung adenocarcinomas have been shown to exhibit similar genomic profiles,[12,15,28] PSCs have unique histological features and are characterized by aggressive behavior and poor clinical outcome. Further, associations between *EGFR* mutations and response to targeted therapy have been established in lung adenocarcinoma, but similar durable responses have not been observed in small series of PSC.[10,30] Herein, two patients with PSC with *EGFR*-mutated tumors had stable disease for approximately 5 months during treatment with erlotinib and afatinib, respectively, but did not achieve a response, and an additional patient with *BRAF* V600E (*TP53-WT*) treated with vemurafenib had rapid progressive disease (see Table 1). Therefore, one should not assume that response rates of 33% to 53% in NSCLC harboring *BRAF* V600E/K can be directly extrapolated to PSC,[29,31,32] as overall responses to targeted therapy in PSC may be somewhat less robust. We recognize that the response in case 1 presented herein should be interpreted with caution, given the failure of another seemingly similar patient, albeit with *TP53*-WT, to respond, and accumulation of additional case studies or results from basket trials accompanied by detailed genomic assessment is necessary to reach more definitive conclusions. Another shortcoming of this study is the lack of available smoking history for most patients.

Our series of PSCs had a median TMB of 8.1 mutations per Mb, which was similar to that in other NSCLCs (median 7.2) but higher than in other solid tumors in our database (median 3.8). A TMB of more than 20 mutations per Mb was observed in 20% of PSC NSCLCs compared with in only 13.6% of non-PSC NSCLCs, which is consistent with data showing that sarcomatoid tumors have an increased number of somatic mutations[12,21]; however, this difference was not statistically significant ($p = 0.054$). A high TMB was not mutually exclusive with known oncogenic drivers, and 23% of *KRAS*-mutant cases had a TMB greater than 20 mutations per Mb. *TP53*-mut tumors were also significantly more likely than *TP53*-WT tumors to have high TMB ($p = 0.010$). Recent studies have suggested that high TMB correlates with positive response to immune checkpoint inhibitors (ICPIs) in multiple tumor types, including NSCLC.[33,34] Recent studies have also reported that PSCs have high PD-L1 expression,[35–37] which has been linked to improved response to ICPIs.[38] However, a subset of patients with low PD-L1 expression by IHC have benefited from ICPIs.[39] These responders may represent a subset of patients with low PD-L1 expression but high TMB, or they may be indicative of inconsistent IHC scoring.[40] Ultimately, a combination of biomarkers may offer the best approach for predicting response to ICPIs. One limitation of this study is the lack of available data on PD-L1 expression for most cases, and future analyses comparing TMB, programmed death ligand 1 gene (*PD-L1*) amplification, and PD-L1 expression levels in tumors of patients treated with ICPIs will be valuable.

To our knowledge, we are presenting the first report of a durable response to an ICPI in a patient with PSC. This patient had a high TMB as well as positive PD-L1 staining. This case, in addition to published reports linking high TMB with response to programmed cell death 1/PD-L1 inhibitors,[33,34] suggests that ICPIs may be beneficial in this population. However, additional clinical cases will need to be evaluated to determine which biomarkers are most predictive of response in PSC.

Importantly, the work presented here is the most detailed genomic characterization of a sarcomatoid tumor across any organ system. This study demonstrates both targetability of GAs, albeit not uniformly and anecdotally, and responsiveness to checkpoint inhibitors for PSC. Analogous studies in other mixed tumor types are needed both to further delineate possible genomic classes of sarcomatoid carcinoma and to predict clinical benefit from targeted and immunotherapies.

In summary, PSC is a unique subtype of NSCLC with an exceptionally poor prognosis. However, oncogenic mutations, fusions, and copy number alterations of driver oncogenes specified in the NCCN NSCLC guidelines were identified 30% of cases, and a TMB greater than 10 mutations per Mb was identified in an additional 34% of cases. We also report the first case of response to matched BRAF-targeted therapy in PSC. A significant fraction of PSCs also harbor a high TMB, and these cases may be particularly responsive to ICPIs, as evidenced by published data in other NSCLCs as well as by our novel report of an excellent response to pembrolizumab in a patient with PSC with a high TMB. Thus, the use of CGP in clinical care may provide important treatment options for a historically poorly characterized and difficult to treat disease.

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at http://dx.doi.org/10.1016/j.jtho.2017.03.005.

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