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KRAS mutation is predictive of outcome in patients with pulmonary sarcomatoid carcinoma

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Abstract

Aims: Pulmonary sarcomatoid carcinoma (PSC) is a poorly differentiated non-small-cell lung carcinoma (NSCLC) with aggressive behaviour. This study aimed to evaluate the prognostic clinicopathological and genetic characteristics of PSCs.

Methods and results: Fifty-three cases of surgically treated PSCs were selected, 23 of which were subjected to mutation and copy number variation analysis using the 50-gene Ion AmpliSeq Cancer Panel. The majority of the patients were male (32 of 53, 60.3%) and smokers (51 of 53, 96.2%). Overall, 25 (47.1%) patients died within 2–105 months (mean = 22.7 months, median = 15 months) after diagnosis, and 28 were alive 3–141 months (mean = 38.7 months, median = 21.5 months) after diagnosis. Five-year overall survival was 12.5%. *KRAS* codon 12/13 mutation in adenocarcinomas ($P = 0.01$), age more than 70 years ($P = 0.008$) and tumour size ≥4.0 cm ($P = 0.02$) were associated strongly with worse outcome. *TP53* (17 of 23, 74.0%) and *KRAS* codon 12 of 13 mutations (10 of 23, 43.4%) were the most common genetic alterations. Potentially actionable variants were identified including *ATM* (four of 23, 17.3%), *MET*, *FBXW7* and *EGFR* (two of 23, 8.7%), *AKT1*, *KIT*, *PDGFRA*, *HRAS*, *JAK3* and *SMAD4* (one of 23, 4.3%). *MET* exon 14 skipping and missense mutations were identified in two (11.1%) cases with adenocarcinoma histology. Copy number analysis showed loss of *RB1* (three of 23, 13%) and *ATM* (two of 23, 8.7%). Copy number gains were seen in *EGFR* (two of 23, 13.0%) and in one (4.3%) of each *PIK3CA*, *KRAS*, *MET* and *STK11*.

Conclusions: Potentially targetable mutations can be identified in a subset of PSC, although most tumours harbour currently untargetable prognostically adverse *TP53* and *KRAS* mutations.

Keywords

copy number analysis; *KRAS*; lung; next-generation sequencing; sarcomatoid carcinoma

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Introduction

Pulmonary sarcomatoid carcinoma (PSC) is a highly aggressive type of non-small-cell lung carcinoma (NSCLC), composed of both epithelial and sarcoma-like components. There are five main histological subtypes in this category: pleomorphic carcinoma, spindle cell carcinoma, giant cell carcinoma, carcinosarcoma and pulmonary blastoma.[1] PSCs are rare and account for fewer than 1% of all pulmonary malignancies; however, compared to other stage-matched NSCLC, they are more resistant to conventional therapies and have poorer prognosis.[2,3]

Although the molecular characteristics of the more common subtypes of NSCLCs, mainly adenocarcinomas, have been studied extensively, the genetic alterations in PSC have only recently become the target of studies.[3–7] This is due perhaps to the rarity of the disease and difficulty in diagnosing PSCs, particularly in small biopsies. The 2015 WHO[1] recommends molecular testing in PSCs according to known genetic abnormalities associated with the histological components in the tumour. *KRAS* mutation has been reported in up to 38% of PSCs and *EGFR* mutations in up to 25%.[6,8–10] Furthermore, few recent studies have identified targetable *MET* exon 14 skipping in a significant fraction of cases,[3,7,9] with a few case reports demonstrating a great response to targeted therapy with MET inhibitors.[11,12] Despite these advancements, there are still limited options available for treatment of these tumours. In addition, there are no clinicopathological or molecular features that could predict outcome reliably in PSC patients. In this work, using a targeted next-generation sequencing approach, we explored the genetic profile and clinicopathological characteristics of a cohort of surgically treated PSC.

Materials and methods

PATIENTS AND SPECIMENS

Of 53 consecutive, surgically treated PSCs during a 10-year period (from 2004 to 2014), 23 were selected based on tissue availability for additional studies. All cases were reviewed to confirm the diagnosis applying the 2015 WHO criteria[1] and were staged according to the American Joint Committee staging manual (8th edn).[13] The study was conducted under an exemption approved by the University of Pittsburgh Institutional Review Board (PRO 12070229).

Clinical information including gender, age, tumour stage and smoking status was obtained from patients' electronic medical records. Follow-up data regarding survival were collected through the institutional Network Cancer Registry.

IMMUNOHISTOCHEMISTRY

Immunohistochemistry (IHC) for Rb1 (Leica, Allendale, NJ, USA; clone 13A10, monoclonal mouse, 1:50) was performed.

FLUORESCENCE *IN - SITU* HYBRIDISATION ASSAYS

Fluorescence *in-situ* hybridisation (FISH) assays for amplification of *KRAS*, *EGFR*, *PIK3CA* and *MET* were performed as described previously.[14,15]

NEXT – GENERATION SEQUENCING

DNA sequencing was performed using the Ion AmpliSeq Cancer Panel (Ion Torrent; Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA), as reported previously.[16] Briefly, 10 ng of DNA was amplified by polymerase chain reaction (PCR) using the AmpliSeq Cancer Panel Primers pool and Ion AmpliSeq Master Mix version 2.0. Multiplexed barcoded libraries were enriched by clonal amplification using emulsion PCR on ion sphere particles (ISPs) (Ion PGM template OT2 200 kit or Ion PI OT2 200 kit version 3) and loaded onto an Ion 318 chip or P1 chip (Thermo Fisher Scientific). Massively parallel sequencing was carried out on a personal genome machine sequencer or ion proton (Thermo Fisher Scientific).

The raw signal data were analysed using Torrent Suite (version 4.0.1; Life Technologies, Carlsbad, CA, USA). The short sequence reads were aligned to the human genome reference sequence (GRCh37 patch 13, GCF_000001405.25). Variant calling was performed using Variant Caller version 4.4.3.3 plugin (integrated with Torrent Suite) that generated a list of identified sequence variations [single nucleotide variants (SNV) and insertions or deletions (indels)] in a variant calling file (VCF version 4.2; https://samtools.github.io/hts-specs/VCFv4.2.pdf). After removing reference calls from the VCF files, variant calls in each VCF files were normalised[17] and sorted based on the chromosome and genomic position. Variant calls were annotated using ANNOVAR[18] and the HGVS python module.[19] Several publically available databases were used for variant annotation: COSMIC version 81 (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/; last accessed 8/30/2017), dbSNP build 137 (http://www.ncbi.nlm.nih.gov/SNP/; last accessed 8/30/2017), 1000 genomes (http://www.1000genomes.org/; last accessed 8/30/2017), Exome Variant Server (http://evs.gs.washington.edu/EVS/; last accessed 8/30/2017), Exome Aggregation Consortium (ExAC) (http://exac.broadinstitute.org/; last accessed 8/30/2017) and in-silico prediction scores (PolyPhen-2 and SIFT).[20,21] Sequence variants with at least 5% allelic fraction and at least ×200 depth of coverage were included for analysis. Integrated Genomics Viewer[22] (IGV; Broad Institute, Cambridge, MA, USA) was used for manual review of the sequence read pile-ups to assess variant call quality. A joint cohort analysis of all variants across all samples were performed to identify recurrent low-frequency false positive variants. Variants were prioritised using the Association for Molecular Pathology, American Society of Clinical Oncology and College of American Pathologists joint consensus guidelines on variant interpretation in cancer.[23] Copy number analysis from next-generation sequencing data was performed using the copy number variation (CNV) kit.[24] A pooled normal reference was generated from targeted sequence analysis of 10 normal peripheral blood samples. Copy number variation (gains or losses) that was supported by deviation of all gene-specific amplicons from the baseline was prioritised and evaluated further. Sequence variants and CNVs were confirmed using DNA Sanger sequencing, FISH and IHC. Visualisation plots were created using JavaScript library jsComut (https://github.com/pearcetm/jscomut; last accessed 8/30/2017).

STATISTICAL ANALYSIS

Categorical data were presented as frequency and percentage, whereas continuous variables were described with mean. Overall survival (OS) was defined as the time from date of

commencement of treatment (either surgical resection or beginning of radiation or chemotherapy) to the date of the last follow-up or death. Survival differences between groups for an individual risk factor were examined by the log-rank test. Statistical tests were performed using GraphPad Prism software version 7.03 (GraphPad Software, Inc., La Jolla, CA, USA). All tests were two-sided, and differences were considered significant at *P*-values ≤0.05.

Results

PATIENT CHARACTERISTICS

Clinicopathological characteristics of the 53 PSC cases are summarised in Table 1. Cases include surgically resected 52 pleomorphic carcinomas (98.2%) and one carcinosarcoma (1.8%), the latter composed of squamous cell carcinoma (SCC) and chondrosarcoma. Overall, adenocarcinoma was found in 35 (66.1%), SCC in 11 (20.7%), adenosquamous carcinoma (AdSC) in five (9.4%) and large cell neuroendocrine carcinoma (LCNEC) in two (3.7%) cases. All 52 cases of PSC had >10% of spindle cell carcinoma and giant cell carcinoma components. Tumours ranged in size from 1.0 to 10.0 cm in diameter with a median of 4.1 cm in greatest dimension.

MUTATIONS AND CNV

A total of 48 mutations (mean = 2.0; range = 0–6) were identified. The most commonly mutated gene was *TP53* (17 of 23, 74.0%) followed by *KRAS* codon 12 of 13 (10 of 23, 43.4%). *KRAS* mutations were all found in smokers, distributed among eight (80%) PSCs with adenocarcinoma morphology, one (10%) AdSC and one (10%) SCC (Table 2). Figure 1 and Table 2 summarise the detected actionable and investigational variants by Ion AmpliSeq Cancer Panel. Only one PSC with adenocarcinoma histology had no identifiable mutation. Cases with frequent mutations (≥5) were all adenocarcinomas with a component of giant cell carcinoma.

Among the 23 cases, Ion AmpliSeq Cancer Panel detected a total of 11 CNV (Figure 1 and Table 3). Gains in *PIK3CA*, *EGFR*, *KRAS* and *MET* were also confirmed by FISH. Additionally, there were copy number losses in *RB1*, confirmed by immunohistochemistry (Figure 1 and Table 3). There was no co-occurrence of *MET* amplification and *MET* exon 14 skipping mutation.

SURVIVAL ANALYSIS

Mean follow-up was 28.8 months (range = 2–141, median = 16 months). Overall, 25 (47.1%) patients died within 2–105 months (mean = 22.7 months, median = 15 months) after diagnosis, and 28 were alive at 3–141 months (mean = 38.7 months, median = 21.5 months) after diagnosis. Five-year overall survival was 12.5% for the whole population. Kaplan–Meier survival analyses showed age greater than 70 years ($P = 0.008$), tumour size ≥4 cm ($P = 0.02$) and *KRAS* mutation ($P = 0.01$) among adenocarcinomas were associated strongly with worse overall survival (Figure 2A–C). There was no significant association between angiolymphatic invasion, visceral pleural invasion, tumour histology and stage with the outcome.

Discussion

Pulmonary sarcomatoid carcinoma is a rare form of NSCLC characterised by high aggressiveness and mortality. The rare occurrence of PSC has restricted the characterisation of its genetic and molecular basis, thus impeding the development of targeted treatment protocols.

In this study, similar to previous reports, most patients were male, in the seventh decade of life and had a history of heavy smoking.[2,25] Tumours were found commonly as large masses, with a median diameter of 4.1 cm. We demonstrated that both older age (greater than 70 years) and large tumour size (greater than 4 cm) were associated with significantly worse survival (P = 0.008 and 0.02, respectively). With a mean follow-up period of 28.8 months the overall survival was poor, and only 12.5% of patients were alive at 5 years. Unlike previous studies,[2,25] we did not find a significant association between clinical stage and prognosis and this is perhaps because, for diagnostic purposes, we sought to include only surgically treated patients.

In our series, we demonstrated that PSCs harbour a broad spectrum of mutations, the most common being *TP53* found in 74.0% of patients. These results are in accordance with those reported by Schrock *et al.*,[3] who also identified *TP53* mutations in 74% of their cases.[3,26] *TP53* mutation often co-occurred with other mutations, with the most common being *KRAS*. We are uncertain about the significance of co-existing alterations in our study, but they were not of prognostic significance.

KRAS codon 12/13 mutations were the second most common mutation in our series, found in 43.4% of the overall cohort and 46.6% of PSC with adenocarcinoma component. This is slightly higher than the overall frequency of 33% in lung adenocarcinoma according to The Cancer Genome Atlas data;[26] however, it is in keeping with previous reports of *KRAS* mutations in PSC.[3,5,6,27] Prognostic significance of *KRAS* mutations in 'pure' lung adenocarcinomas is controversial,[28–32] with larger study cohorts indicating no apparent difference in outcome based on *KRAS* mutation status and subtype. In contrast, PSCs with adenocarcinoma morphology and *KRAS* codon 12/13 mutations in our study had a significantly worse outcome ($P = 0.01$) compared to *KRAS* wild-type. The number of cases is relatively small to make a reliable comparison based on *KRAS* mutation subtype. Interestingly, *KRAS* mutation was also identified in a single case of morphologically and immunohistochemically proven squamous cell carcinoma.

Recent studies indicate that inhibition of *MET*-driven oncogenic pathways has potential as a biomarker-driven targeted approach for PSC therapy.[3,7,26,33–35] *MET* exon 14 mutations have been identified previously in up to 22% of PSC cases,[3,7,36] whereas others[3,4,9,27] have reported infrequent *MET* mutations, which may be due to differences in methodologies. In our series, *MET* amplification was seen in one case (5.5%) with an adenocarcinoma component and *MET* exon 14 skipping and missense mutations were identified in two (11.1%) cases with adenocarcinoma histology. In contrast to other studies, we did not find co-occurrence of *MET* amplification and mutation.[3,37] However, our findings further argue

for the testing for *MET* mutations in PSC, as they may provide therapeutic options with MET inhibitors such as crizotinib in this setting.

Similar to other studies in the western population, the *EGFR*-sensitising mutation p.L858R was found in only one PSC (5.5%) with an adenocarcinoma component. Our data confirm previous observations that *EGFR* mutations are infrequent in PSCs,[3,6,27,37] limiting the clinical benefits from EGFR tyrosine kinase inhibitors in patients with PSC. Other targetable alterations, such as mutations in *BRAF* and *HER2* or *ALK* and *ROS1* gene rearrangements, were not identified in our cohort. Although these findings may be explained by a small number of cases, the rarity of these alterations and their associations with lack of smoking history and patient's relatively younger age may be an alternative explanation. However, our study demonstrates that testing for genes outside the National Comprehensive Cancer Network and the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology guidelines may be potentially beneficial in this aggressive subtype of lung carcinoma.[38]

Additional actionable and investigational variants were detected in *ATM* (17.3%), *FBXW7* (8.7%), *AKT1* (4.3%), *PDGFRA* (4.3%) and *HRAS* (4.3%), providing oncologists with options for potential therapeutic targets. In addition to mutations found in known cancer-associated genes, we detected and validated frequent copy number losses in *RB1* (three of 23, 13.0%). *RB1* deletion in one case was identified as an isolated event, but in the other two cases co-occurred with mutations, particularly *p53* and *KRAS*. While the loss of *RB1* has been reported recently in PSC,[3,7] its significance is uncertain.

Our study has some limitations. To increase the diagnostic accuracy we restricted our cases to only surgically treated patients, therefore decreasing the total number of cases for the study. Also, tissue blocks were not available for a large subset of cases, limiting molecular and statistical analyses. Further-more, the low number of *MET* exon 14 alterations in our study may be due to the limitation in coverage provided by the Ion AmpliSeq Cancer Panel. The majority of *MET* splicing mutations occur at the 3' end of exon 14 in contrast to the 5' end.[35] In our study and the one by Terra *et al.*,[5] the amplicon in the Ion AmpliSeq Cancer Panel for *MET* exon 14 covers only the 5' splice site and some intronic sequence but not the 3' splice site (Supporting information, Figure S1). Therefore, an alternative sequencing approach may be considered if the initial results are negative for *MET* exon 14 alterations.

In summary, this study confirms that PSCs frequently harbour mutations in *TP53* and *KRAS* genes among many others, probably contributing to patients' decreased survival. Furthermore, we identified several actionable and investigational genomic alterations that could potentially increase targeted therapeutic options for these patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG. *WHO* classification of tumours of the lung, pleura, thymus and heart. 4th ed. Lyon, France: IARC, 2015.
2. Yendamuri S, Caty L, Pine M et al. Outcomes of sarcomatoid carcinoma of the lung: a surveillance, epidemiology, and end results database analysis. Surgery 2012; 152; 397–402. [PubMed: 22739072]
3. Schrock AB, Li SD, Frampton GM et al. Pulmonary sarcomatoid carcinomas commonly harbor either potentially targetable genomic alterations or high tumor mutational burden as observed by comprehensive genomic profiling. J. Thorac. Oncol. 2017; 12; 932–942. [PubMed: 28315738]
4. Vieira T, Antoine M, Ruppert A-M et al. Blood vessel invasion is a major feature and a factor of poor prognosis in sarcomatoid carcinoma of the lung. Lung Cancer 2014; 85; 276–281. [PubMed: 24997135]
5. Terra SB, Jang JS, Bi L et al. Molecular characterization of pulmonary sarcomatoid carcinoma: analysis of 33 cases. Mod. Pathol. 2016; 29; 824–831. [PubMed: 27174587]
6. Italiano A, Cortot AB, Ilie M et al. EGFR and KRAS status of primary sarcomatoid carcinomas of the lung: implications for anti-EGFR treatment of a rare lung malignancy. Int. J. Cancer 2009; 125; 2479–2482. [PubMed: 19681124]
7. Liu X, Jia Y, Stoopler MB et al. Next-generation sequencing of pulmonary sarcomatoid carcinoma reveals high frequency of actionable MET gene mutations. J. Clin. Oncol. 2016; 34; 794–802. [PubMed: 26215952]
8. Chang Y-L, Wu C-T, Shih J-Y et al. EGFR and p53 status of pulmonary pleomorphic carcinoma: implications for EGFR tyrosine kinase inhibitors therapy of an aggressive lung malignancy. Ann. Surg. Oncol. 2011; 18; 2952–2960. [PubMed: 21409490]
9. Fallet V, Saffroy R, Girard N et al. High-throughput somatic mutation profiling in pulmonary sarcomatoid carcinomas using the LungCartaTM Panel: exploring therapeutic targets. Ann. Oncol. 2015; 26; 1748–1753. [PubMed: 25969368]
10. Fletcher CDM, Dacic STW. WHO classification of tumours of the lung, pleura, thymus and heart. Lyon: International Agency for Research on Cancer, 2015.
11. Zou F, Xie G, Ma J-A et al. Epidermal growth factor receptor mutation heterogeneity analysis of pulmonary sarcomatoid carcinoma successfully treated with erlotinib: a case report. Oncol. Lett. 2015; 9; 2239–2243. [PubMed: 26137049]
12. Lee C, Usenko D, Frampton GM et al. MET 14 deletion in sarcomatoid non-small-cell lung cancer detected by next-generation sequencing and successfully treated with a MET inhibitor. J. Thorac. Oncol. 2015; 10; e113–e114. [PubMed: 26709483]
13. Amin MB, Edge SB, Greene FL et al. American Joint Committee on Cancer staging manual. 8th ed New York: Springer, 2017.
14. Li Z, Dacic S, Pantanowitz L et al. Correlation of cytomorphology and molecular findings in EGFR+, KRAS+, and ALK+ lung carcinomas. Am. J. Clin. Pathol. 2014; 141; 420–428. [PubMed: 24515771]
15. Schneider F, Derrick V, Davison JM et al. Morphological and molecular approach to synchronous non-small cell lung carcinomas: impact on staging. Mod. Pathol. 2016; 29; 735–742. [PubMed: 27080983]
16. Dacic S, Villaruz LC, Abberbock S et al. ALK FISH patterns and the detection of ALK fusions by next generation sequencing in lung adenocarcinoma. Oncotarget 2016; 7; 82943–82952.
17. Tan A, Abecasis GR, Kang HM. Unified representation of genetic variants. Bioinformatics 2015; 31; 2202–2204. [PubMed: 25701572]
18. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010; 38; e164.

19. Hart RK, Rico R, Hare E et al. A Python package for parsing, validating, mapping and formatting sequence variants using HGVS nomenclature. Bioinformatics 2015; 31; 268–270. [PubMed: 25273102]
20. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat. Protoc. 2009; 4; 1073–1081. [PubMed: 19561590]
21. Adzhubei IA, Schmidt S, Peshkin L et al. A method and server for predicting damaging missense mutations. Nat. Methods 2010; 7; 248–249. [PubMed: 20354512]
22. Thorvaldsdottir H, Robinson JT, Mesirov JP. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. Brief. Bioinform. 2013; 14; 178–192. [PubMed: 22517427]
23. Li MM, Datto M, Duncavage EJ et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: a joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J. Mol. Diagn. 2017; 19; 4–23. [PubMed: 27993330]
24. Talevich E, Shain AH, Botton T et al. CNVkit: genome-wide copy number detection and visualization from targeted DNA Sequencing. PLoS Comput. Biol. 2016; 12; e1004873.
25. Ung M, Rouquette I, Filleron T et al. Characteristics and clinical outcomes of sarcomatoid carcinoma of the lung. Clin. Lung Cancer 2016; 17; 391–397. [PubMed: 27105684]
26. Collisson EA, Campbell JD, Brooks AN et al. Comprehensive molecular profiling of lung adenocarcinoma. Nature 2014; 511; 543–550. [PubMed: 25079552]
27. Lococo F, Gandolfi G, Rossi G et al. Deep sequencing analysis reveals that KRAS mutation is a marker of poor prognosis in patients with pulmonary sarcomatoid carcinoma. J. Thorac. Oncol. 2016; 11; 1282–1292. [PubMed: 27156442]
28. Slebos RJ, Kibbelaar RE, Dalesio O et al. K-ras oncogene activation as a prognostic marker in adenocarcinoma of the lung. N. Engl. J. Med. 1990; 323; 561–565. [PubMed: 2199829]
29. Villaruz LC, Socinski MA, Cunningham DE et al. The prognostic and predictive value of *KRAS* oncogene substitutions in lung adenocarcinoma. Cancer 2013; 119; 2268–2274. [PubMed: 23526491]
30. Tsao M-S, Aviel-Ronen S, Ding K et al. Prognostic and predictive importance of p53 and RAS for adjuvant chemotherapy in non small-cell lung cancer. J. Clin. Oncol. 2007; 25; 5240–5247. [PubMed: 18024870]
31. Johnson ML, Sima CS, Chaft J et al. Association of *KRAS* and *EGFR* mutations with survival in patients with advanced lung adenocarcinomas. Cancer 2013; 119; 356–362. [PubMed: 22810899]
32. Yu HA, Sima CS, Shen R et al. Prognostic impact of KRAS mutation subtypes in 677 patients with metastatic lung adenocarcinomas. J. Thorac. Oncol. 2015; 10; 431–437. [PubMed: 25415430]
33. Paik PK, Drilon A, Fan P-D et al. Response to MET inhibitors in patients with Stage IV lung adenocarcinomas harboring MET mutations causing exon 14 skipping. Cancer Discov. 2015; 5; 842–849. [PubMed: 25971939]
34. Frampton GM, Ali SM, Rosenzweig M et al. Activation of MET via diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET inhibitors. Cancer Discov. 2015; 5; 850–859. [PubMed: 25971938]
35. Awad MM, Oxnard GR, Jackman DM et al. *MET* exon 14 mutations in non-small-cell lung cancer are associated with advanced age and stage-dependent *MET* genomic amplification and c-Met overexpression. J. Clin. Oncol. 2016; 34; 721–730. [PubMed: 26729443]
36. Schrock AB, Frampton GM, Suh J et al. Characterization of 298 patients with lung cancer harboring MET exon 14 skipping alterations. J. Thorac. Oncol. 2016; 11; 1493–1502. [PubMed: 27343443]
37. Ushiki A, Koizumi T, Kobayashi N et al. Genetic heterogeneity of EGFR mutation in pleomorphic carcinoma of the lung: response to gefitinib and clinical outcome. Jpn. J. Clin. Oncol. 2009; 39; 267–270. [PubMed: 19155283]
38. Lindeman NI, Cagle PT, Beasley MB et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. Arch. Pathol. Lab. Med. 2013; 137; 828–860. [PubMed: 23551194]

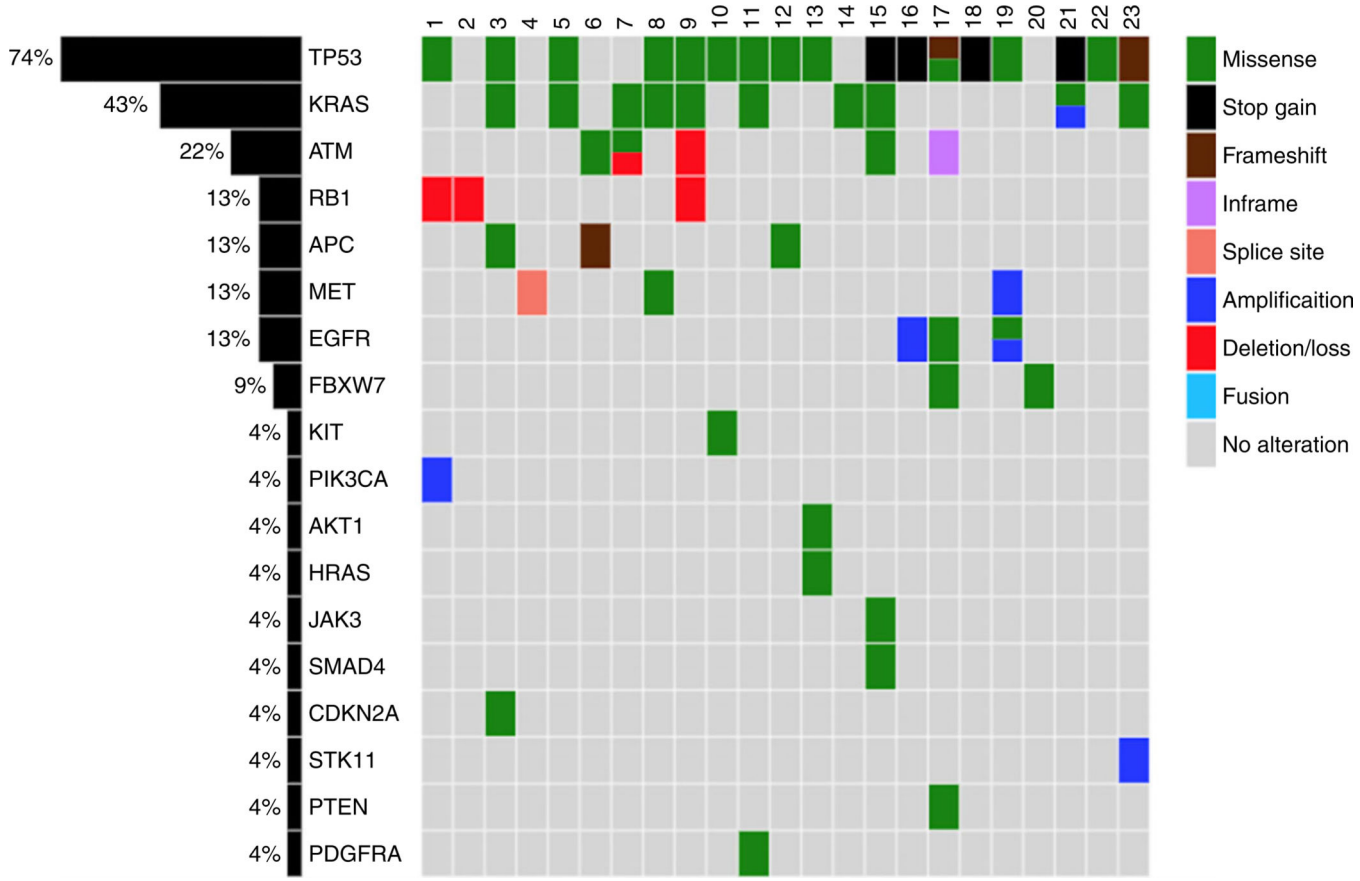

Figure 1.

coMut plot representation of individual mutations and copy number variants (−c) present in 23 cases of pulmonary sarcomatoid carcinoma. Top: cases 1–23; left: percentages of alterations in each gene.

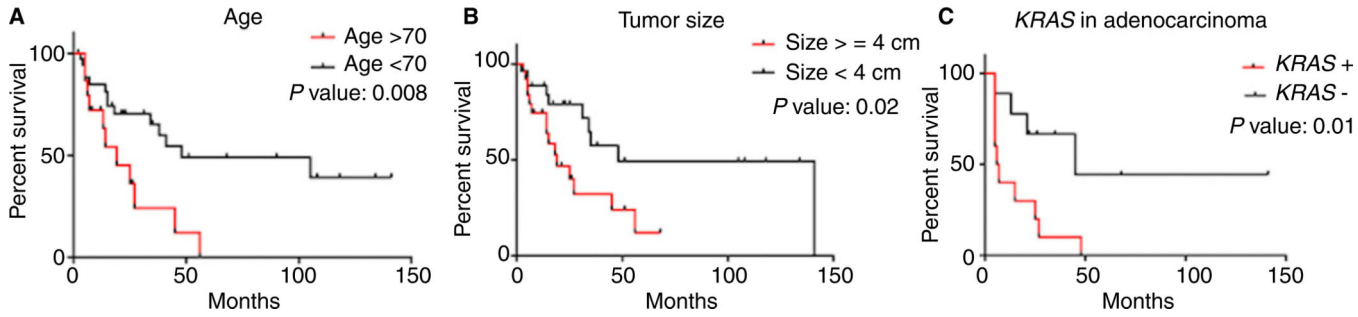

Figure 2.

Kaplan-Meier survival curves. **A**, Patient age >70 years; **B**, tumour ≥4 cm are associated significantly with worse overall survival. **C**, *KRAS* mutation in adenocarcinomas was associated significantly with poor survival.

Table 1.

Clinicopathological characteristics of the study cohort (*n* = 53)

Characteristics	Number (%)
Gender	
Male	32 (60.3)
Female	21 (39.7)
Age range, median (years)	41−84, 67
Smoking history	
Current or former	51 (96.2)
Never smoker	2 (3.8)
Angiolymphatic invasion	
Present	43 (81.1)
Absent	10 (18.9)
Visceral pleural invasion	
Present	24 (45.2)
Absent	29 (54.8)
Histology	
Adenocarcinoma	35 (66.1)
Squamous cell carcinoma	11 (20.7%)
Adenosquamous carcinoma	5 (9.4%)
Large cell neuroendocrine carcinoma	2 (3.7%)
Stage	
I	29 (54.7%)
II	9 (17.0%)
III	7 (13.2%)
IV	8(15.1%)

Table 2.

Actionable and investigational genomic alterations detected by Ion AmpliSeq Cancer Panel* among 23 cases

	Ion AmpliSeq Cancer Panel actionable and investigational genomic alterations					
Tumour type	**Gene**	***n* (%)**	**Exon**	**Protein**	**cDNA**	**Mutation type**
Adenocarcinoma (*n* = 18)	*KRAS*	8 (44.4%)	2	p.G12C (4) p.G12V (3) p.G13D (1)	c.G34T (4) c.G35T (3) c.G38A (1)	SNV missense
	EGFR	2 (11.1%)	21 20	p.L858R p.G779C	c.T2573G c.G2335T	SNV missense
	FBXW7	2 (11.1%)	10 7	p.L459V p.T267K	c.T1375G c.C800A	SNV missense
	CDKN2A	1 (5.5%)	2	p.P81S	c.C241T	SNV missense
	APC	3 (16.6%)	14	p.E1299Q p.T1538fs	c.G3895C (2) c.4613_4614insA (1)	SNV missense (2) Insertion (1)
	ATM	3 (16.6%)	17 9 50	p.F858L p.V410A p.E2446*	c.T2572C c.T1229C c.7335_7336TT	SNV missense (2) Substitution (1)
	MET	2 (11.1%)	14	p.T992I	c.C2975T	Splice (1) Missense (1)
	SMAD4	1 (5.5%)	12	p.I525V	c.A1573G	SNV missense
	JAK3	1 (5.5%)	16	p.V722I	c.G2164A	SNV missense
	PDGFRA	1 (5.5%)	15	p.H687R	c.A2060G	SNV missense
	PTEN	1 (5.5%)	6	p.R173L	c.G518T	SNV missense
Squamous cell carcinoma (*n* = 3)	*HRAS*	1 (33.3%)	3	p.Q61K	c.181A	SNV missense
	AKT1	1 (33.3%)	3	p.E17K	c. G49A	SNV missense
	KRAS	1 (33.3%)	2	p.G12D	c.G35A	SNV missense
Adenosquamous carcinoma (*n* = 2)	*KRAS*	1 (50%)	2	p.G12D	c.G35A	SNV missense
	ATM	1 (50%)	9	p.V410A	c.T1229C	SNV missense
	KIT	1 (50%)	18	p.S850I	c.G2549T	SNV missense

*Ion Torrent, Life Technologies, Thermo Fisher Scientific, Waltham, Massachusetts.

Table 3.

Copy number variants detected by Ion AmpliSeq Cancer Panel * among 23 cases

	Ion AmpliSeq Cancer Panel copy number alterations		
Tumour type	**Gene**	**Gain (%)**	**Loss (%)**
Adenocarcinoma *(n* = 18)	*R81*		2 (11.1)
	EGFR	2 (11.1)	-
	KRAS	1 (5.5)	-
	ATM		1 (5.5)
	MET	1 (5.5)	-
	STK11	1 (5.5)	-
Squamous cell carcinoma *(n* = 3)	*Rs1*	-	1 (33.3)
	P/K3CA	1 (33.3%)	-
Adenosquamous carcinoma *(n* = 2)	*ATM*	-	1 (50.0%)

* ion Torrent, Life Technologies, Thermo Fisher Scientific, Waltham, Massachusetts.