High-throughput somatic mutation profiling in pulmonary sarcomatoid carcinomas using the LungCarta™ Panel: exploring therapeutic targets

V. Fallet1, R. Saffroy2,3, N. Girard4, J. Mazieres5, S. Lantuejoul6, T. Vieira1, I. Rouquette7, F. Thivolet-Bejui8, M. Ung5, V. Poulot1, L. Schlick1, D. Moro-Sibilot8, M. Antoine1,10, J. Cadranel1,11, A. Lemoine2,3 & M. Wislez1,11*

1GRC n°04, Theranoscan, Sorbonne Universités, UPMC Université de Paris 06, Paris; 2INSERM U1004, Université de Paris 11, Institut André Wolf, Villejuif; 3Department of Biochemistry and Molecular Biology, AP-HP, Hôpital Paul Brousse, Villejuif; 4Pulmonary Medicine Unit, Hôpital Louis Pradel, Hospices Civils, Lyon; 5Pulmonary Medicine Unit, Hôpital Larrey, Centre Hospitalier Universitaire, Université de Toulouse III, Toulouse; 6Department of Pathology, Hôpital A. Michallon, Centre Hospitalier Universitaire, Grenoble; 7Department of Pathology, Centre Hospitalier Universitaire Rangueil, Toulouse; 8Department of Pathology, Hôpital Louis Pradel, Lyon; 9Pulmonary Medicine Unit, Hôpital A. Michallon, Centre Hospitalier Universitaire, Grenoble; 10Department of Pathology, AP-HP, Hôpital Tenon, Paris; 11Pulmonary Medicine Unit, AP-HP, Hôpital Tenon, Paris, France

Received 4 January 2015; revised 16 April 2015; accepted 7 May 2015

Background: Pulmonary sarcomatoid carcinomas (SC) are tumors characterized by poor prognosis and resistance to conventional platinum-based chemotherapy. This study sought to describe the mutational profile of SC using high-throughput genotyping technology.

Patients and methods: We used mass spectrometry to test 114 surgical biopsies from 81 patients with SC for 214 mutations affecting 26 oncogenes and tumor suppressor genes.

Results: In total, 75 (92.6%) patients were smokers. Within the total 81 tumors, 67 distinct somatic alterations were identified, with 56 tumors (69.1%) harboring at least one mutation. The most frequent mutations were KRAS (27.2%), EGFR (22.2%), TP53 (22.2%), STK11 (7.4%), NOTCH1 (4.9%), NRAS (4.9%), and PI3KCA (4.9%). The EGFR mutations were almost always rare mutations (89%). In 32 tumors (39.5%), two or more mutations co-existed, with up to four mutations in a single case. In six different cases, comparative genetic analysis of different histological areas from the same tumor (giant, spindle, or epithelial component) revealed a 61% concordance rate for all the mutations with a 10% detection threshold, compared with 91.7% with a 20% detection threshold.

Conclusion: Our results demonstrated a high mutation rate and frequent co-mutations. Despite SC tumors exhibiting a high histological heterogeneity, some intratumoral molecular homogeneity was found. Now with newly developed targeted therapies, SC patients may be eligible for new target mutations, and can now therefore be screened for clinical trials.

Key words: lung sarcomatoid carcinoma, nonsmall-cell lung cancer, EGFR, KRAS, mutation

Introduction

Over the past few decades, the development of the multiplex assay has enabled us to gain knowledge of specific aberrations in genomes of cancer cells. Genomic profiles are becoming increasingly crucial in the management of nonsmall-cell lung cancer (NSCLC). EGFR and ALK tyrosine kinase inhibitors (TKIs) are associated with improved overall survival rates in EGFR-mutated and ALK-rearranged tumors, respectively.

However, targeted therapies primarily benefit adenocarcinoma patients. Pulmonary sarcomatoid carcinomas (SC) are rare
tumors, with <3% of NSCLC [1] cases, and defined as a small subgroup of poorly differentiated tumors containing sarcoma-like or sarcoma elements [2]. They are associated with poor prognosis and resistance to conventional platinum-based chemotherapy [3]. The inherent low frequency and histological heterogeneity of these tumors could account for the lack of driver oncogenes that have been reported in this entity. Previous studies reported KRAS mutation frequency reaching 30%, with that of EGFR mutations at 10% [4–7]. Additional exhaustive genetic testing could help identify new therapeutic targets and develop new therapeutic strategies for managing these tumors.

This study sought to describe the mutational profile of SC using high-throughput genotyping technology. We tested 114 surgical biopsies from 81 patients with SC for activating point mutations or deletions of the main genes known to be altered in adenocarcinomas using mass spectrometry technology. Our results were compared with clinicopathological characteristics and overall survival rates.

**methods**

**patients and tissue tumor collection**

Tissue samples were obtained from the surgical lung biopsies of 81 consecutive patients with pulmonary SC who had undergone surgery between 2005 and 2012 in four referral thoracic oncology centers.

All samples were taken from archived formalin-fixed paraffin-embedded specimens and were centrally reviewed (MA) in order to confirm a pulmonary SC diagnosis in accordance with the 2004 World Health Organization criteria [2]. The presence of an epithelial component and its subtype (adenocarcinoma, squamous, or large-cell carcinoma) were collected. TTF1 and p63 immunohistochemistry were carried out for all the samples. Only samples exhibiting tumor cellularity >50% were utilized.

In one center, biopsies taken from normal adjacent lung tissue (N = 27) were available and used as negative controls. In some cases (N = 6), tumor tissue samples comprising different histological components were available and used to assess intratumoral heterogeneity.

Full clinical data pertaining to demographics, tumor characteristics, and treatment regimens was extracted from the medical records of each patient. Patients who died before 30 days after surgery were excluded from analysis. According to national guidelines, each patient signed a research-approval form. The samples were collected according to French legislation with the respect of ethical rules.

**molecular analysis**

Technics are detailed in supplementary Tables S1 and S2 available at Annals of Oncology online. The LungCarta™ mutation panel was applied (Sequenom, San Diego, CA) in order to detect 214 mutations in 26 oncogenes and tumor suppressor genes (supplementary Table S3, available at Annals of Oncology online). Target genes were selected based on key mutations identified via sequencing discovery studies in lung adenocarcinoma [8]. Mutations were determined using a minimum 10% threshold of the mutant allele peak.

**statistical analysis**

Differences in demographic characteristics and tumor characteristics were analyzed by applying Pearson’s χ² or Fisher’s exact test, as appropriate, to compare mutational status. For continuous variables (age and number of mutations), Student’s t-test was carried out. Overall survival (OS) was calculated from the date of surgery to the date of death from any cause. The censoring date was 30 June 2013. OS was estimated using the Kaplan–Meier method, and comparisons were made using log-rank tests. All P values were based on a two-sided hypothesis. A P value <0.05 was considered statistically significant. Statistical calculations were carried out using SPSS® software (Version 20.0 SPSS, Inc.).

**results**

**patient characteristics**

Our study initially included 97 patients with SC who had undergone surgical lung resection between 2005 and 2012 in four centers. A total of 16 were excluded due to early death (N = 5), missing clinical data (N = 3), unfound block of surgical lung biopsy (N = 3), or absence of DNA amplification (N = 5). Mutation detection by means of the LungCarta™ mutation panel was carried out in 81 patients.

The patient clinical characteristics are presented in Table 1. Median age was 61 years (range 41–79 years). Patients were more frequently males (74.1%), heavy smokers (smokers: 92.6% and median pack-year: 36; range: 1–100). They were almost all Caucasians (80.2%) and none were Asian. In terms of pathological stage, 15 patients were stage I (18.5%), 24 stage II

| Table 1. Clinical characteristics of patients with pulmonary sarcomatoid carcinoma |
|---------------------------------|------------------|
| Variable                        | Total, N (%)     |
| Gender                          |                  |
| Male                            | 60 (74.1%)       |
| Female                          | 21 (25.9%)       |
| Age (years)                     |                  |
| Median (range)                  | 61 (41–79)       |
| Ethnicitya                      |                  |
| Caucasian                       | 65 (80.2%)       |
| Others                          | 10 (13.3%)       |
| Asian                           | 0 (0%)           |
| Smoking historyb                |                  |
| Smoker                          | 75 (92.6%)       |
| Non-smoker                      | 5 (6.2%)         |
| Pack-yearsc                     |                  |
| Median (range)                  | 36 (4–100)       |
| Clinical stage                  |                  |
| I                               | 15 (18.5%)       |
| II                              | 24 (29.6%)       |
| III                             | 35 (43.2%)       |
| IV                              | 7 (8.6%)         |
| Histological subtypes           |                  |
| Pleomorphic carcinomas          | 63 (77.8%)       |
| Spindle-cell carcinomas         | 4 (4.9%)         |
| Giant-cell carcinomas           | 6 (7.4%)         |
| Spindle- and giant-cell carcinomas | 3 (3.7%)       |
| Carcinosarcomas                 | 4 (4.9%)         |
| Pneumoblastoma                  | 1 (1.2%)         |
| Type of surgery                 |                  |
| Pneumonectomy                   | 17 (21%)         |
| Lobectomy                       | 50 (61.7%)       |
| Other                           | 14 (17.3%)       |
| Neoadjuvant chemotherapy        | 16 (19.8%)       |

*aSix data missing.  
bOne data missing.
(29.6%), 35 stage III (43.2%), and 7 stage IV (8.6%). Surgery carried out on stage IV patients was mainly for diagnostic purposes. Pleomorphic carcinoma was found to be the main histological subtype (77.8%) (Table 1). Based on morphological and immunohistochemical analysis, majority of pleomorphic carcinomas (46%) were of adenocarcinoma subtype (supplementary Figure S1, available at *Annals of Oncology* online).

**somatic alterations**

In the total 81 tumors detected, there were 67 distinct somatic alterations identified among the 214 mutations tested (supplementary Table S4, available at *Annals of Oncology* online). We reported 56 tumors (69.1%) harboring at least one mutation. The most frequent mutations were KRAS (*N* = 22, 27.2%), EGFR (*N* = 18, 22.2%), TP53 (*N* = 18, 22.2%), STK11 (*N* = 6, 7.4%), NOTCH1 (*N* = 4, 4.9%), NRAS (*N* = 4, 4.9%), and PI3KCA (*N* = 4, 4.9%) (Figure 1).

In 32 tumors (39.5%), two or more mutations co-existed (co-mutated tumors). EGFR, TP53, and PI3KCA mutations were detected along with other mutations in 72.2%, 83.3%, and 100%, respectively. This was higher than rates observed for other mutations. KRAS mutations were found to be mutually exclusive from EGFR mutations (*P* = 0.019), while EGFR and NOTCH1 mutations were frequently associated with each other (*P* = 0.033).

KRAS mutations were always found to involve codon 12, with KRAS G12C being the most frequently found (63.6%). Most KRAS mutations (72.7%) were found in tumors with adenocarcinoma component (*P* < 0.001) (supplementary Figure S2, available at *Annals of Oncology* online). Almost all EGFR mutations (16/18, 88.9%) were rare mutations (exons 2, 18, and 20), with G719A mutation the most frequent (55.5%) (supplementary Table S5, available at *Annals of Oncology* online). One sample presented complex mutations involving both G719A and insertion exon 20. The two sensitizing EGFR L858R mutations were found in pleomorphic carcinoma with adenocarcinoma component. TP53 mutations were all located within the DNA-binding domain of TP53, yet were very heterogeneous, with TP53 V157F and R273L being revealed as the most frequent, both at 16.7% (*N* = 3). Six different STK11 mutations were found. For NOTCH1, V1672I was the most frequently detected (*N* = 3, 7.5%).

A presence of mutations was not associated with any clinico-pathological factors, nor with overall survival (for Kaplan–Meier curves, see supplementary Figure S3, available at *Annals of Oncology* online). After a median follow-up of 22.1 months (range: 2.7–116.9 months), 35 patients were still alive among the 66 patients with a stage I, II, or IIIA. Median OS was 36.6 months [95% confidence interval (CI) 2.8–70.5]. Median OS for patients with no mutated tumors was 17 months (95% CI 12.1–21.8). Median OS for patients with co-mutated tumors was 47.8 months (95% CI 19.7–76).

**adjacent normal lung**

No mutation was found in 23 of the 27 samples of the adjacent normal lung (85.2%) (Table 2). In three samples, mutations found in the normal lung tissue were concordant with those found in the tumor. In one sample, an EGFR G719A mutation was detected in the tumor sample, while an EGFR R776H mutation was revealed in the normal adjacent lung. However, after blind central pathological review, this particular sample was described as exhibiting an atypical adenomatous hyperplasia (supplementary Figure S4, available at *Annals of Oncology* online). For the other mutations, no KRAS, EGFR, or TP53 mutations were ever found in adjacent normal lung tissues.

**heterogeneity**

Different tumor blocks with different pathological SC subtypes for six cases were available for comparison (Table 3). In these blocks, from one to three mutations were found present in the initial tumor sample. The comparative genomic analysis between the different areas of histological component revealed a 61% concordance rate for all mutations. When the threshold of mutation frequency detection was increased to 20%, the concordance rate reached 91.7%. Almost all mutations harbored in the primary original tumor sample matched the different histological components. In all cases, EGFR and KRAS mutations were found in the different blocks of tumors, regardless of the threshold imposed.

**discussion**

The multiplex high-throughput analysis of gene mutations in 81 pulmonary SC from four thoracic oncology centers revealed a high rate of mutated tumors (69.1%), which corresponded with a high rate of co-mutated tumors (39.5%). The most frequently detected mutations were KRAS (27.2%), EGFR (22.2%), TP53 (22.2%), STK11 (7.4%), NOTCH1 (4.9%), NRAS (4.9%), and PI3KCA (4.9%).

This constitutes the largest multicenter cohort to date of molecular profiling in these rare tumors. A specialized pathologist conducted the central histological review on surgical samples in accordance with WHO classifications. MassARRAY® technology has already proven to display high sensitivity and specificity for detecting molecular abnormalities in samples composed of ≤10% tumor cells [9]. One limitation of the study was the predefined panel of oncogenes and tumor suppressor genes, as imposed by the LungCarta™ panel. Other oncogenes that are potentially involved in sarcoma, such as c-kit, were not investigated.

Furthermore, only the most commonly known mutations in 26 genes were studied, and other mutations or chromosomal
No mutations were found in 23 of the 27 samples of adjacent normal lung. In one sample, an EGFR mutation was observed that differed from that of the tumor area. This sample, however, exhibited an atypical adenomatous hyperplasia on central pathologist review. As described above, EGFR mutations have the potential of being present in early lung preneoplastic lesions, which might favor carcinoma development [10]. In three samples, MET N375S, NOTCH1 V1672I, and STK11 Y272Y mutations were found in both normal adjacent lung and tumor block tissue. One possible hypothesis is that these alterations were single-nucleotide polymorphism. The frequency of MET N375S mutations, as assessed by blood DNA sequencing, did not differ between lung cancer patients and non-cancer controls [11]. Moreover, STK11 Y272Y mutation had been found in lung adenocarcinoma, and not in normal adjacent lung or healthy blood samples [12]. On the other hand, no KRAS, EGFR, or TP53 mutations were ever found in nontumor areas. Given that this was a retrospective study based on surgical tumor biopsies, we were unable to obtain blood samples in order to confirm this hypothesis.

In order to account for tumor heterogeneity, we analyzed samples of different pathological SC subtypes. Despite this histological heterogeneity, we were able to demonstrate a 61% molecular concordance between the samples. When applying a higher cutoff of detection (at least 20% mutated allele/wild-type allele ratio), the concordance rate increased to 91%. This difference proved the inherent difficulty in defining a threshold for mutation detection, and the determination of a clinically significant rate requires further investigation. In contrast, the identification of high percentage of mutated allele present within the same tumor let us hope a good efficiency of the targeted therapies. This high concordance of mutations within the same tumor reinforced the hypothesis of a single cell clone, which is acquired by different, still unknown mechanisms with different histological phenotypes involving epithelial to mesenchymal transition.

Mutations were present in 69.1% of cases, which is higher than expected for other lung neoplasms using same panel-imposed mutation analysis and technology. The rate was 57% in a Western Europe cohort of 139 nonsquamous-cell carcinomas [13] and 44.5% in a French cohort of 382 NSCLC (230 adeno-carcinomas and 152 squamous-cell carcinomas) [14]. Of note, squamous-cell carcinoma cases were not included in their cohort, which could have led to an increase in the rate of mutations, since the LungCarta® Panel was designed based on key mutations identified in adenocarcinoma.

Moreover, the rate of co-mutated tumors (39.5%) in SC cases was compared with the 17.8% rate found in nonsquamous-cell carcinoma of the Tan et al. cohort [13], and to the 8.4% found in the French NSCLC cohort [14]. This high rate of co-mutated tumors may represent a specific characteristic of SC. These results are in line with the high rate of smoker patients in this population [15]. KRAS mutations (72%) were, in fact, transversions, contrary to the transitions described in never-smoker patients [16].

EGFR mutation was revealed in 18 patients (22.2%), though only two exhibited EGFR-sensitizing mutations (2.5%) identified in exon 21 (L858R). These were both found in male smokers with pleomorphic carcinoma with adenocarcinoma component. In the literature, a few cases with EGFR-sensitizing mutations have been treated by EGFR TKIs, providing minimal benefit (four cases with activating EGFR mutations were described, with two...
between 32.7% and 37.5% of NSCLC cases [13, 14]. We found rare EGFR mutations were not routinely detected. Using the same panel-imposed mutation analysis and technology, rare EGFR mutations were detected in 10% of cases [21]. However, rare mutations were not routinely detected. Using the same panel-imposed mutation analysis and technology, rare EGFR mutations were found in 89% of the SC cases. This rate of rare EGFR mutations was higher than those previously reported. In a large European cohort of 1047 EGFR-mutated tumors, rare mutations were detected in 10% of cases [21]. However, rare mutations were not routinely detected. Using the same panel-imposed mutation analysis and technology, rare EGFR mutations were found in between 32.7% and 37.5% of NSCLC cases [13, 14]. We found almost-rare EGFR mutations located in exon 18. Interestingly, as in SC, exon 18 mutations were associated with tobacco use and male gender [21]. Finally, all the rare mutations we found were confirmed by means of an alternative technique.

This high rate of rare EGFR mutations (25%) has been already reported as stable disease and two partial response). Progression-free survival did not reach 5 months in three of these patients [17–20]. Of the EGFR mutations, we found rare mutations (exon 2, 18, or 20) in 89% of the SC cases. This rate of rare EGFR mutations was higher than those previously reported. In a large European cohort of 1047 EGFR-mutated tumors, rare mutations were detected in 10% of cases [21]. However, rare mutations were not routinely detected. Using the same panel-imposed mutation analysis and technology, rare EGFR mutations were found in between 32.7% and 37.5% of NSCLC cases [13, 14]. We found almost-rare EGFR mutations located in exon 18. Interestingly, as in SC, exon 18 mutations were associated with tobacco use and male gender [21]. Finally, all the rare mutations we found were confirmed by means of an alternative technique.

This high rate of rare EGFR mutations (25%) has been already described in SC in a series of 32 Chinese patients [22]. The impact of these rare mutations remains unclear in terms of sensitivity to EGFR TKI [21, 23]. However, when a rare mutation is observed in an NSCLC not otherwise specified, immunohistochemical analysis should be carried out in order to investigate a sarcomatoid component especially in a small biopsy.

Globally, despite SC having a unique histological appearance, their molecular signature might be similar to smokers’ lung adenocarcinomas with high frequency of KRAS G12C mutations and rare EGFR mutations. These rare tumors should be treated as smokers’ lung adenocarcinomas.

Our results demonstrated high mutation rates and frequent co-mutations in SC, probably related to genetic instability caused by tobacco exposure. These tumors exhibited relative molecular homogeneity, as shown by the analyses of different tumor sites, regardless of different histological components. EGFR TKI requires prudent evaluation owing to the associated high rate of rare EGFR mutations. Afatinib or neratinib could be active in lung tumors harboring rare EGFR mutations, such as G719X [24]. Now with the advantage of newly developed therapies targeted against KRAS, PIK3CA, BRAF, and NRAS, patients with SC should be screened for molecular alterations, as they could be eligible for clinical trials.

**Acknowledgements**

The authors thank the following contributors for their assistance in data collection: N. Rabbe (GRC no. 04, Theranoscan, Paris, France) and C. Khoja (Hôpital Paul Brousse, Laboratoire de Biochimie et Oncogénétique, Villejuif, France).

They also thank the Tumorothèque des Hôpitaux Universitaires de l’Est Parisien (HUEP), AP-HP, Hôpital Tenon, Service d’Anatomie Pathologique, Paris, France, for having provided some samples.

**Funding**

This work was supported by the Fond de Dotation ‘Recherche en Santé Respiratoire’ (Appel d’offre 2012 to VF and TV) (no grant number). The funding source had no influence over the conduct, data collection, or analysis of the study.

**Disclosure**

The authors have declared no conflicts of interest.

**References**


