Sarcomatoid lung carcinomas show high levels of Programmed Death Ligand-1 (PD-L1) and strong immune-cell infiltration by TCD3 cells and macrophages.

Thibault Vieira, Martine Antoine, Cécile Hamard, Vincent Fallet, Michael Duruisseaux, Nathalie Rabbe, Anita Rodenas, Jacques Cadranel, Marie Wislez

Original article

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Thibault Vieira, MD[1,2], Martine Antoine, MD[1,3], Cécile Hamard, MD[1,2], Vincent Fallet, MD[1,2], Michael Duruisseaux, MD PhD[1], Nathalie Rabbe[1], Anita Rodenas[3], Jacques Cadranel, MD PhD[1,2], and Marie Wislez, MD PhD[1,2*]marie.wislez@tnn.aphp.fr.

[1]Sorbonne Universités, UPMC Univ. Paris 06, GRC n°04, Theranoscan, F-75252, Paris

[2]AP-HP, Hôpital Tenon, service de Pneumologie, F-75970, Paris

[3]AP-HP, Hôpital Tenon, service d'anatomopathologie, F-75970, Paris

Corresponding author: Prof Marie Wislez , AP-HP, Hôpital Tenon, Department of Pneumology, F-75970, Paris Phone: +33 (0)1 56 01 68 38

Highlights

- **PD-L1, tumor-infiltrating lymphocytes (TIL) were higher in SC than in NSCLC**
- **High levels of TIL constituted a factor of good prognosis**
- **PD-L1 expression distinguishes subpopulations within tumor-infiltrating lymphocytes (CD3+ or CD4+) with different prognosis**
- **This result could represent a justification for the use of PD-L1/PD-1 immunotherapy**

Abstract

Objectives

Pulmonary sarcomatoid carcinomas (SC) are rare tumors, associated with worse prognosis and resistant to platinum-based regimens. Therapies targeting the PD-1/PD-L1 pathway are an emerging treatment for lung cancer. By characterizing intra-tumoral immune infiltration and evaluating PD-L1 expression, it could be possible to predict the efficacy of these new treatments.

Materials and Methods

From 1997 to 2013, data from all patients with SC who underwent lung resection was collected. Tumor-immune infiltration and PD-L1 expression were studied by immunochemistry tests, analyzing CD3 (clone SP7), CD4 (clone 1F6), CD8 (clone C8/144b), CD20 (clone L26), CD163 (clone 10D6), MPO (clone 59A5), and PD-L1 (clone 5H1). Results were compared to those of 54 NSCLC.

Results

In total, 75 SC were included. Forty (53%) SC expressed PD-L1 *vs* 11 NSCLC (20%) ($p<0.0001$). CD3+ tumor-infiltrating lymphocytes and CD163+ tumor-associated macrophages were more important in SC than in NSCLC (median 23%[17-30] of tumoral surface *vs* 17 %[7–27], $p=0.011$ and 23%[17-30] *vs* 20%[13–23], $p=0.002$, respectively). In SC, the presence of Kirsten Ras (KRAS) mutations, blood vessel invasion, and TTF1+ positivity were associated with PDL1 expression. On multivariate analysis, only CD163+ macrophages and blood-vessel invasion were associated with tumoral PD-L1 expression. High levels of tumor-infiltrating lymphocytes (CD3+ or CD4+ and not CD8+) constituted a factor of good prognosis on survival. Interestingly, PD-L1 expression distinguishes subpopulations within tumor-infiltrating lymphocytes (CD3+ or CD4+) with different prognosis

Conclusions

PD-L1 expression was higher in SC than in NSCLC as well as immune-cell infiltration by TCD3 cells and macrophages. This suggests that targeting the PD-1/PD-L1 pathway could represent a new potential therapy.

Keywords: lung sarcomatoid carcinoma, PD-L1, Tumor-Infiltrating lymphocytes, Tumor-Infiltratingmacrophages, non-small-cell lung cancer

Introduction

Pulmonary sarcomatoid carcinomas (SC) are challenging to manage. They probably arise from an epithelial clone with either squamous or glandular features[1]. These tumors share some similarities with non-small-cell lung cancer (NSCLC) in terms of morphology and immunohistochemistry. They may undergo a dedifferentiation process with the activation of an epithelial-mesenchymal transition[2]. Their clinical presentation is usually similar to that of NSCLC, with particularly strong associations observed with tobacco use, frequent atypical locations of metastasis, such as in the peritoneum[3], and highly-prevalent blood-vessel invasion[1]. The prognosis is poor, irrespective of the stage of disease[3,4].

SC exhibit a large number of somatic mutations[5] that may be related to tobacco use. These tumors present high levels of chemoresistance to platinum-based regimens, with disease progression reported in two-thirds of patients on first evaluation[3,6]. New targets must therefore be explored in order to improve the management of these tumors. Recently, MET exon 14 splicing alterations have been described in this histological subtype in patients that which should allow to treat some of these patients with anti-MET therapy [7,8].

We recently showed that these tumors exhibited high mutation rates[5], suggesting a high amount of tumoral neoantigens, which should induce strong tumor immunogenicity. Rich inflammatory and immune infiltrates have been described in these tumors[9], usually consisting of neutrophils in giant-cell carcinomas and lymphoplasmacytic cells in spindle-cell carcinomas, which could prove the existence of a tumor immunoselection phenomenon. Our goal was to investigate whether programmed cell death of Ligand-1 (PD-L1) played a role in sarcomatoid sarcomas. PD-L1 is overexpressed in certain tumors and induces a peripheral blockade of T-cell immune response. In a previous report, Velcheti *et al*. studied PD-L1 expression using immunofluorescence analysis in a retrospective cohort involving 445 NSCLC cases and 13 SC. They reported positive PD-L1 expression in 69.2% of the SC, compared to 27.4% of the NSCLC cases ($p < 0.01$) [10]. More recently, PD-L1 was expressed in 90% of a Korean cohort of 41 SC [11]. We sought to study PD-L1 expression and quantify tumor immune infiltration by macrophages, neutrophils, and T and B lymphocytes in 75 Caucasian patients with SC and compared these findings to those of a cohort of non-small cells lung

cancers (NSCLC). We also analyzed the relevant factors associated with PD-L1 expression and their prognostic value.

Material and methods

Sarcomatoid cohort

From January 1995 to April 2013, we included all patients with pathologically-proven primary pulmonary SC based on surgical formalin-fixed paraffin-embedded tissue samples (FFPE) (Department of Pathology, *Tumorothèque des Hôpitaux Universitaires de l'Est Parisien* [the East Paris University Hospitals' tumor biobank]). Their histology was centrally reviewed (M.A.) and all the clinical data and molecular biomarker analyses (Kirsten Ras [KRAS], epidermal growth factor receptor [EGFR], anaplastic lymphoma kinase [ALK], PIK3CA, human epidermal growth factor receptor 2 [HER2], and BRAF)[1] were taken from previous publications, provided in Supplementary Table 1.

Surgical samples were used to perform tissue micro-array analysis, with 1.0 mm-diameter cores. All the tissue blocks from each surgical specimen were reviewed (median of 26 blocks per case, interquartile range (Q1-Q3): 18-41). Core areas were selected with the aims of collecting data on all the different morphological areas (*i.e.,* epithelial, sarcomatous, and a mix of both) from each tumor (supplementary Figure 1). A median of four (Q1-Q3): (3–5) cores per tumor were extracted. Surgical samples from consecutive NSCLC tumors (arbitrary 20 adenocarcinomas, 19 squamous, and 15 large cell carcinomas) were selected to be compared to the SC cohort. Demographic characteristics did not differ from those of SC cohort except for the age (Table 1 and Supplementary Table 2) with a median of 61 years (53-69) for SC compared to 65 (60-45) for NSCLC (*P=0.002*). For NSCLC, 3 to 4 cores were selected from each tumor, 2 from the center and at least 1 from the periphery of the tumor. According to national guidelines, each patient signed a research consent form.

Analysis of PD-L1 expression

To measure PD-L1 expression, we used a murine anti-B7-H1 (PD-L1) monoclonal antibody (clone 5H1) provided by Dr. Lieping Chen (Yale University, New Haven, CT, USA). Briefly, 3-µm sections were deparaffinized, rehydrated, and washed with phosphate-buffered saline (PBS). The PD-L1 antigen was retrieved by means of a tris-EDTA buffer (pH 9) (Dako,

Courtaboeuf, France) for 40 minutes (min) in a steamer. The samples were blocked for peroxidase endogenous activity using the Dako Dual Endogenous and Serum-free blocks, prior to incubation with the primary antibody (1/500 dilution) overnight at +4°C. Amplification was performed using the Dako EnVision anti-mouse immunoperoxidase method, with diaminobenzidine as the chromogen, for the purposes of detection. Irrelevant mouse IgG1 for mouse antibody (Dako) was used as the negative control. Human placenta cores were included in the tissue micro-array (TMA) as positive controls. PD-L1 tumor positivity was defined as ≥5% tumor cell membrane staining.

Analysis of immune-cell infiltration

To characterize immune-cell populations, immunohistochemistry studies were performed by means of a Benchmark System® (Ventana medical system, Tucson, AZ, USA), using FFPE 3-µm slides to characterize T lymphocytes by CD3 (clone SP7; dilution: 1:200; incubation: 20 min; Labvision, Fremont, CA, USA), CD4 (clone 1F6; dilution: 1:25; incubation: 60 min; Novocastra, Nanterre, France), CD8 (clone C8/144b; dilution: 1:50; incubation: 32 min, DAKO), and CD20 (clone L26, dilution 1:400, 40 min, DAKO); macrophages by CD163 (clone 10D6; dilution: 1:100; incubation: 32 min; Novocastra); and neutrophils by myeloperoxidase (MPO) (clone 59A5; dilution: 1:100; incubation: 32 min; Novocastra). More details have been provided in Supplementary Table 3. The mean percentage of immune-positive cells per tumoral surface was evaluated for each tumor sample.

Statistical analyses

Categorical variables were compared by means of chi-squared or Fisher's exact test. Non-normal continuous variables were expressed as medians (interquartile). The Mann-Whitney U-test was used on non-normal continuous variables. For survival analysis, Cox test was used in univariate and multivariate analyses. For logistic regression, independent variables exhibiting a p <0.1 in the univariate analyses were included in the final model, chosen using a backward-stepwise variable elimination method based on the p-value, as candidate predictors. Redundant variables were not included in the final model. Results were

considered significant if the *p* value was <0.05. Statistical tests were performed using SPSS 20.0 software (IBM Corporation, New York, NY, USA), and graphs were constructed using Graphpad software (GraphpadInc, San diego, CA, USA).

Results

Population characteristics

In total, 77 consecutive patients had pathologically-proven primary pulmonary sarcomatoid carcinoma, based on surgical FFPE tissue samples. All the patients were included in the study, except two for whom not enough tissue was available for testing. The median age was 61 years old (Q1–Q3, 53–69) (Table 1). Most patients were males (79%) and smokers (96%). There were 15 patients presenting with Stage I (20%), 33 with Stage II (44%), 23 with Stage III (31%), and four with Stage IV (5%) (adrenal gland: *n=1*; brain: *n=2*; pleural nodule: *n=1*). Pleomorphic carcinoma (79%) was the most frequent subtype found, followed by carcinosarcoma (12%). Blood-vessel invasion was found in 68 (91%) of the tumors. The molecular biomarker analysis results were published, revealing KRAS mutations in 23 (31%) tumors, PIK3CA in six (8%), and EGFR in one (1%).

Intratumoral PD-L1 expression

Membranous PD-L1 staining was observed in tumor cells in 40 (53%) cases (Table 1). Distribution of PD-L1 expression in tumor cells with a 5 % of increments is given in Supplementary Figure 2. In 27% of cases (*n*=20/75), PD-L1 expression was observed in both the epithelial and sarcomatous areas, in 13% only in the sarcomatous areas (*n*=10/75), and in 13% only (*n*=10/75), in the epithelial areas. PD-L1 expression did not differ between the epithelial and sarcomatoid components (p=1.0, Fisher's exact test). PD-L1 expression was higher in SC than in NSCLC in which PD-L1 was expressed in in 11 (20%) cases (p<0.0001) (5/20 adenocarcinomas (25%), 3/19 squamous carcinomas (16%), large cell carcinomas 3/15 (20%) (Figure 1, Supplementary Table 2). PD-L1 could also be expressed by immune cells as shown in Supplementary Figure 2.

Intratumoral immune-cell infiltration

Intratumoral immune-cell infiltration comprised a large infiltration of CD3+ T-cells (tumoral surface median: 23%; (Q1-Q3)

: [17–30]) including CD8+ T-cells (9%; (Q1-Q3): [7–12]), and CD4+ T-cells (10%; [5–14]). Large amounts of CD163+ macrophages (23%; [17–30]) were observed, though these were still fewer than the number of CD20+B-cells (3.5%; [1–8]) and MPO+ neutrophils (5.5%; [2–11]) found (Supplementary table 4).

Infiltration by T CD3+ cells and CD163+ macrophages were higher in SC than in NSCLC (p=0.011 and p=0.002, respectively) while less TCD8+ and B CD20+ cells were observed (p=0.0002 and p=0.03, respectively). Infiltration by TCD4+ T-cells and neutrophils did not differ between SC and NSCLC (Table 2).

Factors associated with PD-L1 expression

On univariate analysis, neither clinical factors, such as age, gender or tobacco status, nor disease stage, *i.e.,* tumor, node, and metastasis (TNM) classification, tumor diameter or presence of lymph-node metastasis, were associated with PD-L1 expression (Table 1). Blood-vessel invasion and TTF1 positivity were significantly associated with PD-L1 expression (Table 1). Of all the molecular abnormalities tested, KRAS mutations were the only ones associated with PD-L1 expression. Moreover, PD-L1+ tumors exhibited significantly-higher infiltration of T CD8+ cells and CD163+ macrophages compared to PD-L1- tumors, (10% *vs.* 8%, p=0.001 and 27% *vs.* 20% p=0.002, respectively) (Supplementary Table 4, Figure 2).

In the final logistic regression model, blood-vessel invasion and CD163+ cell levels were significant independent factors associated with PD-L1 expression (odds ratio [OR]: 17.05 [1.02-285.74], p=0.049 and 1.13 [1.05–1.22], p=0.001*,* respectively). There was a trend observed towards PD-L1 positivity with the presence of KRAS mutations (OR: 3.3 [0.98–11.04], p=0.053) (Table 3).

Survival analyses

In univariate analyses, pathological stage with T and N status, parietal pleural invasion were associated with poorer overall survival (OS) and T CD3+ and T CD4+ cell infiltration with better OS (Table 4). In multivariate analyses, T and N status, parietal pleural invasion were

associated with poorer OS and T CD4+ cell infiltration with better OS (Table 4). PD-L1 expression did not influence overall survival OS (Figure 3, Table 4). Interestingly, PD-L1 expression distinguishes subpopulations within tumor-infiltrating lymphocytes (CD3+ or CD4+) with different prognosis

Discussion

SC are associated with poor prognosis and high levels of chemoresistance[3], underlining the necessity of finding new potential therapy methods. Therapies targeting immune molecule checkpoints could be particularly interesting in this indication. In our study, we observed PD-L1 expression in 53% of the 75 SC patients that was higher than the 20% observed in the NSCLC patients. SC were also characterized by a strong CD3 T cell and macrophage infiltration compared to NSCLC. Blood-vessel invasion, KRAS mutations, and tumor CD163+ macrophage infiltration were all independently associated with PD-L1 expression. Lastly, tumor-infiltrating CD3+ and CD4 +lymphocytes proved to be a factor of good prognosis.

We found that 53% of patients with SC and 20% of patients with NSCLC exhibited positive PD-L1 expression, which is in the range of the usually reported values for NSCLC (20 to 60%). The large variety in PD-L1 positivity could be related to the heterogeneity of technical requirements for measuring PD-L1 expression. Firstly, the inter-study variations could be related to the different thresholds of positivity used in each, as an optimal threshold has yet to be defined. In line with other studies, we chose a threshold of 5%, while some have opted for 1% [12] or 10% or higher [13–17]. Secondly, inter-study variations could be related to the primary antibody used. We used clone 5H1, which was used in nivolumab Phase I trials[18]. However, for the nivolumab Phase III trial, clone 28–8 (Epitomics, Burlingame, CA, USA) was used[19]. The results of PD-L1 expression measured by either E1L3N clone®(Cell signaling, Saint Quentin, France) or clone 5H1 did not correlate[20]. Stromal PD-L1 expression, measured with clone SP142® (Spring Bioscience, Pleasanton, CA, USA), was predictive of response to anti-PD-L1 therapy in the MPDL3280 Phase I clinical trial [21]. Clone 22C3 (Merck) was used for the pembrolizumab Phase I study, demonstrating that high levels of tumoral PD-L1 expression (>50%) were associated with better response to anti-PD-L1 therapy[22].

PD-L1 expression has been reported to be heterogeneous within a tumor [23,24]. We cannot exclude that the tissue microarray analysis may favor false negativity [24]. However,

in light of this, we chose to conduct a tissue microarray analysis with the aims of collecting a large sample of all possible tumoral morphological features. For each tumor, a median of 26 blocks were reviewed and a median of four cores were tested. Expression levels did not differ between the epithelial and sarcomatoid components which is different from the Korean cohort[11]. However, PD-L1 expression was also really higher in this study, with 90% of positivity in these patients. Several reasons could explain this discrepancy such as the different primary antibody used, the ethnicity of the patients and perhaps the histological subtypes of SC. Our cohort comprised more carcinosarcomas- in which PD-L1 expression was weak (Table 1)- than the Korean study.

Tumor PD-L1 expression was associated with KRAS mutations, probably due to tobacco use. Interestingly, in the pembrolizumab Phase I trial, treatment with the anti-PD-L1 antibody induced higher tumor response rates in the KRAS-mutated cases[22].

Tumor PD-L1 expression was also associated with blood-vessel invasion. As reported by other studies[1,25], we observed a high prevalence of blood-vessel invasion in these tumors, which could be related to hypoxia[26,27]. Interestingly, PD-L1 expression has been shown to be induced by HIF-1[28].

Lastly, we demonstrated that PD-L1 was associated with CD163+ macrophages. Macrophages can express PD-L1 [21], as well as other immune infiltrating cells (Supplementary Figure 3) . We cannot exclude the possibility that this positive association was not due to PD-L1 expression in macrophages.

We found there to be high immune-cell infiltration in these tumors, especially concerning tumor-infiltrating lymphocytes CD3+, at a median of 23% of the tumoral surface. High levels of tumor-infiltrating lymphocytes CD3+ and CD4+ constituted a factor of good prognosis. Interestingly, PD-L1 for tumor-infiltrating lymphocytes CD3+ or CD4+, distinguishes subpopulations with different prognosis. This could suggest that PDL1 expression is associated with the exhaustion of lymphocytes and that PD-L1 activation is an adaptive method of tumor resistance to cytokine-producing tumor-infiltrating lymphocytes rather than an aberrant cell signaling, for example, via PTEN loss or via activation of the MEK/ERK signaling pathways [29] The presence of tumor-infiltrating lymphocytes, as a marker of a

preexisting immune response, associated with positive PD-L1 expression, is currently the best-known predictive factor of efficacy of these therapies [21].

These results provide real justification for the use of PD-L1 or PD-1 immunotherapy in this histological subtype[18,21].

Conflict of Interest statement

None declared

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Figure captions

Figure 1: PD-L1 expression in patients with NSCLC and SC.

For each tumor, PD-L1 expression is represented by a white (NSCLC) or a black circle (SC). P-value by Mann Withney test.

Figure 2: Intratumoral immune cell characterization.

Representative pictures of PD-L1 expression according to immune-cell infiltration (CD3 and CD163). **A:** no PD-L1 expression and almost no infiltration by CD3+ and CD163+ cells (x400 magnification). **B:** intermediate levels of PD-L1 expression with intermediate infiltration by CD3+ and CD163+ cells (x400 magnification). **C:** high levels of PD-L1 expression with high infiltration by CD3 and CD163+ cells (x400 magnification).

Figure 3: Survival analyses.

Overall survival analyses adjusted for PD-L1 positivity **(A)**; CD3 **(B)**; and CD4 **(C)** both PD-L1 and CD3 **(D) and** both PD-L1 and CD4 **(E)**. The Kaplan-Meyer method was used for survival analysis.

Tables

Table 1: Factors associated with PD-L1 expression in univariate analyses in patients with SC (*n*=75)

Factors	**Overall *n*=75 *n* (%)**	**PDL1 (-) *n*=35 *n* (%)**	**PDL1 (+) *n*=40 *n* (%)**	***Two-tailed p-value****
Age				0.647
≤61years	39 (52)	17 (44)	22 (56)	
>61years	36 (48)	18 (50)	18 (50)	
Gender				0.412
Women	16 (21)	9(56)	7 (44)	
Men	59 (79)	26 (44)	33 (56)	
Tobacco use				1
Never	3 (4)	1(33)	2 (67)	
Current/former	72 (96)	34 (47)	38 (53)	
pT				0.362
1	7 (9)	5 (71)	2 (29)	
2	31 (41)	15 (48)	16 (52)	
3	32 (43)	12 (38)	20 (63)	
4	5 (7)	3 (60)	2 (40)	
pN				0.359
N0	39 (52)	16 (41)	23 (59)	
N+	36 (48)	19 (53)	17(47)	
Stage				0.847
I	15 (20)	7 (47)	8 (53)	
II	33 (44)	16 (48)	17 (52)	
III	23 (31)	11 (48)	12 (52)	
IV	4 (5)	1 (25)	3 (75)	
Visceral-pleura invasion				0.169
Negative	34 (45)	19 (56)	15 (44)	
Positive	41 (55)	16 (39)	25 (61)	
Parietal-pleura invasion				0.057
Negative	57 (76)	30 (53)	27 (47)	
Positive	18 (24)	5 (28)	13 (72)	
Blood-vessel invasion				**0.045**
Negative	7 (9)	6 (86)	1 (14)	
Positive	68 (91)	29 (43)	39 (57)	
Histological subtype				**0.01**
Carcinosarcoma	9 (12)	8 (89)	1 (11)	
Other subtypes	66 (88)	27 (41)	39 (59)	
▪ Pleomorphic carcinoma	59 (79)	25 (42)	34 (58)	
▪ Giant-cell carcinoma	4 (5)	2 (50)	2 (50)	
▪ Spindle-cell carcinoma	1 (1)	-	1 (100)	
▪ Spindle-cell carcinoma and giant-cell carcinoma	2 (3)	-	2 (100)	
TTF1 IHC				**0.02**
Negative	40 (53)	24(60)	16 (40)	
Positive	35 (47)	11(31)	24 (69)	
P63 IHC				**0.0003**
Negative	54 (72)	18(33)	36 (67)	
Positive	21 (28)	17 (81)	4 (19)	

KRAS mutations				**0.016**
Wild type	52 (69)	29 (56)	23 (44)	
Mutated	23 (31)	6 (26)	17 (74)	
EGFR mutations				1.0
Wild type	71 (99)	35 (47)	39 (53	
Mutated	1 (1)	-	1 (100)	
PIK3CA mutations				1.0
Wild type	69 (92)	32 (46)	37 (54)	
Mutated	6 (8)	3 (50)	3 (50)	

*Fischer's exact or chi-squared test

pT: pathological T stage; pN: pathological lymph node metastasis; TTF1 IHC: thyroid transcription factor-1 immunohistochemistry; KRAS: Kirsten Ras; EGFR: epidermal growth factor receptor; PIK3CA: phosphatidylinositol-4, 5-bisphosphate 3-kinase, catalytic subunit alpha

Table 2: Intratumoral immune-cell characterization in patients with SC and NSCLC.

Factors	**SC** ***n=75*** ***Median (%) [IQR]***	**NSCLC** ***n=54*** ***Median (%) [IQR]***	***Two-tailed p-value****
% T CD3+ cells	23 [17–30]	17 [7–27]	0.011
% T CD8+ cells	9 [7–12]	13 [8–20]	0.0002
% T CD4+ cells	10 [5–14]	11 [7–17]	0.07
% B CD20+ cells	4 [1–8]	6 [3–12]	0.032
% Macrophage CD163+ cells	23 [17–30]	20 [13–23]	0.002
% Neutrophil MPO+ cells	6 [2–11]	7 [3–12]	0.99

*Mann Whitney test

IQR: interquartile range; MPO: myeloperoxidase

Table 3: Factors associated with PDL1 expression in patients with SC on multivariate analysis (*n=75*).

Factors	OR (95% CI)	*Two tailed p-value**
Parietal-pleura invasion (+ *vs.* -)	-	>0.1
Blood-vessel invasion (+ *vs.* -)	17.05 [1.017-285.74]	0.049
Carcinosarcoma (no *vs.* yes)	-	>0.1
TTF1 (no *vs.* yes)	-	>0.1
KRAS mutations (mutated *vs.* wild type)	3.3 [0.98-11.04]	0.053
% CD8+ cells	-	>0.1
% CD 163+ cells	1.13 [1.05–1.22]	0.001

*logistic regression with backward elimination

OR: odds ratio; CI: confidence interval; TTF1: thyroid transcription factor-1; KRAS: Kirsten ras

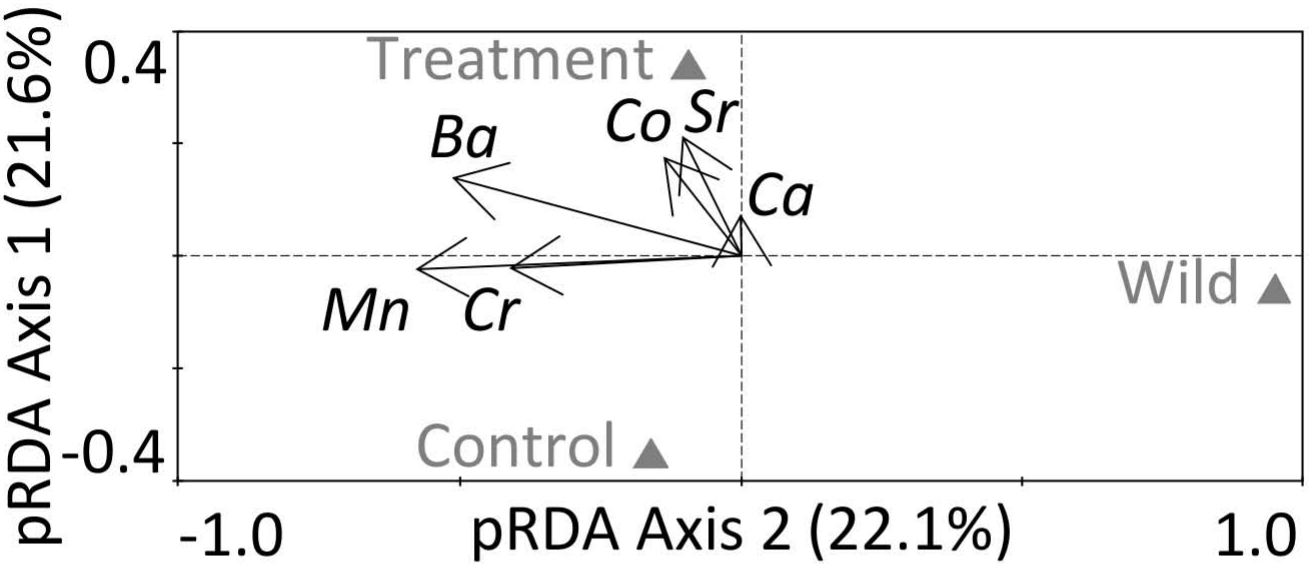
Table 4: Univariate and multivariate analyses of overall survival†

Factors	Univariate analysis		Multivariate analysis	
	HR (CI 95%)	*p**	HR (CI 95%)	*p**
Clinical				
Age (<61 vs. ≥61 years)	0.81 (0.46-1.45)	0.486		
Gender (female vs. male)	1.48 (0.75-2.92)	0.26		
Tobacco status (smoker/former vs. never)	0.88 (0.21-3.67)	0.865		
Pathological				
Pleomorphic carcinomas vs. other subtypes	0.96 (0.20-4.7)	0.961		
Stage (I vs. stage II–III)	5 (2.14-11.7)	**<0.0001**		
Diameter (<5 vs. ≥5 cm)	2.29 (1.26-4.1)	**0.006**	1.89 (0.99-3.62)	**0.055**
Lymph-node metastasis (N0 vs. N+)	2.20 (1.17-4.023)	**0.013**	2.63 (1.35.-5.12)	**0.004**
Blood vessel invasion (no vs. yes)	1.94 (0.69-5.45)	0.211		
Lymphatic emboli (no vs. yes)	0.92 (0.82-1.03)	0.155		
Vascular emboli (no vs. yes)	0.944 (0.85-1.05)	0.272		
Visceral pleural invasion (no vs. yes)	1.67 (0.93-3.01)	0.088		
Parietal pleural invasion (no vs. yes)	2.42 (1.278-4.60)	**0.007**	2.75 (1.31-5.78)	**0.007**
PD-L1 positive vs. negative	1.07 (0.60-2.00)	0.823		
CD3 (>20% vs ≤20%)	0.38 (0.21-0.69)	**<0.0001**		NS
CD8 (>9% vs ≤9%)	0.66 (0.35-1.21)	0.132		NS
CD4 (>10% vs ≤10%)	0.34 (0.17-0.69)	**0.003**	0.34 (0.16-0.72)	**0.005**
CD20 (>4% vs ≤4%)	0.535 (0.284-1.005)	0.052		
CD163 (>23% vs ≤23%)	0.60 (0.33-1.10)	0.099		
MPO (>5% vs ≤5%)	1.01 (0.57-1.79)	0.985		

NS: not significant.

† Analysis of 64 patients, 4 missing data, and 7 patients died in the 30 days after surgery were censored. HR: hazard ratio. An HR >1 indicates a longer OS.

* Cox's model; independent variables with p <0.1 in univariate analyses were included in the multivariate analysis; NS variables were retrieved by a step-by-step backward procedure.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-0.4
pRDA Axis 1 (21.6%)
Treatment
Ba
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pRDA Axis 2 (22.1%)
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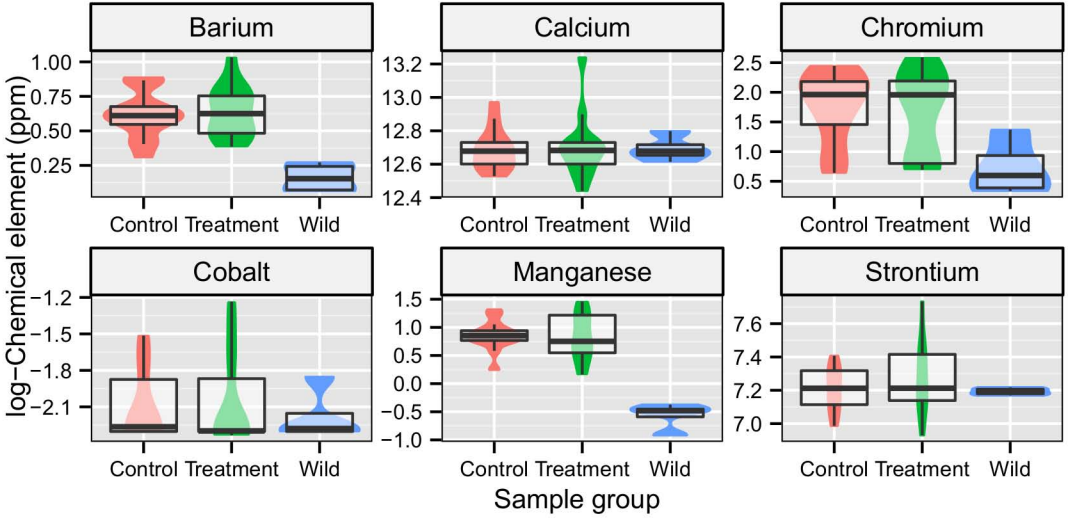
Barium
Calcium
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0.75
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−1.5
−1.8
−2.1
1.5
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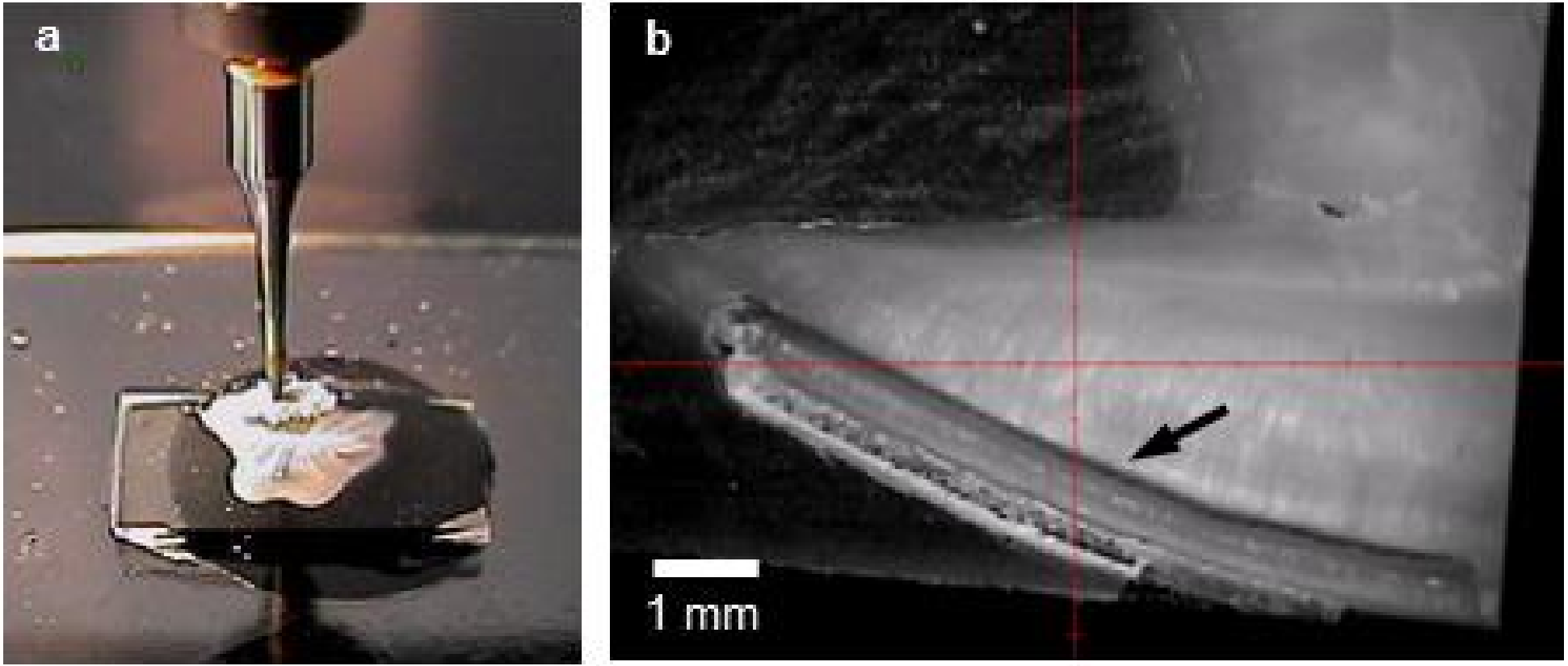
Control
Treatment
Wild
Sample group


a
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