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Case Report

Histological sarcomatoid transformation in a lung adenocarcinoma patient following immune checkpoint blockade

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***Abstract*:** Histological transformation is a phenomenon that is well described as one of the causes of tyrosine kinase inhibitor resistance in oncogene-driven non-small-cell lung cancer (NSCLC). The use of immune checkpoint inhibitors (ICIs) as a potential mechanism of acquired resistance to immunotherapy in NSCLC to small-cell lung cancer was also recently found. Here, we report the histological transformation of sarcomatoid carcinoma and metastasis in a lung adenocarcinoma patient without targetable genetic alterations who experienced long-term disease remission after nivolumab therapy. The patient subsequently developed rapid progression in the mediastinal and retroperitoneal lymph nodes, bones, and small intestine. Surgical resection of the small intestine lesion due to acute small intestine bleeding revealed the transformation of NSCLC to sarcomatoid carcinoma. The patient died 3 months after sarcomatoid carcinoma transformation and extensive disease progression, although he was rechallenged with immunotherapy. Genomic and immunohistochemical analyses revealed a comparable abundance of gene mutations and a limited number of immune cells in the tumor microenvironment, with low infiltration of $CD8^+$ T cells, $CD4^+$ T cells, regulatory T cells, and PD-$L1^+$ macrophages in metastatic tumors, revealing a noninflamed immune microenvironment for ICI-resistant tumors.

Keywords: acquired resistance, histological transformation, immune checkpoint inhibitor, non-small-cell lung cancer, programmed cell death protein-1, sarcomatoid carcinoma

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Background

Immunotherapy with immune checkpoint inhibitors (ICIs), including cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and programmed cell death protein-1 (PD-1)/programmed cell death ligand-1 (PD-L1) inhibitors, has been demonstrated to improve outcomes as a promising strategy for multiple cancer types, including non-small-cell lung cancer (NSCLC).[1] Several PD-1/PD-L1 inhibitors, such as pembrolizumab, atezolizumab, and nivolumab, have been approved for the treatment of advanced or metastatic NSCLC without epidermal growth factor receptor (EGFR) or anaplastic lymphoma kinase (ALK) alterations in first-line or late-line settings.[2] A subgroup of patients can benefit from ICI treatment and have a durable response for 1 year or even longer. Nevertheless, resistance to immunotherapy inevitably occurs. The mechanisms of primary or acquired resistance to immunotherapy with ICIs have yet to be fully elucidated.[3] Previous reports have shown that histological transformation is a well-described phenomenon and is one of the causes of tyrosine kinase inhibitor (TKI) resistance in NSCLC with EGFR- or ALK-rearranged adenocarcinoma.[4–6] The potential mechanism of acquired resistance to immunotherapy in NSCLC to small-cell lung cancer (SCLC) patients treated with ICIs has also been reported recently.[7–9] However, there have been no reports of histological transformation to pulmonary sarcomatoid carcinoma in NSCLC patients after immunotherapy.

Here, we report the histological transformation to sarcomatoid carcinoma and disease progression in one lung adenocarcinoma patient who experienced long-term disease remission after nivolumab treatment [Figure 1(a)]. The histological, genomic, and immune features of these patients were comprehensively analyzed *via* immunohistochemistry (IHC), next-generation sequencing, and multiplex immunofluorescence staining to explore the possible mechanism underlying acquired resistance to PD-1 inhibitors.

Materials and methods

For next-generation sequencing analysis, formalin-fixed paraffin-embedded (FFPE) tumor tissue was extracted using the GeneRead DNA FFPE Kit (Qiagen, 180134, Hilden, Germany). All sample capture libraries were prepared using YuceOne Plus v1.0 (YuceBio, Shenzhen, China) for panel sequencing.

IHC staining was performed according to our previously published procedures.[10] The tumor specimens were reacted overnight at 4°C with primary mouse anti-human monoclonal CD8 and CD4 antibodies (1:200 solution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and with the PD-L1 antibody (22C3 pharmDx assay; Agilent, Carpinteria, CA, USA). After incubating with a biotin-conjugated secondary antibody for 30 min at room temperature, the sections were further treated with an avidin–biotin–peroxidase complex system (RTU VECTASTAIN Kit;

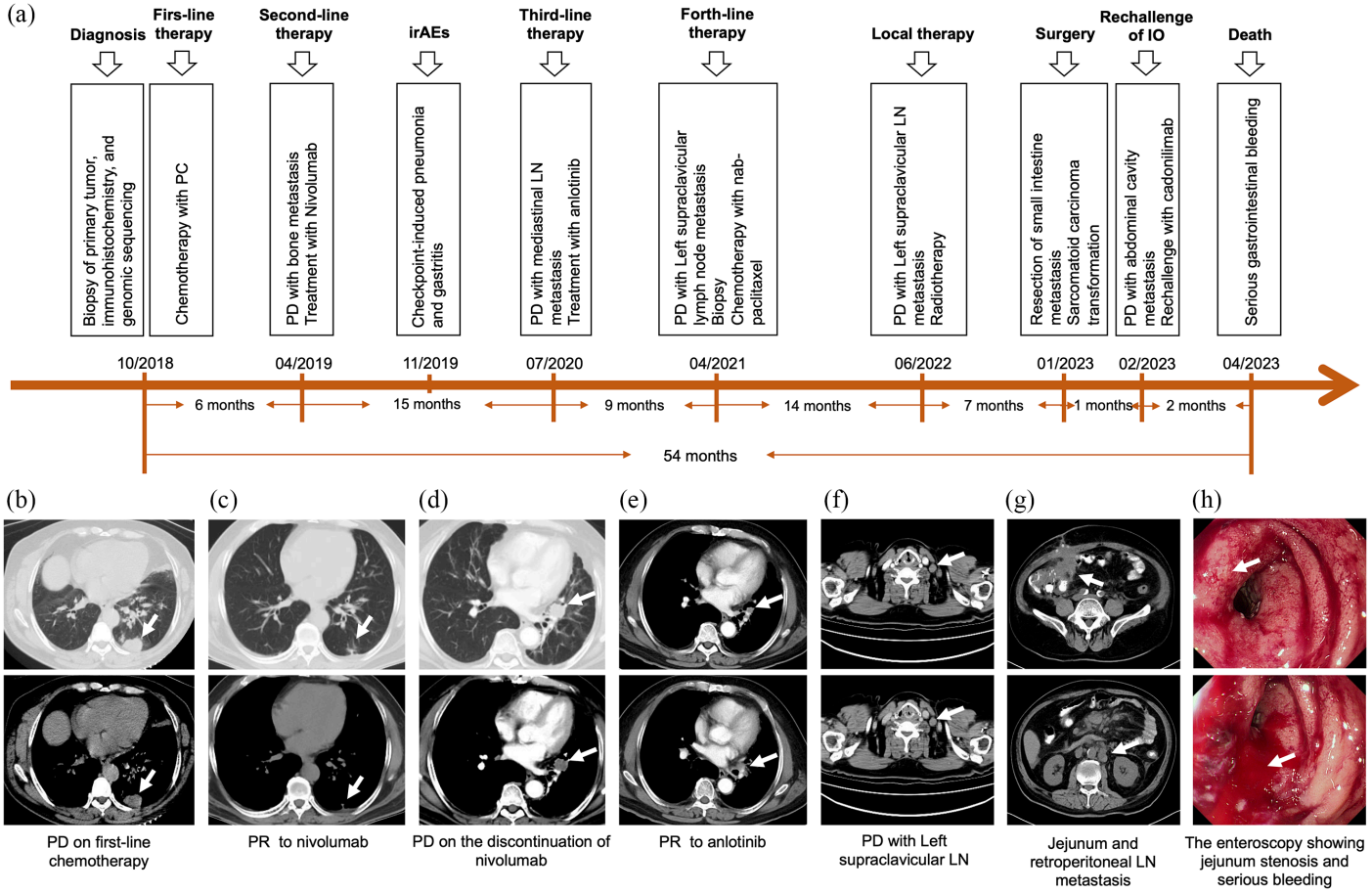

Figure 1. Timeline of anticancer treatment and radiographical evaluations. (a) The timeline of diagnosis and anticancer treatment. (b) PR after first-line treatment with pemetrexed and carboplatin. (c) PR to second-line treatment with nivolumab monotherapy. (d) Mediastinal lymph node metastasis after the discontinuation of immunotherapy due to immune-related pneumonia and gastritis. (e) PR to third-line treatment with anlotinib. (f) Progressed lesions in the left supraclavicular lymph nodes with biopsy-confirmed infiltrating adenocarcinoma. (g) Extensive tumor metastasis in the small intestine and retroperitoneal lymph nodes. (h) Enteroscopy shows jejunum stenosis and severe bleeding in the patient's intestinal tract. The white arrow indicates the specific masses.
irAEs, immune-related adverse effects; LN, lymph node; PC, pemetrexed and carboplatin; PD, progressive disease; PR, partial response.

Vector Laboratories, Burlingame, CA, USA). Finally, the signal was developed with 3,3′-diaminobenzidine tetrahydrochloride (1:50 solution; DAB Substrate Kit; Abcam, Cambridge, MA, USA). All sections were then counterstained with hematoxylin and mounted.

For multiplex immunofluorescence staining, FFPE blocks were first heated at 65°C for 3 h according to the previous procedures.[10] The slides were then dewaxed, rehydrated, and fixed using a Leica BOND RX Auto Stainer (Leica Biosystems, YTAn00042, Buffalo Grove, IL) Subsequently, the slides were stained with six antibodies: anti-CD4 (ab133616; Abcam), anti-CD8 (MA1-80231; Thermo Fisher Scientific), anti-CD68 (MA5-12407; Thermo Fisher Scientific), anti-FoxP3 (ab215206; Abcam), anti-PD-L1 (13684S; Cell Signaling Technology), and anti-pan keratin (sc-81714; Santa Cruz Biotechnology), followed by incubation with horseradish peroxidase-conjugated secondary antibody and tyramide signal amplification. Finally, the slides were stained with 4′-6′-diamidino-2-phenylindole for 10 min at a 9:125 dilution. The slides were air-dried and photographed with an Akoya Vectra Polaris (YTAn00041). Images were analyzed using Indica Halo software. The results of this study conform to the CARE statement (Supplemental Material).[11]

Case presentation

An adult patient with a history of tobacco use for 30 pack-years was found to have a left lower lobe-occupying lesion of the lung by computed thermotropy (CT) scan during his routine physical examination in October 2018. Enhanced CT of the chest and abdomen showed a lesion located in the left lower lobe of the lung, measuring up to 3 cm with left hilar and mediastinal lymph node metastasis. Pulmonary biopsy revealed a diagnosis of typical infiltrating adenocarcinoma in the lower lobe of the left lung [Figure 2(a) and (b)]. The final clinical stage was stage IIIB. Initial genomic analysis by next-generation sequencing showed no somatic mutations in EGFR, ALK, kirsten rat sarcoma (KRAS), or ROS proto-oncogene 1 (ROS1).

The patient was partially responsive to pemetrexed and carboplatin [Figure 1(b)]. Unfortunately, 6 months later, the patient presented with disease progression accompanied by enlargement of the primary pulmonary lesion and mediastinal lymph nodes and diffuse spinal metastasis involving the T11, L2, and L5 vertebral bodies. In April 2019, this patient was switched to second-line monotherapy with nivolumab (200 mg q2 W) in combination with spinal stereotactic radiotherapy when additional IHC confirmed that 30% of the cancer cells were positive for PD-L1. The patient responded well to immunotherapy but developed immune-related pneumonia and gastritis after 18 cycles of nivolumab [Figure 1(c)]. Immunotherapy was discontinued, and anlotinib was started when the disease progressed with mediastinal lymph node metastasis [Figure 1(d) and (e)]. In July 2020, the patient had new progressive lesions in the left supraclavicular lymph nodes and received chemotherapy with nab-paclitaxel and local radiotherapy [Figure 1(f)]. The tumor responded well to these treatments until January 2023, when the patient complained of bloody stools and progressive abdominal pain. A CT scan indicated extensive tumor metastasis in the mediastinal lymph nodes, retroperitoneal lymph nodes, bones, and small intestine [Figure 1(g)]. Enteroscopy revealed jejunum stenosis and severe bleeding in the intestinal tract [Figure 1(h)]. The patient underwent surgical resection of the small intestine lesion, the bleeding was relieved, and pathology confirmed sarcomatoid carcinoma [Figure 2(a)] considering the following histologic features: more undifferentiated, giant cells, loose cellular cohesion, and strong and diffuse vimentin expression. Unfortunately, the patient experienced disease progression with abdominal cavity metastasis after surgery and was rechallenged with cadonilimab (6 mg/kg q2 W), a PD-1/CTLA-4 bispecific antibody. After two cycles of cadonilimab, his abdominal distension and pain were not relieved, and the patient developed progressive gastrointestinal bleeding. Subsequent CT indicated extensive cancer spread in the abdominal cavity. The patient died in April 2023, and the overall survival time was 4.5 years.

We explored the potential mechanism of this patient's rapid progression by analyzing progressive tumors resected surgically. Immunohistochemical staining revealed that the intestinal lesion was CK7 and TTF-1 negative and vimentin positive, and the primary lung tumor was CK7 and TTF-1 positive and vimentin positive, which suggested the transformation of NSCLC to pulmonary sarcomatoid carcinoma. Ki-67, a tumor cell proliferation and growth marker, increased from 30% to 75% after transformation [Figure 2(a)]. We also analyzed the genomic features of

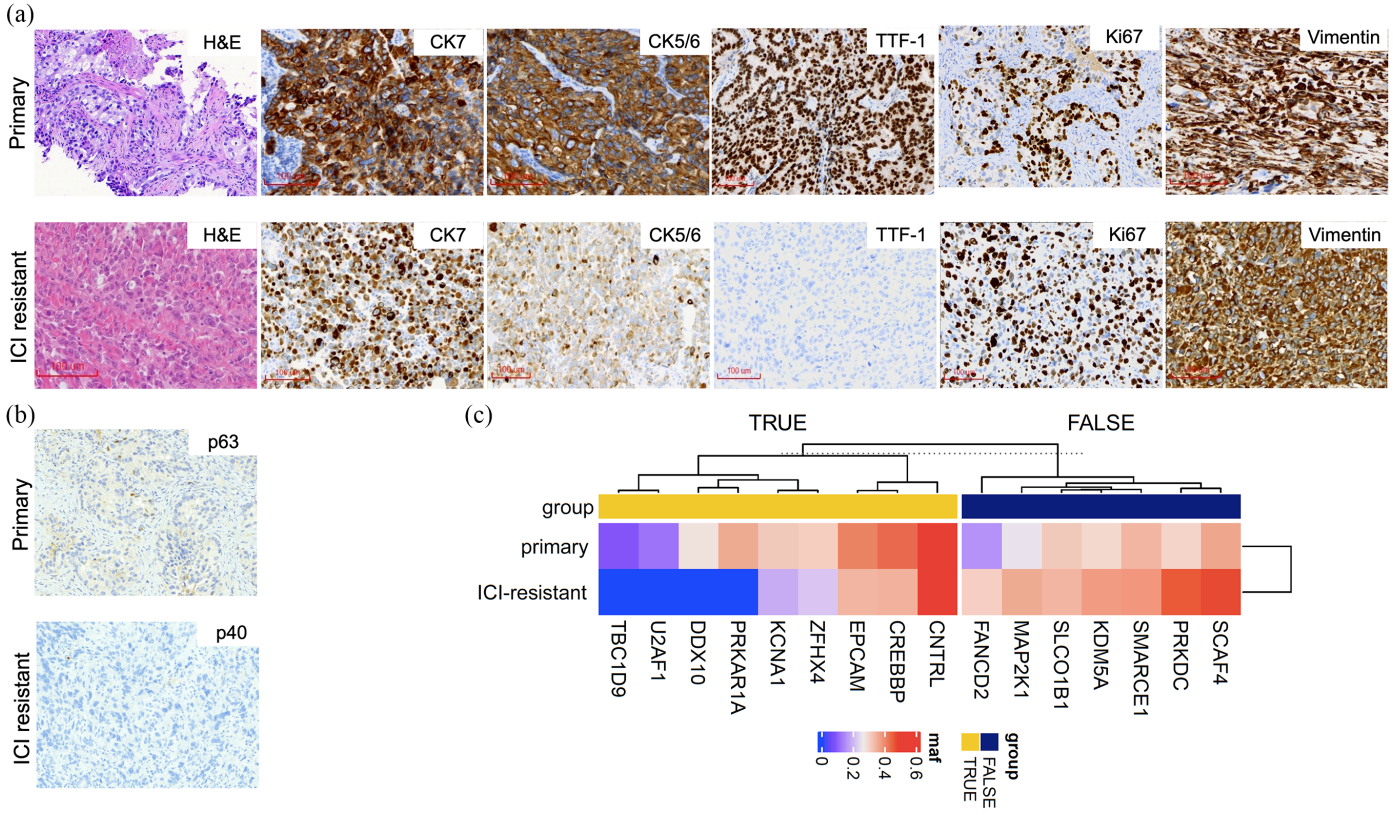

Figure 2. Histopathological analysis and next-generation sequencing results for tumors before and after nivolumab treatment. (a) Representative images of primary pulmonary and ICI-resistant metastatic intestinal tumor tissues stained with hematoxylin plus eosin (H&E), NSCLC markers, including cytokeratin (CK), CK5/6, and thyroid transcription factor-1 (TTF-1); the cell proliferation marker ki67; and the sarcomatoid carcinoma marker vimentin. (b) Representative images of p63 expression in primary pulmonary tumor tissues and p40 expression in resistant intestinal tumor tissues. (c) Comparison of the abundance of gene mutations between primary pulmonary and ICI-resistant metastatic intestinal tumor tissues. The mutation abundance was calculated from alternating allelic observations divided by the read depth at each position. The figure shows the clustering analysis of the data obtained from the exonic regions of the genome using the 'Euclidean' distance metric and the 'complete' clustering method for rows. The color scheme used in the figure is based on the 'maf' of the data.
ICI, immune checkpoint inhibitors; maf, minor allele frequency; NSCLC, non-small-cell lung cancer.

both primary lung tumors and metastatic intestinal tumors by next-generation sequencing using YuceOne Plus v1.0 (YuceBio).

The gene profiling results revealed that a total of 16 genes were mutated in primary and ICI-resistant tumors [Figure 2(c)]. The calculated tumor mutational burden (TMB) was 9.58 mut/Mb. The tumor was TMB-H according to precious findings showing that the optimal cutoff of TMB was 7 mut/Mb from panel sequencing in East Asian populations and the survival period of patients with TMB $\geqslant$7 mut/Mb was longer than those with TMB $<$7 mut/Mb.[12] The mutation abundance was calculated from alternating allelic observations divided by the read depth at each position.[11] The abundance of gene mutations in metastatic tumors was comparable to that in primary tumors. In metastatic ICI-resistant tumors, alterations in human leukocyte antigen (HLA) genes, *JAK1*, *JAK2*, *B2M*, *PTEN*, or *TAP1*, which are associated with acquired resistance to immunotherapy, were not observed. Furthermore, immunohistochemical staining revealed that the proportion of $CD8^+$ T cells, but not $PD\text{-}L1^+$ cells, was greater in the primary tumor than in the metastatic tumor [Figure 3(a) and (b)]. Multiplex immunofluorescence staining also revealed that a limited number of immune cells were present in the tumor microenvironment, with low infiltration of $CD8^+$ T cells, $CD4^+$ T cells, and $PD\text{-}L1^+$ macrophages, and absence of regulatory T cells (Tregs) in metastatic tumors, revealing a noninflamed immune microenvironment for ICI-resistant tumors [Figure 3(c)].

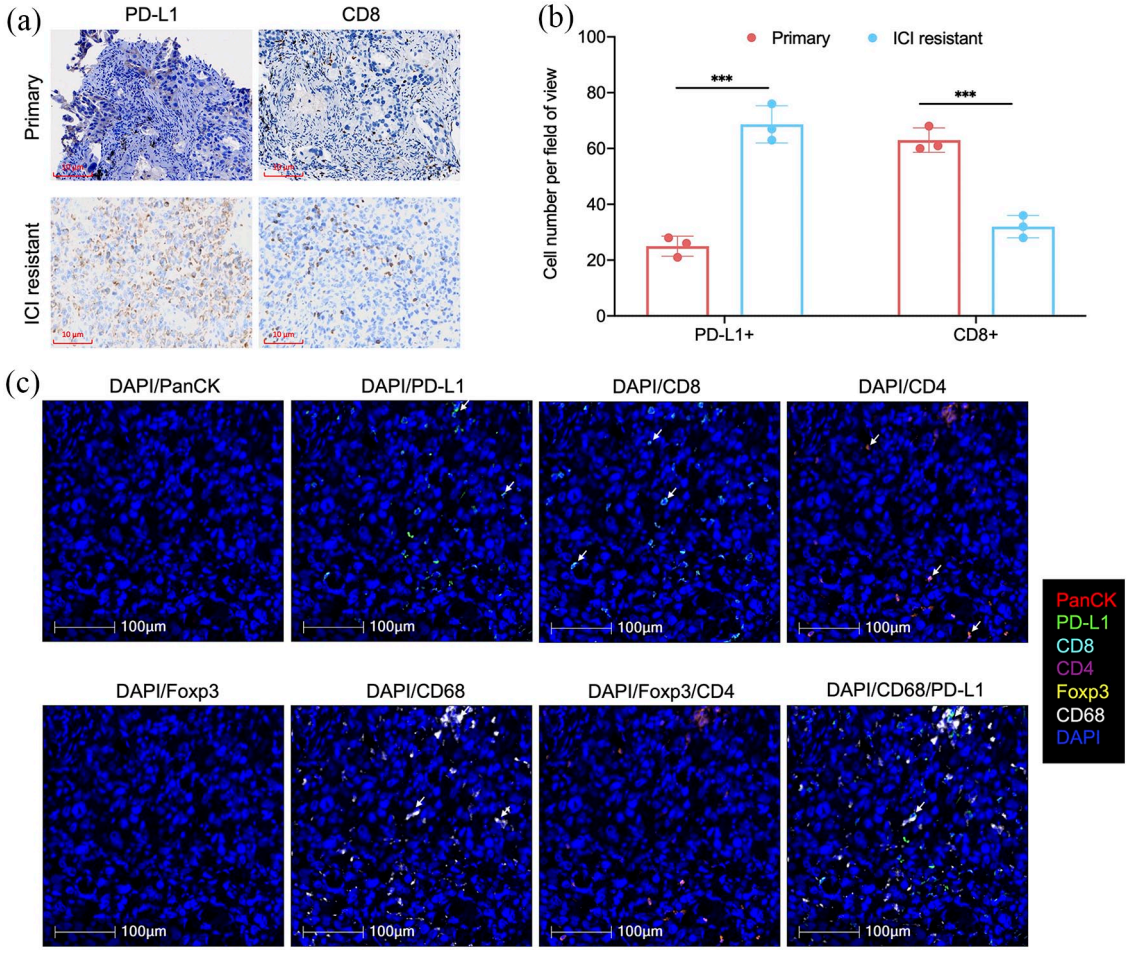

Figure 3. Immune-related microenvironment analysis of primary pulmonary and ICI-resistant metastatic intestinal tumor tissues. (a) PD-L1 expression and $CD8^+$ cell infiltration were determined by immunohistochemical staining in primary pulmonary and ICI-resistant metastatic intestinal tumor tissues. (b) Quantitative analysis of $PD\text{-}L1^+$ and $CD8^+$ cells. (c) Multiplex immunofluorescence staining showing the infiltration of $PD\text{-}L1^+$ cells, Tregs, $CD8^+$ T cells, $CD4^+$ T cells, and $PD\text{-}L1^+$ macrophages in ICI-resistant metastatic intestinal tumor tissues. The staining intensity of the samples was analyzed *via* a tissue cytometry analysis system. For each group, three regions of interest were chosen for analysis, and the number of targeted cells was calculated. The significance of the difference in the means between two groups of compared cells was analyzed by a two-tailed Student's *t*-test. The white arrow indicates positive cells with specific markers.
***$p < 0.001$.
ICI, immune checkpoint inhibitor; PD-L1, programmed cell death ligand-1; Tregs, regulatory T cells.

Discussion

This is the first reported case of an NSCLC patient who developed histological transformation to sarcomatoid carcinoma and acquired resistance to immunotherapy after he experienced long-term disease remission after nivolumab therapy. Although the use of ICIs as a potential mechanism of acquired resistance to immunotherapy in NSCLC to SCLC has also recently been found, the mechanism of histological transformation of NSCLC and ICI resistance is not clear.[7–9,13,14] Here, the interval from the initial diagnosis of lung adenocarcinoma to pathologically confirmed sarcomatoid transformation for immunotherapy in this patient was 51 months, whereas the median interval for TKI treatment was 31.5 months.[15] A poor prognosis is noted for NSCLC patients who develop sarcomatoid transformation. The median survival after sarcomatoid transformation was only 2.5 months for patients receiving TKI treatment and 3 months for our patients receiving ICI treatment.[15] Before TKI therapy and sarcomatoid transformation, EGFR-positive patients exhibited typical adenocarcinoma features, negative or weak

focal vimentin staining, and strong E-cadherin expression.[15] Our patient also demonstrated typical adenocarcinoma features but it had medium vimentin staining at the initial diagnosis. The typical characteristics of pulmonary sarcomatoid carcinoma according to IHC include a lack or absence of CK7 and TTF-1 and increased expression of vimentin. Previous studies have shown that when comparing immunohistochemical results before and after sarcomatoid transformation, the expression of TTF-1 decreases but vimentin expression is positive. When pulmonary sarcomatoid carcinoma (PSC) was diagnosed, the immunohistochemical examination suggested a weakly positive CK7 result, a negative TTF-1 result, and aberrant vimentin expression. All of the above results indicate the process of epithelial–mesenchymal transition.[15] In line with these results, our case showed that the metastatic intestinal tumor was weakly positive for CK5/6 and CK7, negative for TTF-1, and strongly positive for vimentin. Therefore, we believe that the dynamic changes in CK7 and vimentin expression may support the formation of lesions by metastatic tumor cells from primary lung tumors.

The gene profiling results showed that both primary and ICI-resistant tumors were tumor mutational burden-high and had a high number of gene alterations. Among these mutated genes, the MAP2K1 mutation was detected in both sets of gene sequencing results. MAP2K1 mutation is associated with a high objective response rate, long progression-free survival, and long overall survival in melanoma patients treated with immunotherapy,[16] which could explain why this patient responded well to initial nivolumab monotherapy. However, the overall abundance of gene mutations in metastatic tumors was similar to that in primary tumors. In metastatic tumors, alterations in the HLA gene, *JAK1*, *JAK2*, *B2M*, *PTEN*, or *TAP1* that are associated with acquired resistance to immunotherapy have not been observed.[17,18] A previous study indicated that mesenchymal-epithelial transition factor (MET) amplification or MET overexpression might be related to pathological sarcomatoid carcinoma transformation after TKI resistance in NSCLC patients with targetable genetic alterations but these findings were not present in our patient.[6,14] In particular, HLA class I antigen loss and a lack of β2-m expression are associated with decreased recognition of cancer cells by cognate $CD8^+$/granzyme B^+ T cells. HLA class I downregulation is widely recognized as a mechanism of tumor immune escape and has been associated with cancer immunotherapy resistance.[19,20] Here, we did not analyze HLA class I antigen or β2-m expression in cancer cells but at least no mutations in *B2M* genes that have been documented as a mechanism of resistance to PD-1 inhibitors were found. Therefore, we do not know whether HLA class I antigen and β2-m expression reflect a specific mechanism of immunotherapy resistance.

PD-L1 expression in tumor and immune cells is a useful biomarker for predicting the efficacy of PD-1/PD-L1 inhibitors in NSCLC patients.[21] This patient had a high-to-medium PD-L1 tumour proportion score (TPS) of 30% and showed a partial response to initial nivolumab therapy. However, many more metastatic tumor tissues than nonmetastatic tissues had PD-$L1^+$ cells and a high PD-L1 TPS (60%), which could confer acquired resistance to ICIs. A gene expression profile of patients with high PD-L1 NSCLC refractory to pembrolizumab showed that tumors with high PD-L1 expression exhibited activation of pathways associated with cancer stem cells (the Hedgehog, Notch, and Hippo pathways); loss of *PTEN* and *JAK2*; and inhibition of both the priming and effector phases of the immune response.[22] Although baseline vimentin expression is associated with ICI efficacy in NSCLC, the phosphorylation of vimentin activates the transforming growth factor beta β (TGF-β) signaling pathway and upregulates the expression of PD-L1 by recruiting p-Smad2/3 to the PD-L1 promoter, leading to accelerated metastasis and immune escape.[23,24] Overall, PD-L1 is considered a surrogate biomarker that can be used to predict survival in NSCLC patients who are more likely to benefit from anti-PD-1/PD-L1 immunotherapy but it does not have predictive value after sarcomatoid transformation.

Tumor-infiltrating immune cells, including $CD8^+$ and $CD4^+$ T cells, in the tumor microenvironment are associated with favorable prognosis and treatment response to ICIs.[25] Here we found that the percentage of infiltrating $CD8^+$ T cells was lower in metastatic ICI-resistant tumors than in primary tumors by IHC. Furthermore, there was a limited number of $CD8^+$ and $CD4^+$ T cells in metastatic tumors by multiplex immunofluorescence staining. We also found a limited number of infiltrated PD-$L1^+$ macrophages in metastatic tumors, which was in line with previous findings showing low PD-$L1^+$ macrophage infiltration may affect the efficacy of immunotherapy in NSCLC.[26] Interestingly, no Tregs

were observed in metastatic tumors. Basically, tumor-associated Tregs are associated with a suppressive tumor microenvironment.[27] Maybe other cell subtypes rather than Tregs contribute to ICI treatment resistance in transformed sarcomatoid carcinoma.

As a rare lung cancer subtype, primary pulmonary sarcomatoid carcinoma has a poor prognosis, and treatment options for advanced or metastatic disease are extremely limited.[28] Recently, a majority of primary pulmonary sarcomatoid carcinoma patients have been shown to have a high tumor mutational burden, high PD-L1 expression, excellent response rates, and prolonged overall survival when ICIs are used as second-line treatments or beyond.[29] Patients with primary pulmonary sarcomatoid carcinoma are characterized by an immune-inflamed phenotype, which explains the intrinsic reasons for the high efficacy of immunotherapy.[30] However, our patient exhibited high PD-L1 expression in tumor cells after transformation to sarcomatoid carcinoma and was resistant to rechallenge with immunotherapy. These results indicate that the immune features of primary pulmonary sarcomatoid carcinoma differ from those of secondary pulmonary sarcomatoid carcinoma due to histological transformation. Thus, unlike for primary sarcomatoid carcinoma, other alternative treatments for transformed sarcomatoid carcinoma should be established. Although our patient failed to respond to a PD-1/CTLA-4 bispecific antibody, cervical cancer patients with microsatellite instability high (MSI-H) tumors have been reported to exhibit a notable response to dual combination immunotherapy with nivolumab and ipilimumab after progression on prior anti-PD-1 monotherapy.[31]

Conclusion

In conclusion, we report a patient with NSCLC who developed histological transformation of sarcomatoid carcinoma and died of rapid progression after long-term remission with anti-PD-1 monotherapy. The transformation of sarcomatoid carcinoma could be associated with tumor immune escape and could act as an acquired resistance mechanism that determines the response to checkpoint blockade-based immunotherapy. The possibility of histological transformation and rebiopsy or surgery should be taken into consideration following disease progression after immunotherapy. An increased percentage of PD-L1^{+} tumor cells and decreased infiltration of immune cell subgroups in the tumor microenvironment might be responsible for subsequent resistance to immunotherapy rechallenge. The precise cellular and molecular mechanisms of resistance to acquired immunotherapy in NSCLC-to-sarcomatoid carcinoma transformation should be investigated more thoroughly in a larger sample cohort by comprehensive comparative multi-omics analysis of the genetic, epigenetic, and transcriptional profiles of primary NSCLC and transformed sarcomatoid carcinoma tissues.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of The First Affiliated Hospital of Shandong First Medical University (No.: YXLL-KY-2022–059).

Consent for publication

The patient's next of kin provided written informed consent to publish his clinical information and images in this case report.

Author contributions

Xiuju Liang: Conceptualization; Data curation; Writing – original draft.

Yaping Guan: Investigation; Writing – review & editing.

Baocheng Wang: Supervision; Writing – review & editing.

Xiaohong Liu: Conceptualization; Writing – review & editing.

Jun Wang: Conceptualization; Data curation; Writing – review & editing.

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Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

Not applicable.

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Supplemental material

Supplemental material for this article is available online.

References

1. Postow MA, Callahan MK and Wolchok JD. Immune checkpoint blockade in cancer therapy. *J Clin Oncol* 2015; 33: 1974–1982.
2. Hirsch FR, McElhinny A, Stanforth D, *et al*. PD-L1 immunohistochemistry assays for lung cancer: results from phase 1 of the blueprint PD-L1 IHC assay comparison project. *J Thorac Oncol* 2017; 12: 208–222.
3. Passaro A, Brahmer J, Antonia S, *et al*. Managing resistance to immune checkpoint inhibitors in lung cancer: treatment and novel strategies. *J Clin Oncol* 2022; 40: 598–610.
4. Shao Y and Zhong DS. Histological transformation after acquired resistance to epidermal growth factor tyrosine kinase inhibitors. *Int J Clin Oncol* 2018; 23: 235–242.
5. Levacq D, D'Haene N, de Wind R, *et al*. Histological transformation of ALK rearranged adenocarcinoma into small cell lung cancer: a new mechanism of resistance to ALK inhibitors. *Lung Cancer* 2016; 102: 38–41.
6. Xie X, Chen X, Luo N, *et al*. Transformation of invasive lung adenocarcinoma with ALK rearrangement into pulmonary sarcomatoid carcinoma. *J Cancer Res Clin Oncol* 2022; 148: 2165–2168.
7. Shao Y, Sehgal K, Varkaris A, *et al*. Small cell transformation of non-small cell lung cancer on immune checkpoint inhibitors: uncommon or underrecognized? *J Immunother Cancer* 2020; 8: e000697.
8. Bar J, Ofek E, Barshack I, *et al*. Transformation to small cell lung cancer as a mechanism of resistance to immunotherapy in non-small cell lung cancer. *Lung Cancer* 2019; 138: 109–115.
9. Imakita T, Fujita K, Kanai O, *et al*. Small cell transformation of non-small cell lung cancer under immunotherapy: case series and literature review. *Thorac Cancer* 2021; 12: 3062–3067.
10. Cui Y, Han X, Liu H, *et al*. Impact of endogenous glucocorticoid on response to immune checkpoint blockade in patients with advanced cancer. *Front Immunol* 2023; 14: 1081790.
11. Gagnier JJ, Kienle G, Altman DG, *et al*.; CARE Group. The CARE guidelines: consensus-based clinical case reporting guideline development. *J Med Case Rep* 2013; 7: 223.
12. Sun D, Xu M, Pan C, *et al*. Systematic assessment and optimizing algorithm of tumor mutational burden calculation and their implications in clinical decision-making. *Front Oncol* 2022; 12: 972972.
13. Chen J, Quan M, Chen Z, *et al*. Camrelizumab in advanced or metastatic solid tumor patients with DNA mismatch repair deficient or microsatellite instability high: an open-label prospective pivotal trial. *J Cancer Res Clin Oncol* 2020; 146: 2651–2657.
14. Hsieh MS, Lin MW and Lee YH. Lung adenocarcinoma with sarcomatoid transformation after tyrosine kinase inhibitor treatment and chemotherapy. *Lung Cancer* 2019; 137: 76–84.
15. Yang Z, Shen Y, Jiang J, *et al*. Transformation of two cases of lung adenocarcinoma into pulmonary sarcomatoid carcinoma following treatment. *Can Respir J* 2021; 2021: 6661772.
16. Ye T, Zhang JY, Liu XY, *et al*. The predictive value of MAP2K1/2 mutations on efficiency of immunotherapy in melanoma. *Front Immunol* 2022; 12: 785526.
17. Torrejon DY, Abril-Rodriguez G, Champhekar AS, *et al*. Overcoming genetically based resistance mechanisms to PD-1 blockade. *Cancer Discov* 2020; 10: 1140–1157.
18. Zaretsky JM, Garcia-Diaz A, Shin DS, *et al*. Mutations associated with acquired resistance to PD-1 blockade in melanoma. *N Engl J Med* 2016; 375: 819–829.
19. Hurkmans DP, Kuipers ME, Smit J, *et al*. Tumor mutational load, CD8+ T cells, expression of PD-L1 and HLA class I to guide immunotherapy

decisions in NSCLC patients. *Cancer Immunol Immunother* 2020; 69: 771–777.

20. Zhao Y, Cao Y, Chen Y, *et al*. *B2M* gene expression shapes the immune landscape of lung adenocarcinoma and determines the response to immunotherapy. *Immunology* 2021; 164: 507–523.
21. Ren D, Hua Y, Yu B, *et al*. Predictive biomarkers and mechanisms underlying resistance to PD1/PD-L1 blockade cancer immunotherapy. *Mol Cancer* 2020; 19: 19.
22. Talb J, Takam Kamga P, Mayenga M, *et al*. Gene expression profile of high PD-L1 non-small cell lung cancers refractory to pembrolizumab. *Cancer Immunol Immunother* 2022; 71: 2791–2799.
23. Nakahama K, Izumi M, Yoshimoto N, *et al*. Vimentin expression correlates with immune checkpoint inhibitor efficacy in non-small cell lung cancer. *Cancer* 2023; 129: 2297–2307.
24. Jang HR, Shin SB, Kim CH, *et al*. PLK1/vimentin signaling facilitates immune escape by recruiting Smad2/3 to PD-L1 promoter in metastatic lung adenocarcinoma. *Cell Death Differ* 2021; 28: 2745–2764.
25. Lopez de Rodas M, Nagineni V, Ravi A, *et al*. Role of tumor infiltrating lymphocytes and spatial immune heterogeneity in sensitivity to PD-1 axis blockers in non-small cell lung cancer. *J Immunother Cancer* 2022; 10: e004440.
26. Liu Y, Zugazagoitia J, Ahmed FS, *et al*. Immune cell PD-L1 colocalizes with macrophages and is associated with outcome in PD-1 pathway blockade therapy. *Clin Cancer Res* 2020; 26: 970–977.
27. Liang J, Bi G, Shan G, *et al*. Tumor-associated regulatory T cells in non-small-cell lung cancer: current advances and future perspectives. *J Immunol Res* 2022; 2022: 4355386.
28. Sun L, Dai J, Chen Y, *et al*. Pulmonary sarcomatoid carcinoma: experience from SEER database and Shanghai Pulmonary Hospital. *Ann Thorac Surg* 2020; 110: 406–413.
29. Domblides C, Leroy K, Monnet I, *et al*. Efficacy of immune checkpoint inhibitors in lung sarcomatoid carcinoma. *J Thorac Oncol* 2020; 15: 860–866.
30. Ma Y, Li W, Li Z, *et al*. Immunophenotyping of pulmonary sarcomatoid carcinoma. *Front Immunol* 2022; 13: 976739.
31. Gim G, Kim Y, Park Y, *et al*. Response to nivolumab and ipilimumab in microsatellite instability-high (MSI-H) cervical carcinoma with acquired resistance to pembrolizumab: a case report and literature review. *Oncologist* 2022; 27: 525–531.