

[CASE REPORT]

A Long-term Response to Nivolumab in a Case of PD-L1-negative Lung Adenocarcinoma with an *EGFR* Mutation and Surrounding PD-L1-positive Tumor-associated Macrophages

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Abstract:

Anti-programmed cell death 1 (PD-1) antibodies have poor efficacy in epidermal growth factor receptor (*EGFR*)-mutated lung cancer. We herein report a 72-year-old man with programmed cell death-ligand 1 (PD-L1)-negative lung adenocarcinoma harboring an *EGFR* mutation that responded to nivolumab for more than 2 years. A pathological examination revealed infiltration of CD8-positive lymphocytes and macrophages expressing CD68, CD206, and PD-L1 into the PD-L1-negative tumor; CD206 expression is a marker of immunosuppressive tumor-associated macrophages (TAMs). The presence of PD-L1-positive TAMs in the tumor environment might be a predictor of a positive response to anti-PD-1 antibodies.

Key words: lung adenocarcinoma, *EGFR* mutations, nivolumab, tumor-associated macrophages

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Introduction

Immune checkpoint inhibitors (ICIs) have fundamentally changed the lung cancer treatment strategy. However, anti-programmed cell death 1 (PD-1) antibodies have poor efficacy in epidermal growth factor receptor (*EGFR*)-mutated lung cancer (1-3). Possible explanations for the poor efficacy are a low tumor mutation burden (TMB), low expression of programmed cell death-ligand 1 (PD-L1), or low immunogenicity, although the precise mechanisms remain to be clarified.

We herein report a case of PD-L1-negative lung adenocarcinoma harboring an *EGFR* mutation that responded to nivolumab for more than two years.

Case Report

A 72-year-old man who was a former smoker (48 pack-

years) was diagnosed with locally advanced lung adenocarcinoma (cT1bN2M0, cStage IIIA) harboring an *EGFR* exon 19 deletion. He underwent right upper lobectomy after neoadjuvant chemoradiotherapy with cisplatin plus docetaxel and 46 Gy of thoracic radiation. Twelve months after the surgery, brain magnetic resonance imaging indicated recurrence of multiple brain metastases; subsequently, the brain lesions were treated with γ -knife therapy. Thirteen months after radiosurgery, a computed tomography (CT) scan revealed multiple lung metastases.

Salvage chemotherapies, including *EGFR* tyrosine kinase inhibitors (TKIs), erlotinib, carboplatin plus paclitaxel, and docetaxel, were then administered consecutively. Erlotinib as second-line therapy was continued for seven months with a partial response. After docetaxel therapy, erlotinib was re-administered and generated a response for four months, but eventually, multiple lung metastatic lesions regrew. At the time of progression, osimertinib was not approved for clinical practice; therefore, the patient did not agree to a re-

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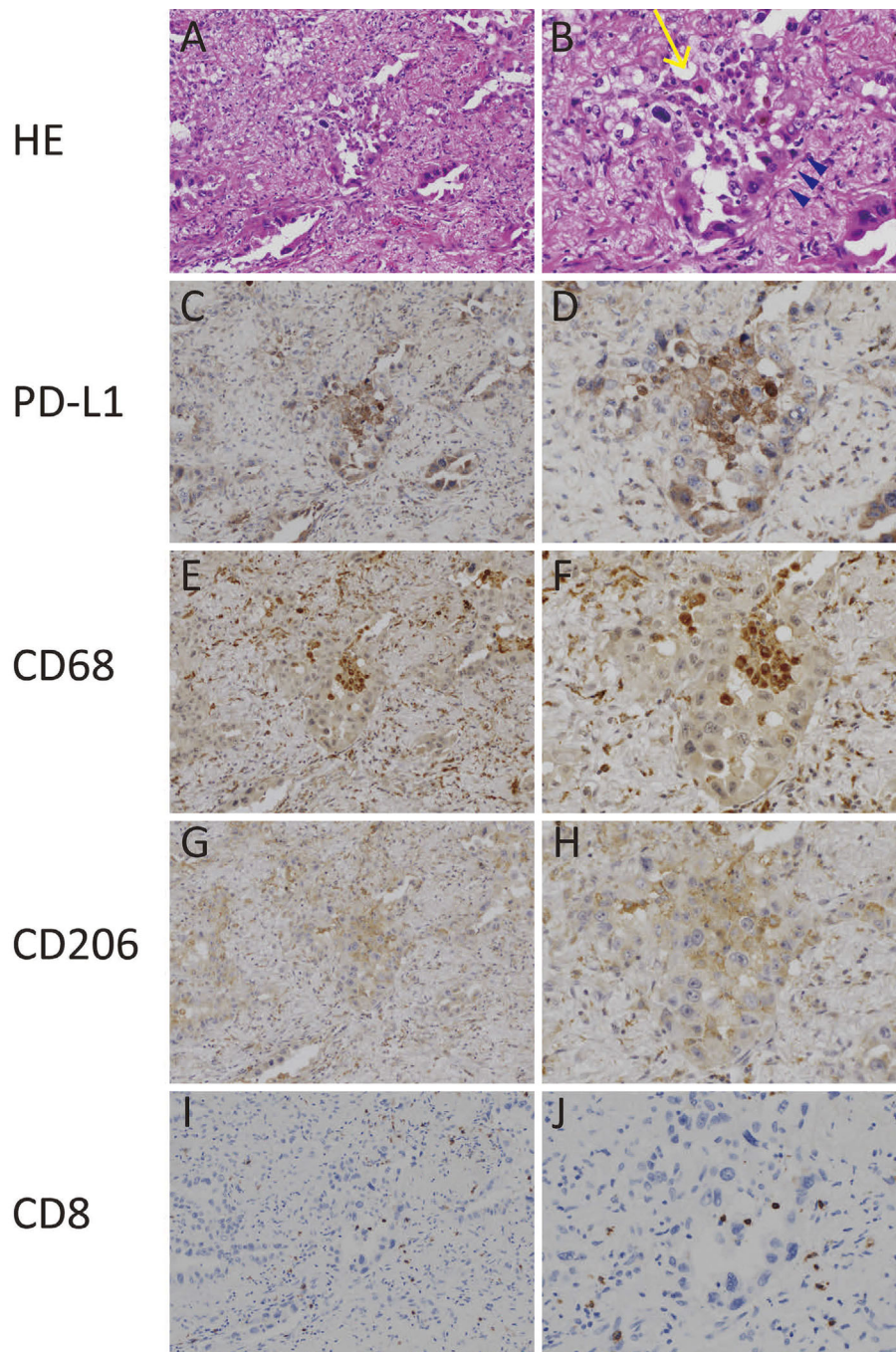


Figure 1. PD-L1-positive M2 macrophages surrounding PD-L1-negative tumors. Microscope magnification: (A, C, E, G, I: 200-fold; B, D, F, H, J: 400-fold). Anti-PD-L1 antibody (28-8) (Dako SK005), anti-CD68 antibody (Abcam Cat# ab955), anti-CD206 antibody (Abcam Cat# ab64693) and anti-CD-8 antibody (Roche Cat# 7904460) were used for immunostaining. A, B: Hematoxylin and Eosin staining. The blue arrowhead indicates lung cancer cells. The yellow arrow indicates macrophages. C, D: The tumor did not express PD-L1. Instead, the surrounding cells showed high PD-L1 expression. E, F: The PD-L1-positive cells were CD68-positive macrophages. G, H: CD206 staining was also positive in the macrophages. I, J: CD8-positive lymphocytes were observed in the vicinity of the cancer cells.

biopsy for testing of his *EGFR* mutation status. Although the PD-L1 expression result for the surgical specimen tumors was negative (Fig. 1A-D), nivolumab was administered as sixth-line therapy. After seven cycles of nivolumab administration, CT revealed that the metastatic lung tumors

and the mediastinal and subclavian lymph node tumors had almost disappeared. Currently, he has continued treatment with nivolumab for more than two years (Fig. 2). Thus far, there has been no evidence of tumor regrowth or serious immune-related adverse events.

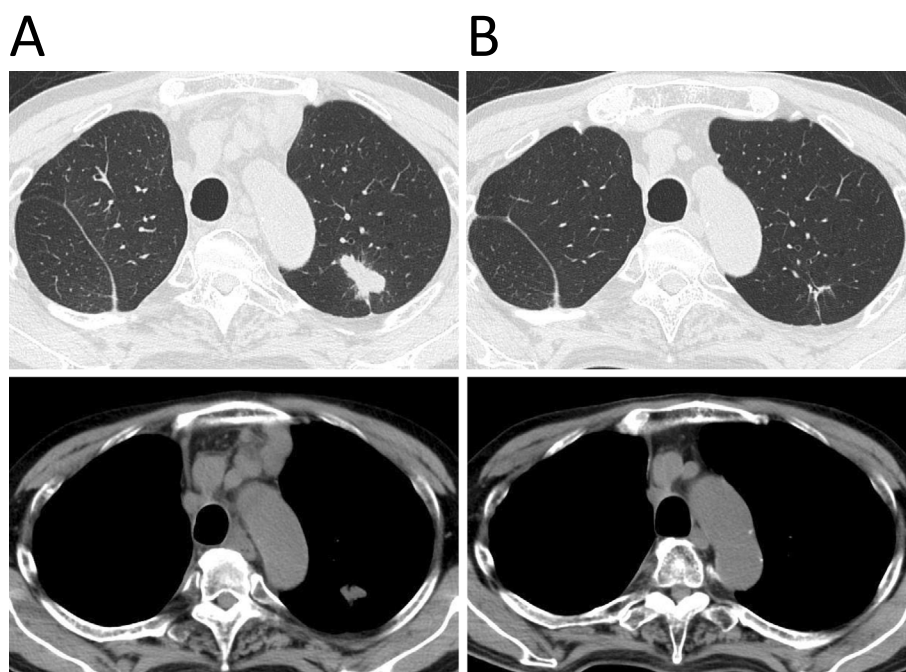


Figure 2. Chest computed tomography (CT) of the metastatic lung tumors and mediastinal and subclavian lymph node tumors. A: Chest CT scan at the beginning of nivolumab treatment. B: Chest CT scan two years after starting nivolumab treatment.

A further pathological examination revealed CD8-positive lymphocytes and macrophages expressing CD68 and PD-L1 that infiltrated the PD-L1-negative tumor (Fig. 1E-F and 1I-J). Some of the CD68-positive macrophages were also positive for CD206, which is a marker of immunosuppressive tumor-associated macrophages (TAMs) (Fig. 1G-H, 3).

Discussion

Nivolumab was found to produce a long-term response in lung adenocarcinoma harboring *EGFR* exon 19 deletion. The lung tumors did not express PD-L1 but were surrounded with CD8-positive T-lymphocytes and immune suppressive TAMs expressing PD-L1. At present, no marker has been established for predicting a positive effect of anti-PD-1 antibody in lung cancer harboring *EGFR* mutations; however, our case suggested that the presence of PD-L1-positive TAMs might predict positive effects of nivolumab.

EGFR-TKIs are a standard treatment option for lung cancer with *EGFR* mutations. Osimertinib has been approved for EGFR-TKI naïve lung tumors; therefore, the role of EGFR-TKIs is more important than ever. However, unlike ICIs, EGFR-TKIs do not result in deep remission in advanced lung cancers. Unfortunately, a recent clinical trial showed that the anti-PD-1 antibody pembrolizumab had a limited effect on EGFR-TKI-naïve lung cancers harboring *EGFR* mutations, even if the tumors had a high PD-L1 expression (4). The treatment sequence might be one of the influencing factors; however, there is thus far no evidence that prior therapy influences the treatment effect of

nivolumab (1, 5).

A recent report showed that a Brinkman index ≥ 600 , duration of EGFR-TKI administration < six months, and uncommon *EGFR* mutations were correlated with a longer progression-free survival with nivolumab in *EGFR*-mutant lung cancers (6). Our patient was a heavy smoker, but the tumor harbored a common activating *EGFR* mutation and responded well to EGFR-TKI for more than six months.

The tumor microenvironment may also influence the effects of ICI treatment (7). A recent report showed that large numbers of CD8-positive lymphocytes and small numbers of forkhead box protein 3 (FOXP3)-positive tumor-infiltrating lymphocytes were related to the effect of nivolumab in *EGFR*-mutant lung cancer that developed resistance to EGFR-TKIs (8). The presence of immune suppressive M2 macrophages in the tumor microenvironment has been reported to be correlated with a poor prognosis of lung cancer (9, 10). Another study showed that macrophages impede CD8 T-cells from reaching tumor cells and limit the efficacy of anti-PD-1 treatment against lung squamous cell cancers in humans and mice models (11). Furthermore, the presence of PD-L1-positive tumor-infiltrating immune cells, including macrophages, showed a correlation with positive effects of anti-PD-L1 antibody in clinical trials involving lung cancers (12). The positive correlation between the effect of anti-PD-1 antibody and the presence of PD-L1-positive macrophages in the tumor microenvironment has also been reported in melanoma (13, 14). Taken together, these data may suggest that immune suppressive TAMs expressing PD-L1 negatively affect cytotoxic lymphocytes, and this negative interaction may be a potential therapeutic target of

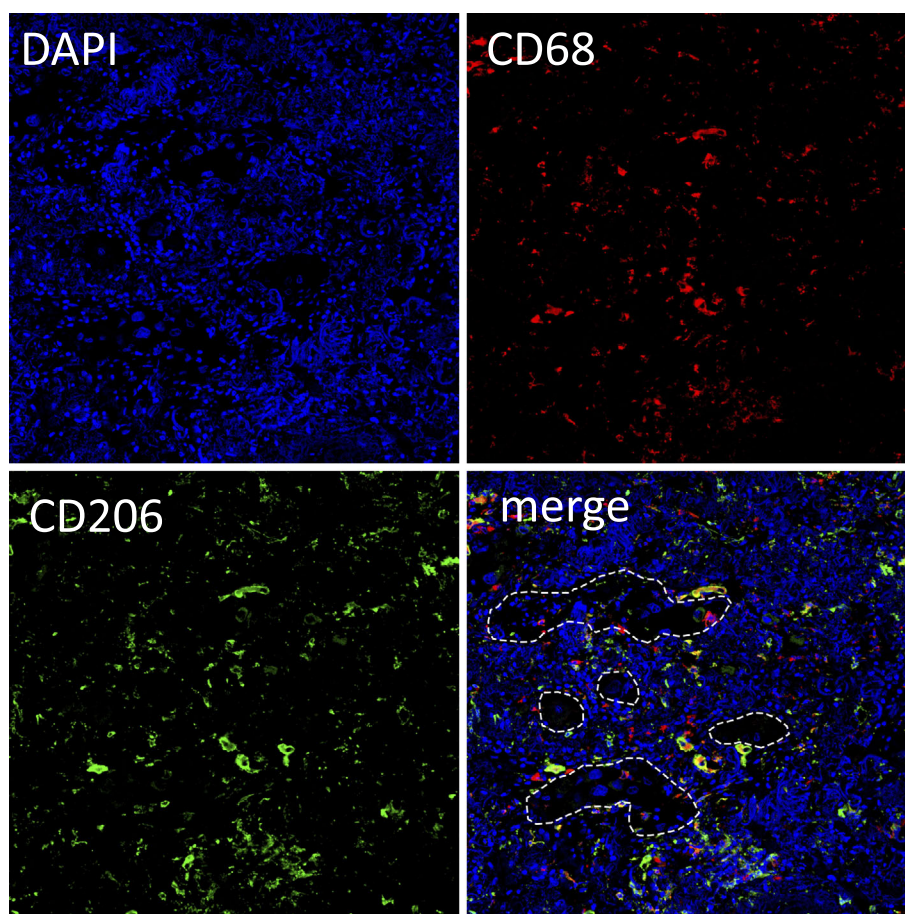


Figure 3. M2 macrophages surrounding the lung tumors. Double staining with anti-CD68 antibody (Abcam Cat# ab955) and anti-CD206 antibody (Abcam Cat# ab64693) was performed. The anti-CD68 antibody appeared red due to the secondary fluorescent conjugated antibody (Alexa Fluor® 594 Abcam ab150116). The anti-206 antibody appeared green due to the secondary fluorescent conjugated antibody (Alexa Fluor® 488 Abcam ab150077). Images were overlaid to demonstrate co-localization. Blue: DAPI, Red: CD68, Green: CD206. The white dotted line indicates tumor cells.

ICIs (15).

At present, macrophages are thought to be a promising therapeutic target (16, 17). One strategy involves the direct inhibition of immunosuppressive TAMs (11, 16), while another involves inducing polarization from immunosuppressive M2 phenotype to immune stimulative M1 phenotype (18, 19). Recent studies have shown that anti-PD-1 or anti-PD-L1 antibodies activate the ability of macrophages on phagocytosis to promote polarization to the M1 phenotype (20-22). Therefore, the immune activation of macrophages by anti-PD-1 or PD-L1 antibody might be involved in patients with PD-L1-negative tumors showing unexpected response to ICIs.

In our case, a pathological examination revealed CD8-positive lymphocytes and macrophages expressing CD68 and PD-L1, which infiltrated the PD-L1-negative tumor (Fig. 1E-F and I-J). The CD68-positive macrophages were also CD206-positive, which is a marker of immunosuppressive TAMs (Fig. 1G-H). Therefore, we speculate that the following scenarios might explain the mechanisms underlying the excellent therapeutic effect in our case: 1) TAMs

suppressed CD8-positive lymphocytes, 2) nivolumab inhibited the negative interaction of M2 macrophages, and 3) CD 8-positive lymphocytes were released and attacked the tumors. Alternatively, nivolumab might promote polarization from the M2 phenotype to the M1 phenotype, at which point the M1 macrophages suppress the tumors.

In addition to the PD-L1 status of tumors, the presence of PD-L1-positive TAMs in the tumor environment might be a predictive biomarker of a positive response to anti-PD-1 antibodies. Further studies are warranted to identify clues about which patients would benefit from ICIs for lung cancers harboring *EGFR* mutations.

The authors state that they have no Conflict of Interest (COI).

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