CASE REPORT

CD103+CD8+ Lymphocytes Characterize the Immune Infiltration in a Case With Pseudoprogression in Squamous NSCLC

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Case

A 65-year-old male patient, current smoker, was diagnosed with stage IV squamous cell lung cancer (multiple central nervous system lesions) (Fig. 1*A* and *B*). He received whole-brain radiotherapy followed by chemotherapy with carboplatin (area under the curve [AUC] of 5) – vinorelbine (25 mg/m^2). After 3 cycles, disease progression was confirmed, with an increase of the lung tumor and development of new liver lesions (Fig. 1*C* and *D*). Nivolumab was then initiated. At radiologic evaluation, after 5 cycles of nivolumab, a discordant response was observed with partial response in the central nervous system and stable lung disease but a significant increase of one liver lesion (Fig. 1*E* and *F*). The marked discrepancy between the clinical benefit and the radiological findings prompted us to perform a liver biopsy. The pathologic findings revealed extensive areas of necrosis, no viable tumor cells, and the presence of a lymphohistiocytic infiltrate.

Immune biomarkers were compared between the lung biopsy at diagnosis and the liver biopsy after 5 cycles of nivolumab by immunohistochemistry (IHC) (Fig. 2*A* images 1 and 2). All tumor cells (100%) expressed programmed death ligand 1 pretreatment and were necrotic in the on-treatment biopsy (Fig. 2*A* images 3 and 4). Lymphocyte characterization revealed increased numbers of CD4 (Fig. 2*A* images 7 and 8) and CD8 (Fig. 2*A* images 9 and 10) in the on-treatment biopsy, with a change in the ratio of CD4/CD8 (at diagnosis 1.25, and 0.875 after treatment with immune checkpoint inhibitors). CD103-positive cells (Fig. 2*A* images 11 and 12) were also increased in the liver biopsy, and CD68 staining showed a higher proportion of macrophages in the liver biopsy (Fig. 2*A* images 13 and 14). Programmed death 1 expression was observed in macrophages and lymphocytes and was also enhanced in the on-treatment liver biopsy (Fig. 2*A* images 17 and 18). Double staining with Ki67/CD3 showed a marked increase in the lymphocytes in liver biopsy compared to pre-treatment lung specimen (Fig. 2*A* images 19 and 20).

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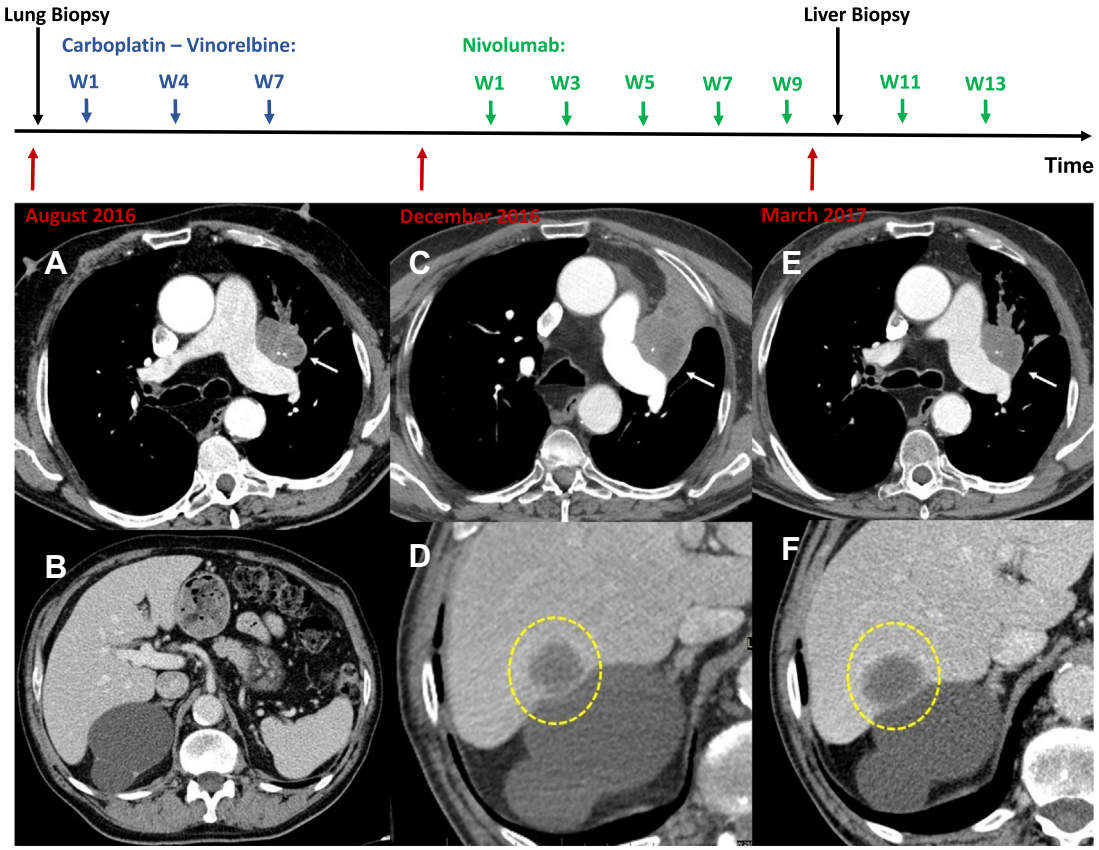

Figure 1. (*A*,*B*) Computed tomographic (CT) scan at diagnosis (August 2016). (*C*,*D*) Pre-nivolumab treatment. (*E*,*F*) After 5 cycles of nivolumab. *A* and *B* show a lung mass of 45 mm (*A*, *arrow*), localized in the upper left upper lobe. This lesion presents extensive contact with the pulmonary artery and compromises the distal airway. In the abdominal CT scan from the same date (*B*) no liver lesions were observed. The radiologic evaluation made in December 2016 revealed an increase in the size of the lung lesion (*C*) (45 mm to 48 mm) and greater obstruction of the distal airway. In the same study, abdominal CT scan revealed the appearance of a hepatic lesion (*D*) (*yellow circle*) of 20 mm, accounting for progressive disease. Subsequent CT scan after 5 cycles of nivolumab, showed a decrease in the size of lung mass (*E*) of 48 mm to 45 mm, and increase in size of the hepatic metastatic lesion (*F*) (*yellow circle*) from 20 to 33 mm. Response Evaluation Criteria in Solid Tumors show 1.1 + 14.7% (two target lesions). There is an increase of 65% at the liver lesion.

To further characterize the lymphocyte populations observed in the on-treatment liver biopsy by IHC, we performed fluorescence-activated cell sorting analysis. Of the live lymphocytes (Fig. 2*B* image I), 55% were T cell receptor–positive (Fig. 2*B* image II) and within this gate 65.2% expressed CD8a and 29.8% CD4 (Fig. 2*B* image III). Consistent with the IHC staining, the majority of CD8 T cells (69.6%)

Figure 2. (*A*) Histologic assessment of lung and liver biopsy. Images 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19 (*left column*) correspond to lung biopsy results at diagnosis. Images 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 (*right column*) correspond to liver biopsy results after 5 cycles of nivolumab. Images 1 and 2 used hematoxylin and eosin stain. Image 1 reveals a poorly differentiated squamous carcinoma. Image 2 reveals a dense lymphohistiocytic infiltrate surrounding a totally necrotic metastatic tumor. Images 3 and 4 show programmed death ligand 1 (PD-L1) staining 100% in tumor cells of lung biopsy and high expression in macrophages and lymphocytes. Images 5 and 6 used CD3 staining. Image 5 shows 75 lymphocytes/high power field (HPF). Image 6 shows 250 lymphocytes/HPF Images 7 and 8 show staining for CD4 marker. Image 7 shows 50 lymphocytes/HPF. Image 8 shows 140 lymphocytes/ HPF. Images 9 and 10 show CD8 staining. Image 9 shows 40 lymphocytes/HPF. Image 10 shows 160 lymphocytes/HPF. Images 11 and 12 show CD 103 staining. Image 11 shows 15 lymphocytes/HPF. Image 12 shows 100 lymphocytes/HPF. Images 13 and 14 show CD68 staining. Image 13 shows 30 lymphocytes/HPF. Image 14 shows 100 lymphocytes/HPF. Images 15 and 16 show CD56 marker. Image 15 shows 10 lymphocytes/HPF. Image 16 shows 10 lymphocytes/HPF. Images 17 and 18 show programmed death1 (PD-1) staining, with 10 cells/HPF at lung biopsy and 20 cells/HPF at liver biopsy. Images 19 and 20 show CD3 (membrane staining in red) Ki67 (nucleus staining in brown) double immunostaining. Image 19 shows 2 lymphocytes/HPF. Image 20 shows 18 lymphocytes/HPF. Original magnification 400. (*B*) Representative flow cytometry plot and analysis of liver biopsy, performed during treatment with PD-1 blockade (nivolumab). Image I show a selection of lymphocytes in the flow cytometry plot. Image II shows an expression of pan-T cell receptor (TCR) (a marker that identified the T cell population). Image III shows flow cytometry showing expression of CD4 and CD8 of the pan-TCR population. Image IV shows expression of CD103 in the selected CD8 T cell population.