

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. 1A and B). He received whole-brain radiotherapy followed by chemotherapy with carboplatin (area under the curve [AUC] of 5) – vinorelbine (25 mg/m²). After 3 cycles, disease progression was confirmed, with an increase of the lung tumor and development of new liver lesions (Fig. 1C 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. 1E 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. 2A images 1 and 2). All tumor cells (100%) expressed programmed death ligand 1 pretreatment and were necrotic in the on-treatment biopsy (Fig. 2A images 3 and 4). Lymphocyte characterization revealed

increased numbers of CD4 (Fig. 2A images 7 and 8) and CD8 (Fig. 2A 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. 2A 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. 2A 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. 2A 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. 2A images 19 and 20).

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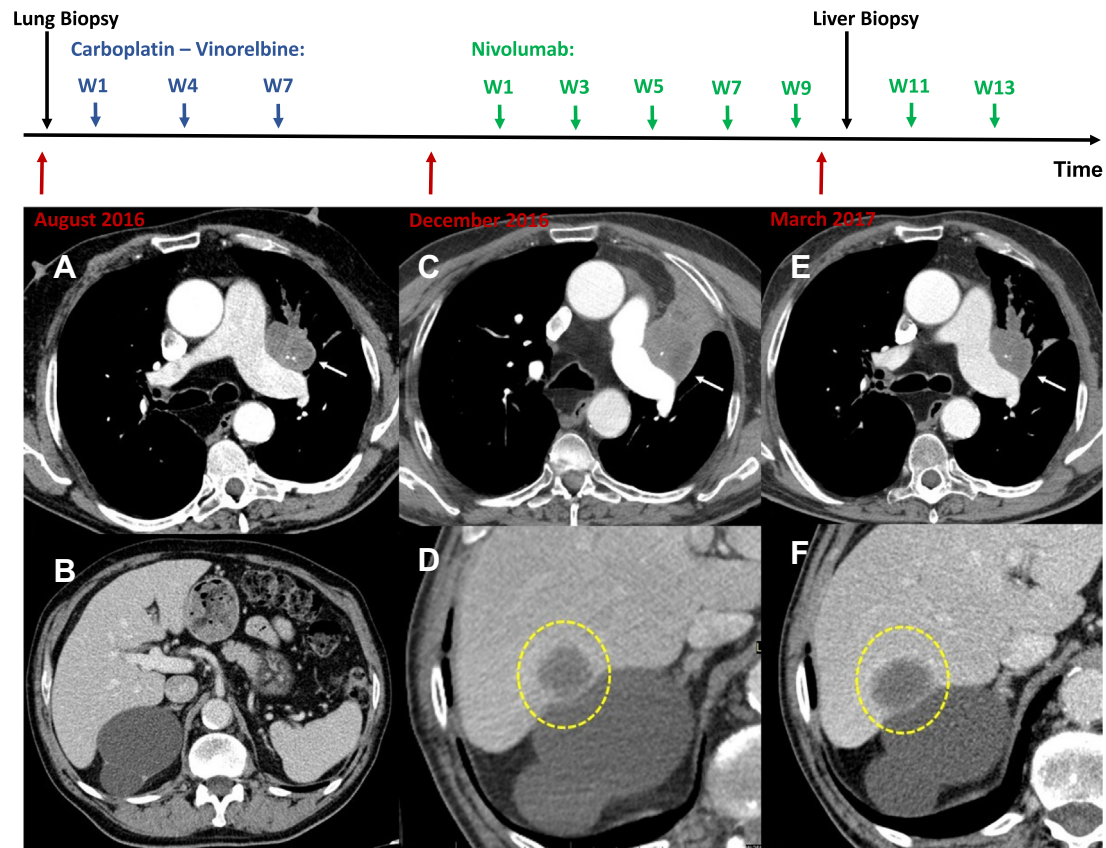
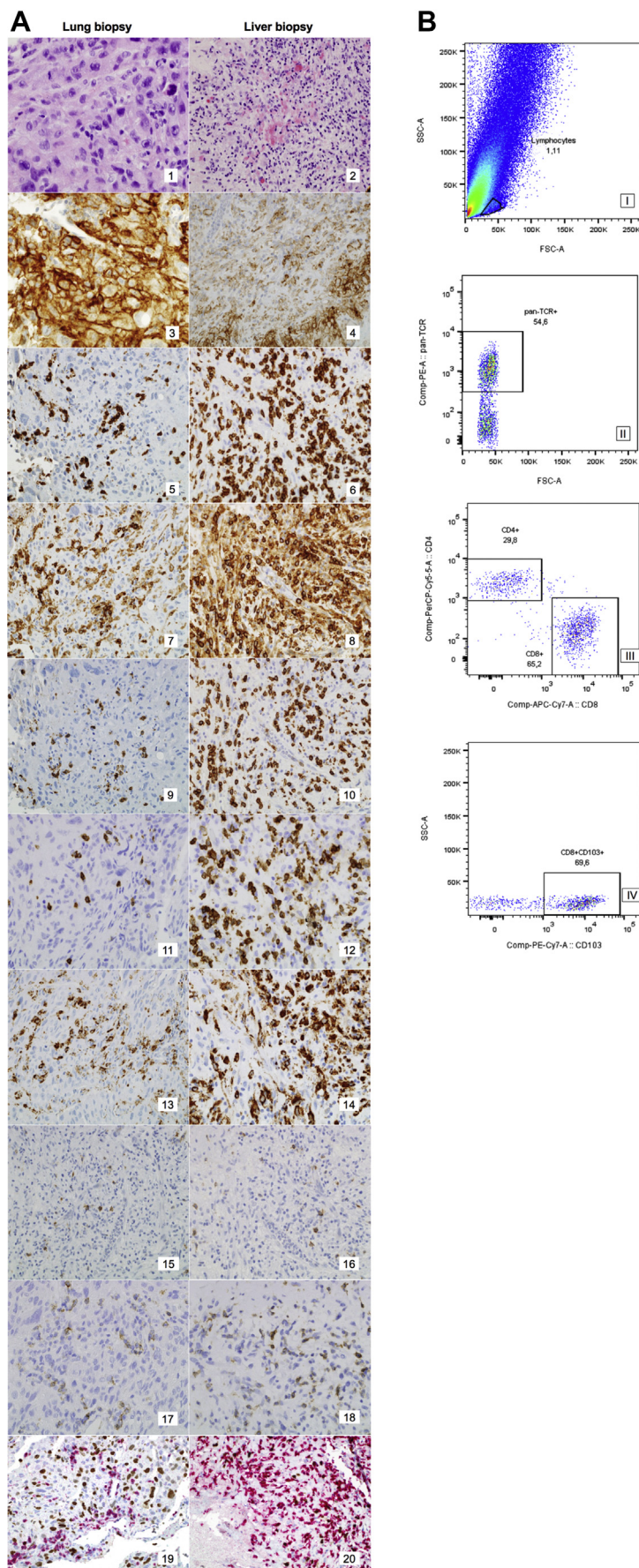


Figure 1. (A,B) Computed tomographic (CT) scan at diagnosis (August 2016). (C,D) Pre-nivolumab treatment. (E,F) After 5 cycles of nivolumab. A and B show a lung mass of 45 mm (A, arrow), localized in the upper left upper lobe. This lesion presents extensive contact with the pulmonary artery and compromises 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. 2B image I), 55% were T cell receptor-positive (Fig. 2B image II) and within this gate 65.2% expressed CD8a and 29.8% CD4 (Fig. 2B 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.



co-expressed the tissue residency marker CD103 (Fig. 2B image IV).

Discussion

New patterns of radiologic response have been described with immune checkpoint inhibitors such as pseudoprogression and mixed responses.^{1,2} Biopsy specimens of the “growing” lesion have shown lymphocyte infiltration as a cause of this initial increase in size.^{3,4}

We have recently reported that CD103+ tissue resident memory CD8+ T cells are protective in early lung cancer.⁵ The pretreatment biopsy of our patient showed the presence of CD8+CD103+ T cells. Characterization of immune population by IHC and florescent-activated cell sorting in the on-treatment sample showed a substantial increase in CD8+CD103+ T cells in the liver metastasis, compared to pretreatment density.

Our data show for the first time that programmed death 1 blockade might therefore not only enhance the activation and proliferation of immune competent T cells globally, but may also contribute to effective responses through release and expansion of CD103+ tissue residual memory cells as shown by increase of Ki67 marker in lymphocytes. The influx or intratumoral expansion of protective immune cells presented clinically as tumor

volume increase. Our data offer a morphological insight to understand clinical correlates of an antitumor response that can be released in lung cancer through checkpoint blockade.

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