Supplementary material

MATERIALS AND METHODS

Immunohistochemistry for PDCD1 (PD-1)

Tissue sections were deparaffinised, rehydrated, and heated in Antigen Retrieval Citra Solution, pH 6 (BioGenex Laboratories, San Ramon, CA, USA). Sections were incubated with Dual Endogenous Enzyme Block (Dako, Glostrup, Denmark), and were treated with Protein Block Serum-Free (Dako). Slides were then incubated at room temperature for 1 hour with a mouse monoclonal anti-PDCD1 antibody (Clone EH33; dilution, 1:1000) that was obtained from the Gordon J. Freeman's laboratory at Dana–Farber Cancer Institute. The primary antibody for PDCD1 was validated in a previous study.[1] The primary antibody was visualised using EnVision+ System-HRP (Dako) for 30 minutes with diaminobenzidine, and counterstained with hematoxylin. Sections processed with replacement of the primary antibody by tris-buffered saline were used as a negative control. Normal human tonsil tissue was used as a positive control.

PDCD1 expression was observed in stromal cells including tumour-infiltrating lymphocytes (TIL), not in colorectal carcinoma cells. Immunohistochemical expression for PDCD1 was interpreted by a pathologist (Y.M.) blinded to other data. The number of $PDCD1^+$ cells in a high-power microscopic field (hpf; diameter, 0.55 mm) was counted in a hot spot area for each tumour. $PDCD1^+$ cell density was scored as absent (no detectable $PDCD1^+$ cells), very

low (1-2 cells/hpf), low (3-5 cells/hpf), intermediate (6-10 cells/hpf), or high (>10 cells/hpf) (supplementary figure 2).

REFERENCE

1 Ansell SM, Lesokhin AM, Borrello I, *et al.* PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med* 2015;372:311-9.