Appendix S1

Supplementary methods

Immunohistochemistry

Five µm sections were prepared from FFPE tumour blocks and immunohistochemistry (IHC) for indoleamine 2,3-dioxygenase (IDO), PD-1 ligand 1 (PD-L1), programmed cell death protein 1 (PD-1), lymphocyte-activation gene 3 (LAG-3) and T cell immunoglobulin and mucin-domain containing-3 (TIM-3) was performed. The antibodies and staining conditions are included in the Table S2. Photographs of staining were visualized with AxioVision (Zeiss, Feldbach, Switzerland). Three observers who were blinded to the clinical parameters scored the IHC stained sections independently. Histology score (H-Score) was calculated for IHC markers IDO, PD-1, TIM-3 and LAG-3 as a semi quantitative approach using the following formula: H-Score = [1 × (% cells 1+) + 2 × (% cells 2+) + 3 × (% cells 3+)]. Membrane staining intensity (0 = no staining, 1+ = weak staining, 2+ = moderate staining, or 3+ = strong staining[18]). PD-L1 staining was measured using three different scores: CPS = combined positive score (number of PD-L1 positive tumour cells and lymphocytes, divided by the total number of viable tumour cells, multiplied by 100) TPS = tumour proportion score (number of PD-L1 positive tumour cells, divided by the total number of viable tumour cells, multiplied by 100) IC = immune cell score (number of PD-L1 positive lymphocytes, divided by the tumour area, multiplied by 100).

Table SI Immunohistochemical staining reagents and methods

Primary antibody	Source	Antigen retrieval	Primary antibody incubation	Detection
IDO	Cell Signaling Technology	TRS, pH 9.0 (Dako)	60 min, RT	Biotin-conjugated secondary antibody (Dako REAL™ Link, Biotinylated Secondary Antibodies) visualized with a alkaline phosphatase/RED chromogen (Dako REAL™ Detection System, Rabbit/Mouse (Code K5005))
PD-1	R&D	TRS, pH 6.0 (Dako)	60 min, RT	HRP-conjugated antibody visualized by a DAB chromogen (Dako Envison FLEX Mini Kit).
PD-L1	Cell Signaling Technology	TRS, pH 9.0 (Dako)	60 min, RT	Biotin-conjugated secondary antibody (Dako REAL™ Link, Biotinylated Secondary Antibodies) visualized with a alkaline phosphatase/RED chromogen (Dako REAL™ Detection System, Rabbit/Mouse (Code K5005)
LAG-3	Novus Biologicals	TRS, pH 9.0 (Dako)	60 min, RT	Biotin-conjugated secondary antibody (Dako REAL™ Link, Biotinylated Secondary Antibodies) visualized with a alkaline phosphatase/RED chromogen (Dako REAL™ Detection System, Rabbit/Mouse (Code K5005)
TIM-3	Cell Signaling Technology	TRS, pH 9.0 (Dako)	60 min, RT	Biotin-conjugated secondary antibody (Dako REAL™ Link, Biotinylated Secondary Antibodies) visualized with a alkaline phosphatase/RED chromogen (Dako REAL™ Detection System, Rabbit/Mouse (Code K5005)

Footnotes: HRP, horse radish peroxidase; min, minutes; TRS, target retrieval solution

Table SII Semi-quantitative measurements of Immunohistochemistry markers. Table shows histology scores for the markers IDO, PD-1, TIM-3 and LAG-3.

Marker	Patient 1	Patient 2	
Tumour site (grading)	SCC right hand (G1)	SCC right forearm (G1)	SCC right foot (G3)
IDO (tumour)	150	15	0
IDO (stroma)	200	160	70
PD-1	190	170	60
PD-L1 (CPS)	70	30	5
PD-L1 (TPS)	50	20	0
PD-L1 (IC)	20	10	5
TIM-3	110	150	60
LAG-3	150	40	60

Legend: Histology score (H-Score) was calculated as a semiquantitative approach using the following formula: H-Score = [1 × (% cells 1+) + 2 × (% cells 2+) + 3 × (% cells 3+)]. Membrane staining intensity (0 = no staining, 1+ = weak staining, 2+ = moderate staining, or 3+ = strong staining. Mean values of 3 blinded observers were calculated. For PD-L1 staining the CPS = combined positive score (number of PD-L1 positive tumour cells and lymphocytes, divided by the total number of viable tumour cells, multiplied by 100), TPS = tumour proportion score (number of PD-L1 positive tumour cells, divided by the total number of viable tumour cells, multiplied by 100) and the IC = immune cell score (number of PD-L1 positive lymphocytes, divided by the tumour area, multiplied by 100) was measured. G1 = well differentiated, G3 = poorly differentiated. SCC = squamous cell carcinoma.