Supplemental material

BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s)

J Immunother Cancer

Supplemental Figure 7. Characterization of CD14⁺CD1c⁺ DC and CD14⁺CD1c- monocytes/macrophages in the TME of HPV16⁺IR- and HPV16⁺IR⁺ OPSCC tumors.

Freshly dissociated OPSCC tumor tissue from 9 HPV16⁺ OPSCC patients was analyzed by 13-parameter flow cytometry analysis with antibodies directed against CD3/CD19/CD20/CD56, CD11c, HLA-DR, CD14, CD11b, CD163, CD141, CLEC9A, CD1c, CD16, CD123, CD36 and CD32B. **A, B)** The gating strategy is depicted for a representative OPSCC sample. **A)** Dot plot showing expression of CD14 and CD1c within lineage-negative (LIN-), CD11c⁺ and HLA-DR⁺ myeloid cells. Singlets were gated on FSC-H/FSC-A properties, after which dead cells were excluded through gating on yellow amine reactive dye-negative cells. Next, CD3-CD19-CD20-CD56-HLA-DR⁺CD11c⁺ myeloid cells were selected, which were subsequently divided based on CD14 and CD1c expression. **B)** Histogram plots showing CD16, CD163, CD32B, CD36, CD123, CD141 and CLEC9A expression for CD14-CD1c- (black), CD14⁺CD1c- (blue) and CD14⁺CD1c⁺ cells (red). **C)** Histogram plots showing CD40 and CD86 expression for CD45⁺lin- (black), CD11c-HLA-DR- (blue) and CD14⁺CD11c⁺HLA-DR⁺ cells (red). **D)** Box plots depicting the distribution of the identified CD14⁺CD1c⁺ DC (left) and CD14⁺CD1c- monocytes/macrophages (right) among HPV16⁺IR- (blue, n=3) and HPV16⁺IR⁺ (green, n=6) OPSCC tumors. Data is represented as percentage of live cells.