Supplemental material

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Supplemental Table 1: R/M HNSCC patient characteristics. ISH = in situ hybridization. PD-1 = Programmed cell death protein 1. Immunotherapy combinations received included: Nivolumab plus lirilumab, Nivolumab plus ipilimumab, Nivolumab plus urelumab, durvalumab plus tremelimumab.

Supplemental Table 1. Patient Characteristics

	N	%
Age		
Median age	59	
Range	40-80	
Race		
Caucasian	32	89
African American	3	8
Other	1	3
Gender		
Male	25	70
Female	11	30
Smoking History		
Yes	21	58
No	15	42
Primary Site		
Oropharynx	10	28
Oral Cavity	15	42
Nasopharynx	2	5
Hypopharynx	2	5
Larynx	6	17
Parotid	1	3
HPV in OPC (p16 and/or HPV ISH)		
Positive	4	40
Negative	5	50
Unknown	1	10
Type of immunotherapy		
Ant-Pd-1 mAb monotherapy	28	78
Anti-PD-1 mAb + other immunotherapy	7	19
Anti-PD-1 mAb + chemo (platinum/5FU)	1	3
Indication for immunotherapy		
Front-line	14	39
Platinum Failure	22	61

Supplemental Table 2. Summary of correlation results for immune biomarkers in R/M HNSCC patients. * Top value is Spearmen correlation coefficients, below is the p-value.

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Correlations*					
	%CAIX+	%CAIX/int	Average CD8/ PanCK Area	Average Treg/ PanCK Area	%PD-L1+
%CAIX+	1.000	0.840 <0.001	0.117 0.495	0.330 0.049	-0.241 0.162
%CAIX/int	0.840 <0.001	1.000	0.012 0.945	0.384 0.021	-0.135 0.058
Average CD8/ PanCK Area	0.117 0.495	0.012 0.945	1.000	0.278 0.101	0.324 0.058
Average Treg/ PanCK Area	0.330 0.049	0.384 0.021	0.278 0.101	1.000	-0.019 0.912
%PD-L1+	-0.241 0.162	-0.135 0.439	0.324 0.058	-0.019 0.912	1.000

Supplemental Figure 1. (a) Representative immunofluorescence of Foxp3, carbonic anhydrase IX (CAIX) and pan cytokeratin (PanCK) at 20X magnification of formalin-fixed paraffin-embedded (FFPE) sections from HNSCC patients treated with either pembrolizumab or nivolumab. Scale bar, 200µm (b) Tabulated results of Foxp3^{+} T cells normalized to the PanCk[+] area from HNSCC patients that progressed on (P) or had disease control (DC; defined as stable disease, partial or complete response) immunotherapy. (c) Overall survival of patients treated with either pembrolizumab or nivolumab monotherapy based on Foxp3^{+} T cells. (d) Progression free survival of patients as in c. (e) Tabulated Programmed death-ligand 1 (PD-L1) scoring of immunohistochemistry from FFPE sections of patients as in b. (f) Overall survival of patients treated with either pembrolizumab or nivolumab monotherapy based on PD-L1 score. (g) Progression free survival of patients as in f. ns not significant. Error bars indicate s.e.m.