Data Supplement – Online Only Appendix

Ferris RL, et al. Neoadjuvant nivolumab for patients with resectable HPV-positive and HPV-negative squamous cell carcinomas of the head and neck in the CheckMate 358 trial

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This supplementary material has been provided by the authors to give readers additional information about their work.

Supplementary methods 1. Sample size determination

Enrollment of ≥42 evaluable patients was planned (≥21 patients each for the human papillomavirus [HPV]-positive and HPV-negative cohorts). Evaluable patients were defined as those with available paired pre-treatment (screening) and post-treatment (day 29) tissue samples. The minimum sample size of 21 patients per HPV cohort was not based on a statistical power calculation but was determined to detect, with greater than 66% or 89% probability, safety events occurring at an incidence rate of 5% or 10%, respectively. Assuming pathologic complete response rates of 10%, 15%, or 20%, this sample size could detect with greater than 89%, 97%, or 99% probability, respectively, at least one pathologic complete response.

Supplementary methods 2. Definitions of censoring for recurrence-free and overall survival

For analysis of recurrence-free survival, the following censoring rules were defined in the statistical analysis plan:

- Patients who remain alive and whose disease has not recurred will be censored on the date of last known alive date.
- Patients who do not have any post-surgery disease assessments and who remain alive will be censored on the date of surgery.
- Patients who receive subsequent anticancer therapy without recurrence or death will be censored at the date of last evaluable disease assessment before initiation of subsequent therapy or on the date of initiation of subsequent therapy. Note that protocol-allowed standard of care adjuvant therapy is not counted as subsequent therapy for this rule.

In relation to the analysis of overall survival, the following censoring rules were defined in the statistical analysis plan:

- Patients who remain alive will be censored on the date of last known alive.

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Table S1 Treatment-related adverse events and select treatment-related adverse events in 26 treated patients with HPV-positive HNSCC

TRAEs, No. (%)	HPV-positive HNSCC (N=26)	
	Any grade	Grade 3–4
Any TRAE[a]	19 (73.1)	5 (19.2)
Fatigue	6 (23.1)	0
Amylase increased	3 (11.5)	0
Localized edema	3 (11.5)	0
Lipase increased	2 (7.7)	2 (7.7)
Chills	2 (7.7)	0
Headache	2 (7.7)	0
Pruritis	2 (7.7)	0
Pyrexia	2 (7.7)	0
Colitis	1 (3.8)	1 (3.8)
Dehydration	1 (3.8)	1 (3.8)
Glossodynia	1 (3.8)	1 (3.8)
Myasthenia gravis	1 (3.8)	1 (3.8)
Abdominal pain	1 (3.8)	0
Asthenia	1 (3.8)	0
Back pain	1 (3.8)	0
Blood thyroid stimulating hormone decreased	1 (3.8)	0
Diarrhea	1 (3.8)	0
Flushing	1 (3.8)	0
Formication	1 (3.8)	0
Hot flush	1 (3.8)	0
Impetigo	1 (3.8)	0
Mouth hemorrhage	1 (3.8)	0
Myalgia	1 (3.8)	0
Nausea	1 (3.8)	0
Radiation skin injury	1 (3.8)	0
Rash macular	1 (3.8)	0
Skin exfoliation	1 (3.8)	0
Tendon disorder	1 (3.8)	0
TRAEs leading to discontinuation	0	0
Any treatment-related serious AE[b]	2 (7.7)	2 (7.7)
Dehydration	1 (3.8)	1 (3.8)

Glossodynia	1 (3.8)	1 (3.8)
Myasthenia gravis	1 (3.8)	1 (3.8)
Mouth hemorrhage	1 (3.8)	0
Any treatment-related serious AE leading to discontinuation	0	0
Select TRAEs, No. (%)[c]	**Any grade**	**Grade 3–4**
Any select TRAE	4 (15.4)	1 (3.8)
Skin	2 (7.7)	0
Pruritus	2 (7.7)	0
Rash macular	1 (3.8)	0
Skin exfoliation	1 (3.8)	0
Endocrine	1 (3.8)	0
Blood thyroid stimulating hormone decreased	1 (3.8)	0
Gastrointestinal	2 (7.7)	1 (3.8)
Colitis	1 (3.8)	1 (3.8)
Diarrhea	1 (3.8)	0
Hepatic	0	0
Hypersensitivity	0	0
Pulmonary	0	0
Renal	0	0

AE, adverse event; HNSCC, head and neck squamous cell carcinoma; HPV, human papillomavirus; TRAE, treatment-related adverse event.

[a]Includes events reported between first dose of neoadjuvant nivolumab and 100 days after the last dose. Individual patients may have had more than one TRAE.
[b]Individual patients may have had more than one treatment-related serious AE.
[c]AEs deemed by the investigator to have a potential immunologic cause. Individual patients may have had more than one select TRAE.

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Table S2 Treatment-related adverse events and select treatment-related adverse events in 26 treated patients with HPV-negative HNSCC

TRAEs, No. (%)	HPV-negative HNSCC (N=26)	
	Any grade	Grade 3–4
Any TRAE[a]	14 (53.8)	3 (11.5)
Fatigue	5 (19.2)	0
Lipase increased	2 (7.7)	2 (7.7)
Rash maculo-papular	2 (7.7)	1 (3.8)
Amylase increased	2 (7.7)	0
Hyperthyroidism	2 (7.7)	0
Pyrexia	2 (7.7)	0
Arthralgia	1 (3.8)	0
Dermatitis	1 (3.8)	0
Diarrhea	1 (3.8)	0
Eosinophilia	1 (3.8)	0
Facial pain	1 (3.8)	0
Hyperhidrosis	1 (3.8)	0
Hypothyroidism	1 (3.8)	0
Influenza-like illness	1 (3.8)	0
Infusion-related reaction	1 (3.8)	0
Lymphocyte count decreased	1 (3.8)	0
Monocytosis	1 (3.8)	0
Mouth hemorrhage	1 (3.8)	0
Myalgia	1 (3.8)	0
Nausea	1 (3.8)	0
Night sweats	1 (3.8)	0
Pancreatitis	1 (3.8)	0
Platelet count increased	1 (3.8)	0
Pruritis	1 (3.8)	0
Salivary hypersecretion	1 (3.8)	0
TRAEs leading to discontinuation	0	0
Any treatment-related serious AE[b]	4 (15.4)	0
Facial pain	1 (3.8)	0
Fatigue	1 (3.8)	0
Hyperhidrosis	1 (3.8)	0

Mouth hemorrhage	1 (3.8)	0
Pancreatitis	1 (3.8)	0
Pyrexia	1 (3.8)	0
Any treatment-related serious AE leading to discontinuation	0	0
Select TRAEs, No. (%)[c]	**Any grade**	**Grade 3–4**
Any select TRAE	5 (19.2)	1 (3.8)
Skin	4 (15.4)	1 (3.8)
Rash maculo-papular	2 (7.7)	1 (3.8)
Dermatitis	1 (3.8)	0
Pruritus	1 (3.8)	0
Endocrine	2 (7.7)	0
Hyperthyroidism	2 (7.7)	0
Hypothyroidism	1 (3.8)	0
Gastrointestinal	1 (3.8)	0
Diarrhea	1 (3.8)	0
Hepatic	0	0
Hypersensitivity	1 (3.8)	0
Infusion-related reaction	1 (3.8)	0
Pulmonary	0	0
Renal	0	0

AE, adverse event; HNSCC, head and neck squamous cell carcinoma; HPV, human papillomavirus; TRAE, treatment-related adverse event.

[a]Includes events reported between first dose of neoadjuvant nivolumab and 100 days after the last dose. Individual patients may have had more than one TRAE.
[b]Individual patients may have had more than one treatment-related serious AE.
[c]AEs deemed by the investigator to have a potential immunologic cause. Individual patients may have had more than one select TRAE

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Table S3 Death summary

Deaths, No.	HPV-positive HNSCC (n=26)	HPV-negative HNSCC (n=26)
Any cause	**1**	**13**
Disease progression	1	8
Median TTD (range), days[a]	557 (NA–NA)	413.5 (64–1500)
AEs unrelated to neoadjuvant nivolumab or protocol surgery[b]	0	5[c]
Median TTD (range), days[a]	–	122 (16–363)

AE, adverse event; HNSCC, head and neck squamous cell carcinoma; HPV, human papillomavirus; NA, not applicable; TTD, time to death.

[a]Time to death calculated from date of last dose of nivolumab.
[b]AE causality for deaths was determined in relation to neoadjuvant nivolumab treatment and the protocol surgery only. Any subsequent treatments or medical procedures were not directly assessed for relationship to deaths.
[c]One patient who received two doses of nivolumab and did not undergo surgery or biopsy died from multiple organ dysfunction syndrome following coronary artery bypass surgery 16 days after their last dose; one patient who received two doses of nivolumab and underwent surgery died from febrile neutropenia 96 days after their last dose; one patient who received two doses of nivolumab and underwent surgery died from septic shock and pulmonary embolism 363 days after their last dose; one patient who received two doses of nivolumab and underwent surgery died from arterial injury (injury to carotid artery during unrelated surgery) 306 days after their last dose; one patient who received two doses of nivolumab and did not undergo surgery or biopsy died from sepsis 122 days after their last dose.

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Table S4 Pathologic and radiographic outcomes and baseline tumor PD-L1 expression for 26 treated patients with HPV-positive HNSCC

Patient No.	Procedure	Pathologic response		% Target lesion change	RFS[b]		OS[c]		% Tumor PD-L1 expression[d]	PD-L1 CPS[d]
		Site review[a]	Central review		Status	Months	Status	Months		
1	Surgery	Non-pCR	NR	–20.0	No event	51.4	In follow-up	52.3	1	1
2	Surgery	Non-pCR	NR	–6.7	No event	38.4	In follow-up	39.4	10	12
3	Surgery	Non-pCR	MPR	–9.5	No event	37.6	In follow-up	38.7	100	100
4	Surgery	Non-pCR	pPR	+16.7	No event	36.7	In follow-up	37.7	10	1
5	Surgery	Non-pCR	NR	–15.2	No event	33.0	In follow-up	34.0	60	60
6	Surgery	Non-pCR	NR	–56.3	No event	32.4	In follow-up	33.3	*NA*	*NA*
7	Surgery	Non-pCR	NR	–26.7	No event	30.1	In follow-up	31.2	40	41
8	Surgery	Non-pCR	NR	0.0	No event	30.4	In follow-up	31.5	1	10
9	Surgery	Non-pCR	NR	0.0	No event	31.7	In follow-up	32.7	40	65
10	Surgery	Non-pCR	NR	0.0	No event	30.9	In follow-up	31.9	0	0
11	Surgery	Non-pCR	pPR	–66.7	Recurrence	7.2	In follow-up	30.4	20	12
12	Surgery	Non-pCR	NR	+9.6	No event[e]	0.0	In follow-up	30.7	5	15
13	Surgery	Non-pCR	pPR	–15.0	Recurrence	3.6	In follow-up	34.5	*NA*	*NA*
14	Surgery	Non-pCR	NR	–1.8	No event	40.3	In follow-up	41.2	0	2
15	Surgery	Non-pCR	NR	–75.0	No event	42.7	In follow-up	43.7	40	40
16	Surgery	Non-pCR	*NA*	–12.0	No event	39.1	In follow-up	40.0	10	10
17	Surgery	Non-pCR	NR	+32.4	WC	18.2	WC	19.1	3	2
18	Surgery	Non-pCR	NR	+54.3	No event	44.0	In follow-up	44.8	1	15
19	Biopsy	*NA*	NR	–26.4	*NA*	*NA*	In follow-up	52.5	30	50
20	Biopsy	*NA*	pPR	–8.7	*NA*	*NA*	In follow-up	51.0	*NA*	*NA*
21	Biopsy	*NA*	NR	+2.6	*NA*	*NA*	In follow-up	51.0	25	10
22	Biopsy	*NA*	NR	–5.9	*NA*	*NA*	In follow-up	49.7	*NA*	*NA*
23	Biopsy	*NA*	NR	*NA*	*NA*	*NA*	Death	18.8	1	0
24	Biopsy	*NA*	pPR	0.0	*NA*	*NA*	In follow-up	48.1	0	10
25	Biopsy	*NA*	NR	+19.4	*NA*	*NA*	In follow-up	42.8	2	5
26	Biopsy	*NA*	NR	0.0	*NA*	*NA*	LTFU	26.2	*NA*	*NA*

CPS, combined positive score; HNSCC, head and neck squamous cell carcinoma; HPV, human papillomavirus; LTFU, lost to follow-up; MPR, major pathologic response; NA, not available; NR, no response (i.e. no pCR, MPR, or pPR); OS, overall survival; pCR, pathologic complete response; pPR, pathologic partial response; PD-L1, programmed cell death ligand 1; RFS, recurrence-free survival; WC, withdrew consent.

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[a]Represents individual investigative site's pathologic analysis of complete specimens only in patients with a complete surgical resection.
[b]RFS was measured from the date of surgery.
[c]OS was measured from the date of start of neoadjuvant nivolumab therapy.
[d]"NA" (not available) refers to one of the following situations: (i) a sample was not taken for PD-L1 testing or a test was not performed, or (ii) a sample was collected and tested, but the result was not interpretable.
[e]Patient was censored for RFS on the date of surgery due to no post-surgery disease assessments and no death.
All patients received two neoadjuvant doses of nivolumab.

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Table S5 Pathologic and radiographic outcomes and baseline tumor PD-L1 expression for 26 treated patients with HPV-negative HNSCC

Patient No.	Procedure	Pathologic response		% Target lesion change	RFS[b]		OS[c]		% Tumor PD-L1 expression[d]	PD-L1 CPS[d]
		Site review[a]	Central review		Status	Months	Status	Months		
27	Surgery	Non-pCR	NR	–6.5	Death	2.7	Death	3.6	20	45
28	Surgery	Non-pCR	NR	+10.0	Recurrence	6.2	Death	16.5	0	0
29	Surgery	Non-pCR	NR	+11.1	Recurrence	8.1	Death	43.9	80	80
30	Surgery	Non-pCR	NR	–42.0	No event[e]	0.0	In follow-up	33.6	3[f]	1
31	Surgery	Non-pCR	NR	*NA*	Recurrence	4.8	Death	12.4	*NA*	*NA*
32	Surgery	Non-pCR	NR	–3.6	No event[e]	0.0	In follow-up	43.8	1	5
33	Surgery	Non-pCR	NR	+17.7	Recurrence	2.8	Death	11.6	5	2
34	Surgery	Non-pCR	NR	+15.7	Recurrence	13.5	Death	22.1	0	7
35	Surgery	Non-pCR	NR	–7.8	No event	51.1	In follow-up	51.8	40	90
36	Surgery	Non-pCR	NR	+111.9	Death	1.9	Death	2.7	35	35
37	Surgery	Non-pCR	pPR	–19.6	No event	45.4	In follow-up	46.6	40	56
38	Surgery	Non-pCR	NR	0.0	WC	6.2	WC	7.2	50	60
39	Surgery	Non-pCR	*NA*	–24.0	No event	53.3	In follow-up	53.9	35	40
40[g]	Surgery	Non-pCR	*NA*	0.0	No event	33.5	In follow-up	34.4	50	53
41	Surgery	Non-pCR	NR	+5.4	Death	9.6	Death	10.5	0	0
42	Surgery	Non-pCR	NR	+16.9	No event	43.8	In follow-up	44.8	0	0
43	Surgery	Non-pCR	NR	–24.0	No event	42.4	In follow-up	43.3	*NA*	*NA*
44	Surgery	Non-pCR	NR	0.0	No event	41.4	In follow-up	42.2	25	35
45	Surgery	Non-pCR	NR	–2.8	No event	39.2	In follow-up	39.9	5	6
46	Biopsy[h]	*NA*	pPR	-13.3	No event[i]	14.5	Death	49.8	0	0
47	Biopsy	*NA*	NR	+40.0	*NA*	*NA*	Death	9.9	5	15
48	Biopsy	*NA*	pCR	–54.5	*NA*	*NA*	In follow-up	49.7	*NA*	*NA*
49	None	*NA*	*NA*	*NA*	*NA*	*NA*	Death	1.0	100	100
50	None	*NA*	*NA*	+30.2	*NA*	*NA*	Death	7.8	10	25
51	None	*NA*	*NA*	+32.6	*NA*	*NA*	WC	1.8	100	100
52	None	*NA*	*NA*	+40.0	*NA*	*NA*	Death	4.4	40	5

CPS, combined positive score; HNSCC, head and neck squamous cell carcinoma; HPV, human papillomavirus; MPR, major pathologic response; NA, not available; NR, no response (i.e. no pCR, MPR, or pPR); OS, overall survival; pCR, pathologic complete response; pPR, pathologic partial response; PD-L1, programmed cell death ligand 1; RFS, recurrence-free survival; WC, withdrew consent.

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[a]Represents individual investigative site's pathologic analysis of complete specimens only in patients with a complete surgical resection.
[b]RFS was measured from the date of surgery.
[c]OS was measured from the date of start of neoadjuvant nivolumab therapy.
[d]"NA" (not available) refers to one of the following situations: (i) a sample was not taken for PD-L1 testing or a test was not performed, or (ii) a sample was collected and tested, but the result was not interpretable.
[e]Patient was censored for RFS on the date of surgery due to no post-surgery disease assessments and no death.
[f]Tumor PD-L1 expression was confirmed after database lock.
[g]Patient received only one neoadjuvant dose of nivolumab; all other patients received two neoadjuvant doses.
[h]Patient was captured in database lock as receiving complete surgical resection; however, the study site subsequently indicated that they had received a planned post-nivolumab biopsy instead.
[i]Patient was censored at last tumor assessment.

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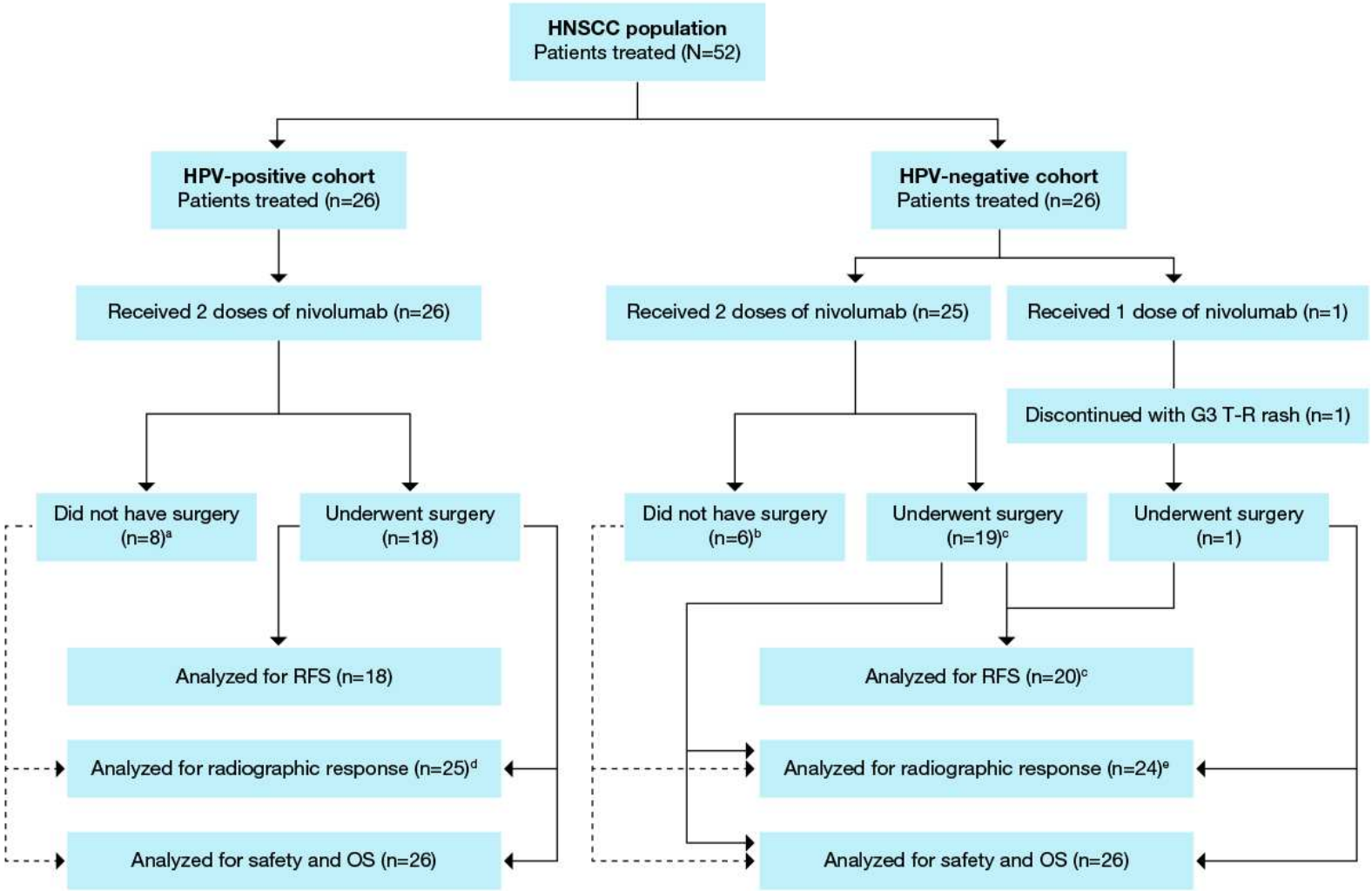
Figure S1 Patient treatment and disposition



HPV, human papillomavirus; G3, grade 3; OS, overall survival; RFS, recurrence-free survival; T-R, treatment-related.

[a]All eight patients received a planned post-nivolumab biopsy instead of surgery due to misinterpretation of the study protocol. Surgery is defined as complete surgical resection of all disease sites with intent to cure.
[b]Four patients did not receive surgery or biopsy due to non-treatment-related multiple organ dysfunction syndrome (n=1), consent withdrawal before surgery (n=1), or rapid tumor progression (n=2); two other patients received a planned post-nivolumab biopsy instead of surgery due to misinterpretation of the study protocol.
[c]One patient initially reported as having received surgery at database lock was subsequently found to have received a planned post-nivolumab biopsy instead, not complete surgical resection; this patient is categorized as having received surgery in the RFS outcomes.
[d]One patient who had a planned post-nivolumab biopsy instead of surgery did not have available data for assessing radiographic response.
[e]One patient who underwent surgery and one who did not undergo surgery or biopsy did not have available data for assessing radiographic response.

The numbers of patients in each cohort who underwent site and/or central pathology review are shown in Tables S4 and S5 in this supplement.

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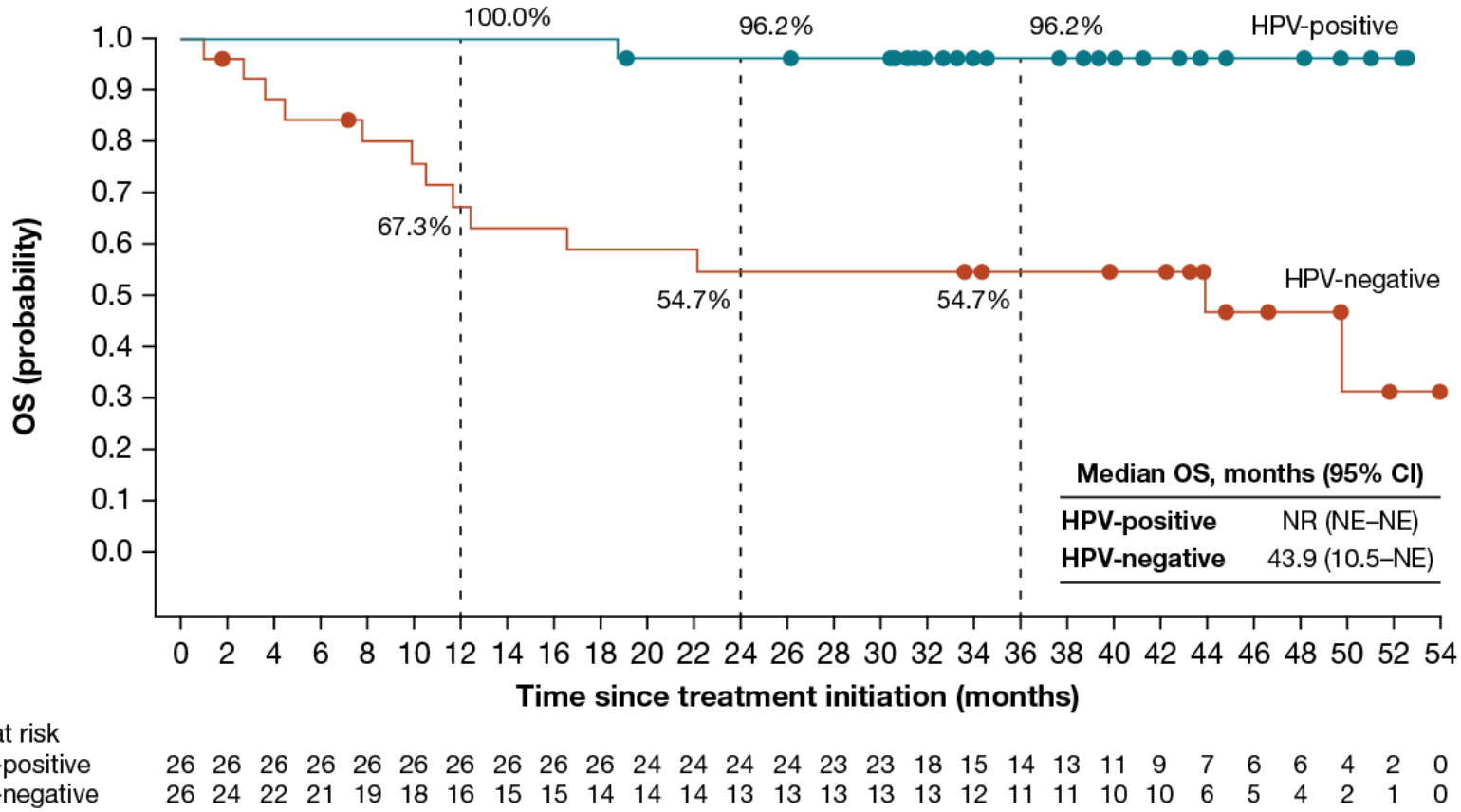
Figure S2 Overall survival in all treated patients with HNSCC in the HPV-positive (n=26) and HPV-negative (n=26) cohorts. Median follow-up for these cohorts was 38.2 months (range, 18.8–52.5) and 27.9 months (range, 1.0–53.9), respectively. The single death in the HPV-positive cohort was due to tumor progression. There were 13 on-study deaths in the HPV-negative cohort, eight due to disease progression and five due to AEs unrelated to neoadjuvant nivolumab or protocol surgery.



HPV, human papillomavirus; NE, not estimable; NR, not reached; OS, overall survival.

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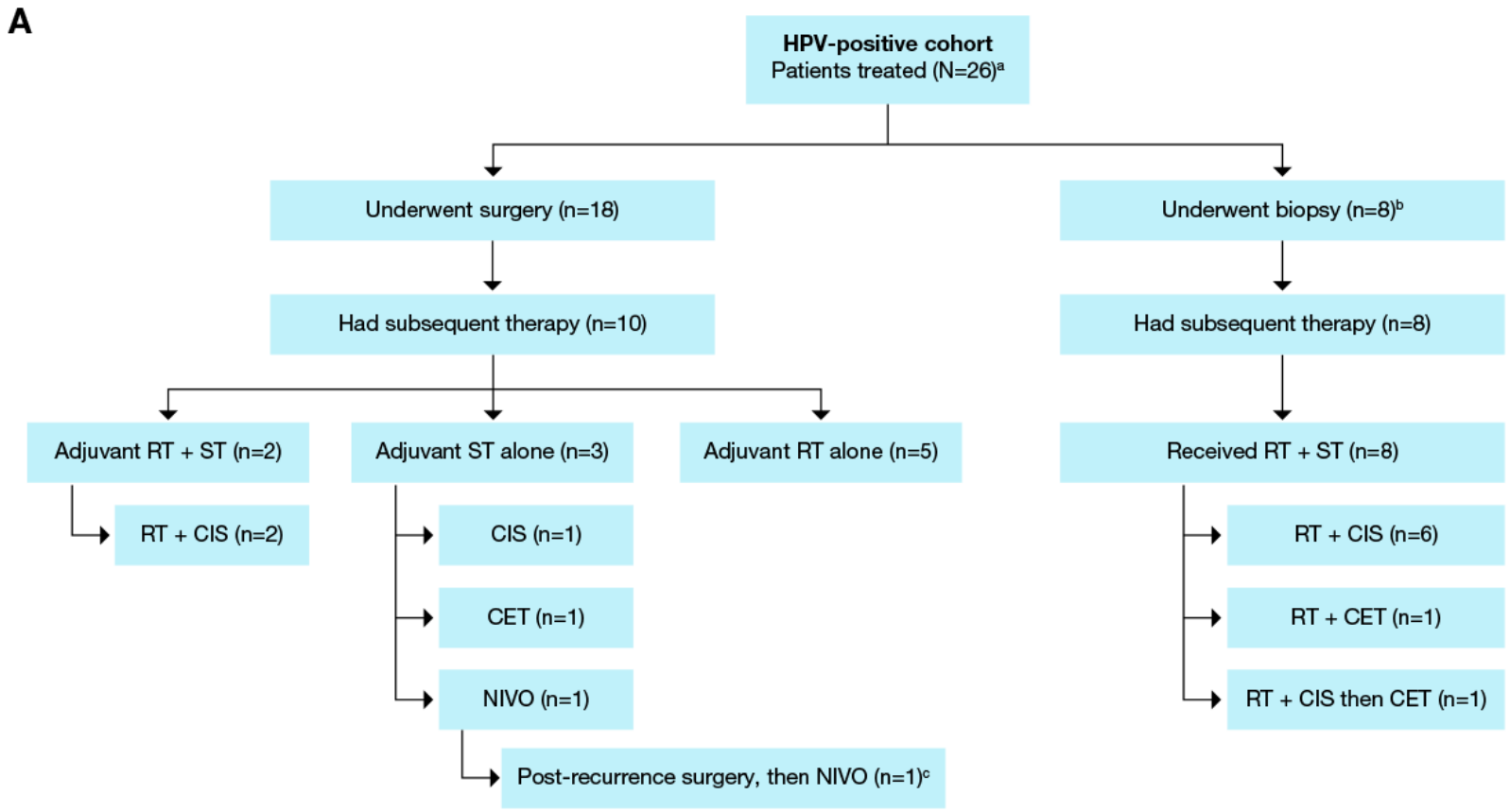
Figure S3 Flow chart of anticancer therapies subsequent to neoadjuvant nivolumab for the (A) HPV-positive and (B) HPV-negative HNSCC cohorts



CET, cetuximab; CIS, cisplatin; HPV, human papillomavirus; NIVO, nivolumab; RT, radiotherapy; ST, systemic therapy.

[a]All patients received two doses of neoadjuvant nivolumab.
[b]All eight patients received a planned post-nivolumab biopsy instead of complete surgical resection due to misinterpretation of the study protocol.
[c]Patient received off-study post-recurrence nivolumab (i.e. outside of the CheckMate 358 trial).

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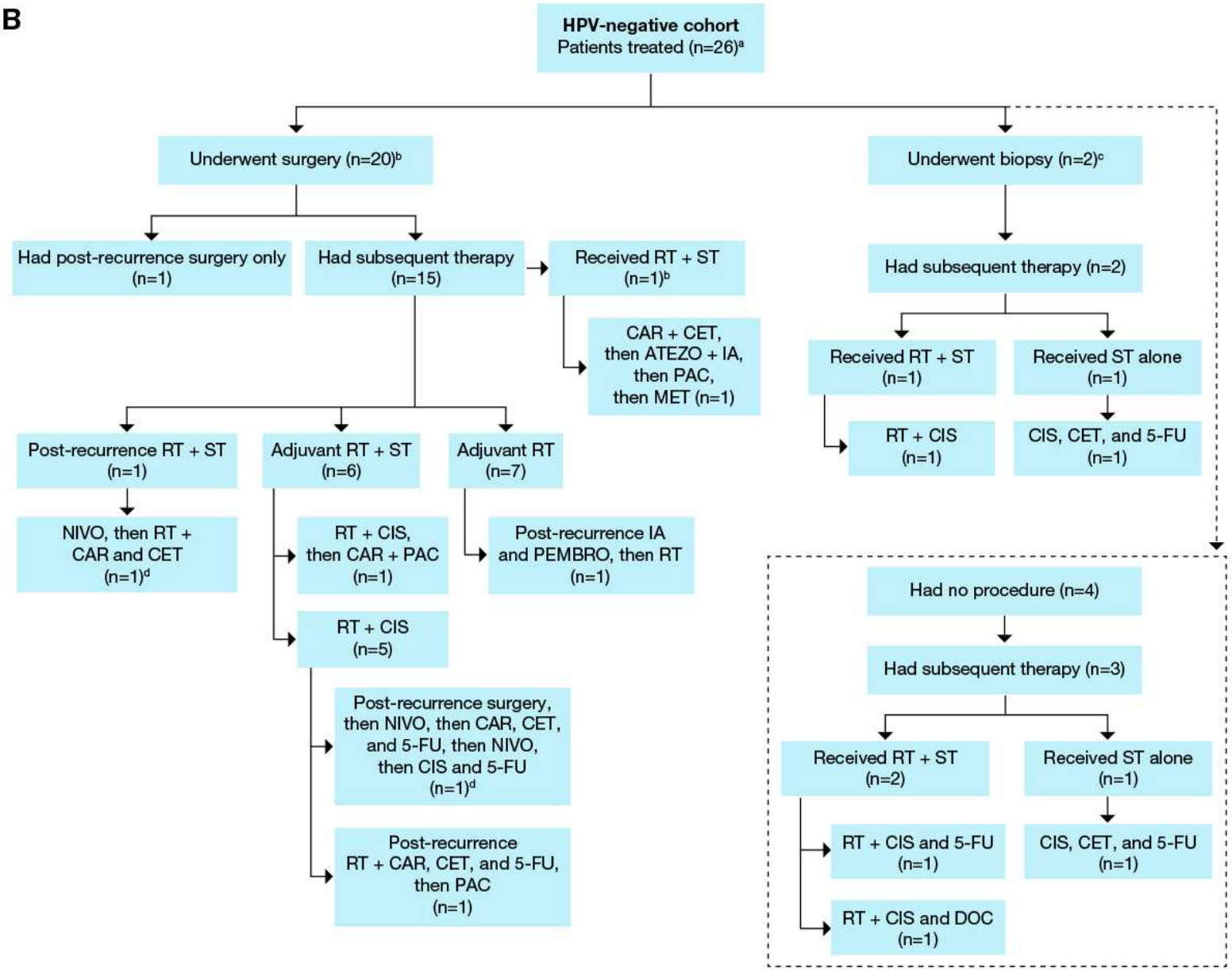
B



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5-FU, fluorouracil; ATEZO, atezolizumab; CAR, carboplatin; CET, cetuximab; CIS, cisplatin; DOC, docetaxel; HPV, human papillomavirus; IA, investigational agent; MET, methotrexate; NIVO, nivolumab; PAC, paclitaxel; PEMBRO, pembrolizumab; RT, radiotherapy; ST, systemic therapy.

[a]25 patients received two doses of neoadjuvant nivolumab; one patient received only a single neoadjuvant dose.
[b]One patient was reported as having received surgery at database lock but was subsequently found to have received a planned post-nivolumab biopsy instead, not complete surgical resection.
[c]Both patients received a planned post-nivolumab biopsy instead of complete surgical resection due to misinterpretation of the study protocol.
[d]Patient received on-study post-recurrence nivolumab per the CheckMate 358 protocol.

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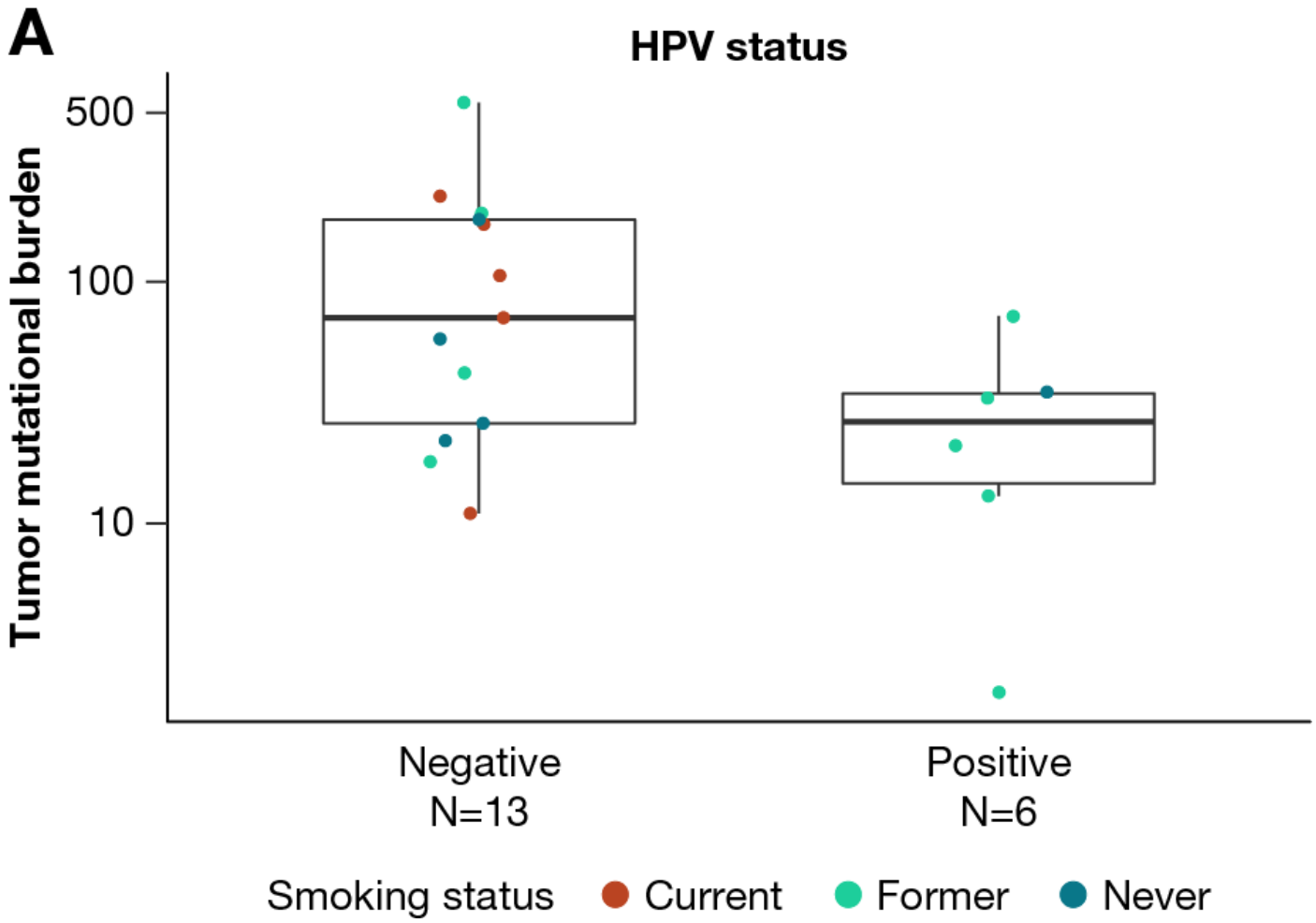
Figure S4 Association of TMB with (A) HPV status and smoking status or (B) pathologic response by central review in patients with relevant available data. HPV-positive tumors appeared to have lower TMB than HPV-negative tumors. None of six patients with HPV-positive tumors had TMB >100, versus six of 13 patients with HPV-negative tumors. There was no clear relationship between TMB and smoking status in these samples. Because there were only two pathologic responses among these samples (both in HPV-positive tumors), no conclusions can be drawn about a potential relationship between TMB and pathologic response. Of the 19 patients with TMB data, 16 had surgery, one had a planned post-nivolumab biopsy instead, and two had neither surgery nor biopsy.



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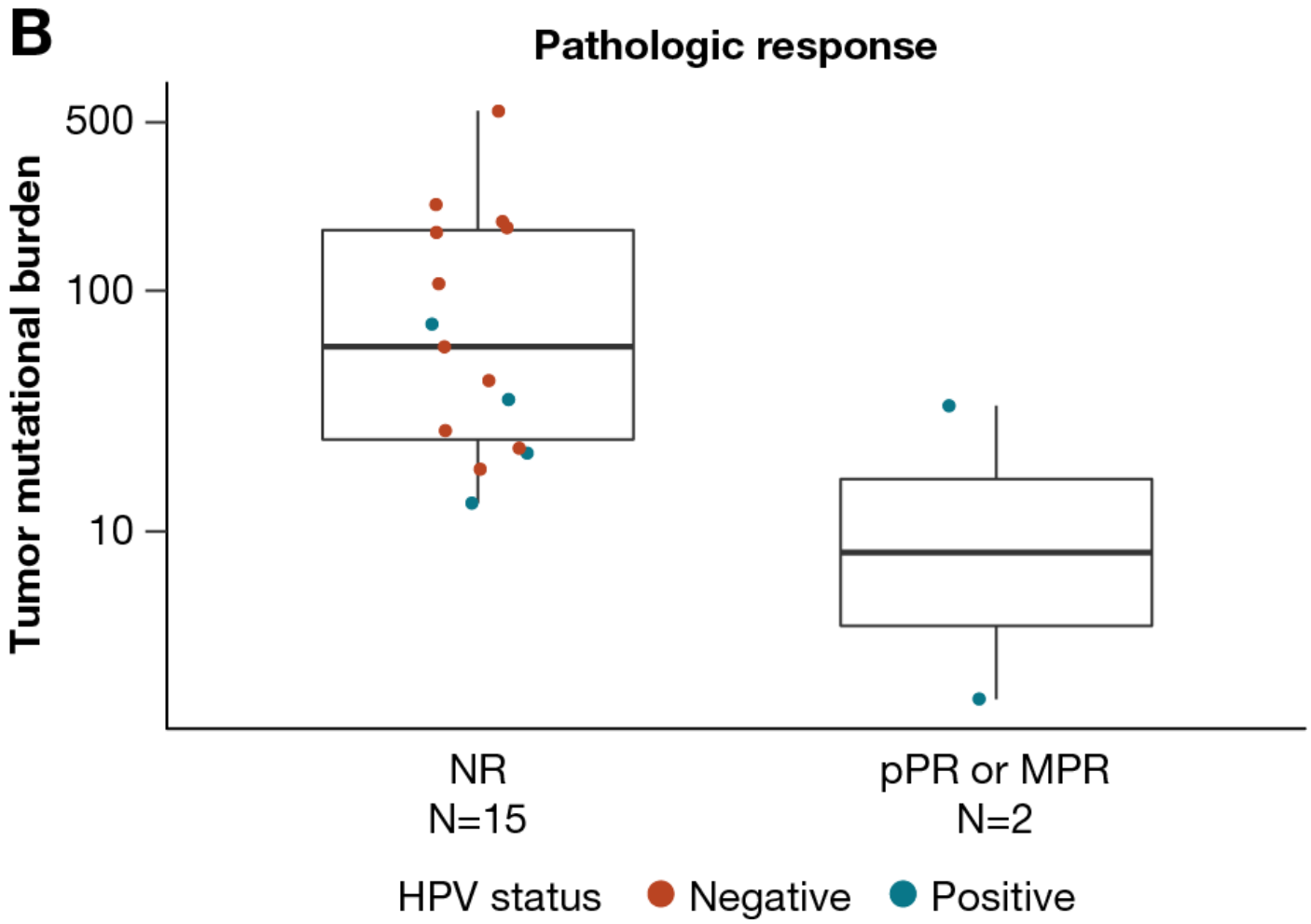

HPV, human papillomavirus; MPR, major pathologic response; NR, no response (i.e. no pCR, MPR, or pPR); pPR, pathologic partial response; TMB, tumor mutational burden.

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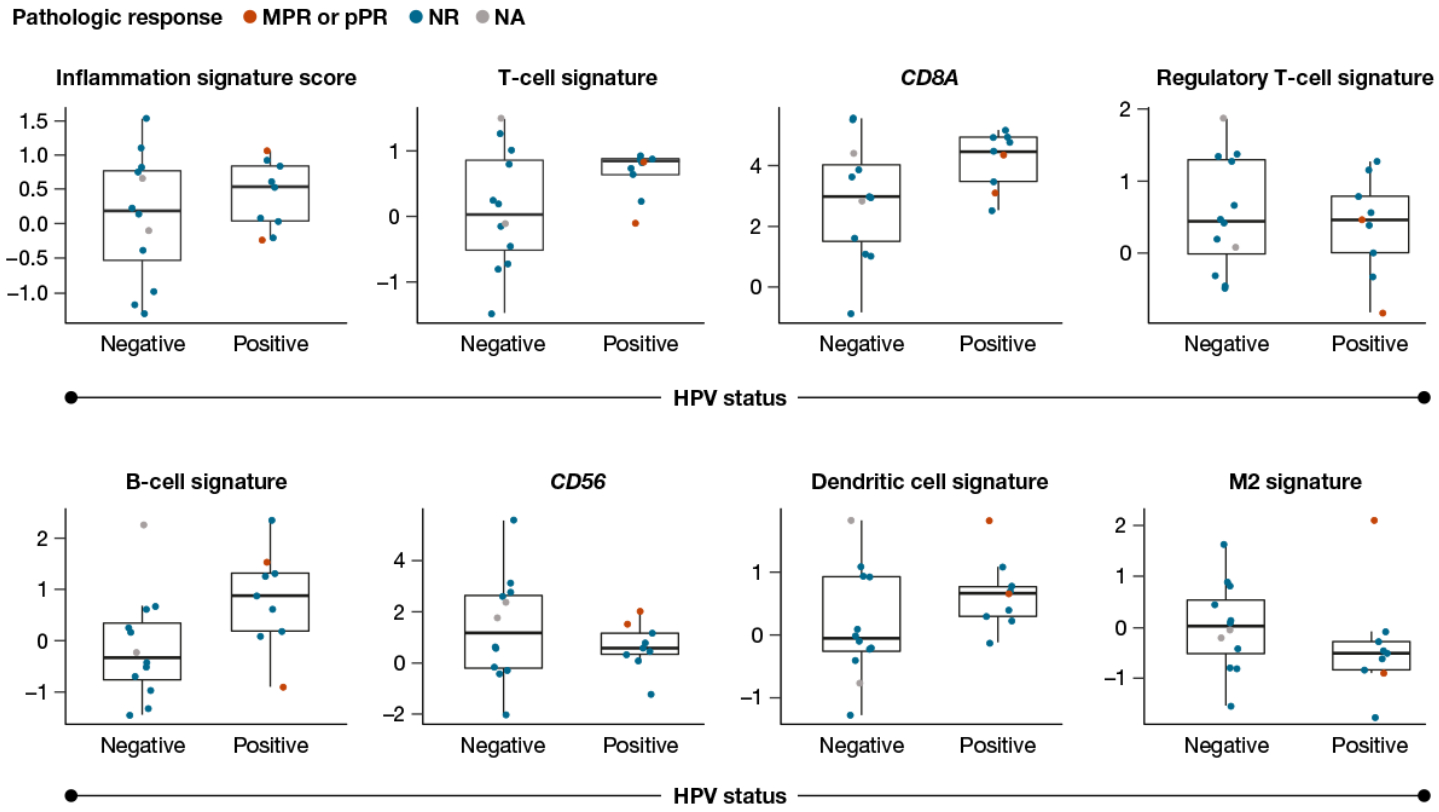
Figure S5 Gene expression profiling (RNAseq) for immune cell signatures and individual genes in patients with relevant available data (HPV-positive, n=9; HPV-negative, n=12). Inflammation gene signature scores, T-cell signature, and *CD8A* gene expression, as well as B-cell and dendritic cell signature scores appeared to be higher in HPV-positive tumors. In contrast, M2 macrophage signature and *CD56* gene expression were slightly lower in HPV-positive tumors. Regulatory T-cell signature expression appeared to be similar between the two groups. Because there were only two pathologic responses among these samples (both in HPV-positive tumors), no conclusions can be drawn about a potential relationship between immune cell signatures and pathologic response. Of the 21 patients with RNAseq data, 19 had surgery and two had neither surgery nor biopsy.



HPV, human papillomavirus; MPR, major pathologic response; NA, not available; NR, no response (i.e. no pCR, MPR, or pPR); pPR, pathologic partial response.

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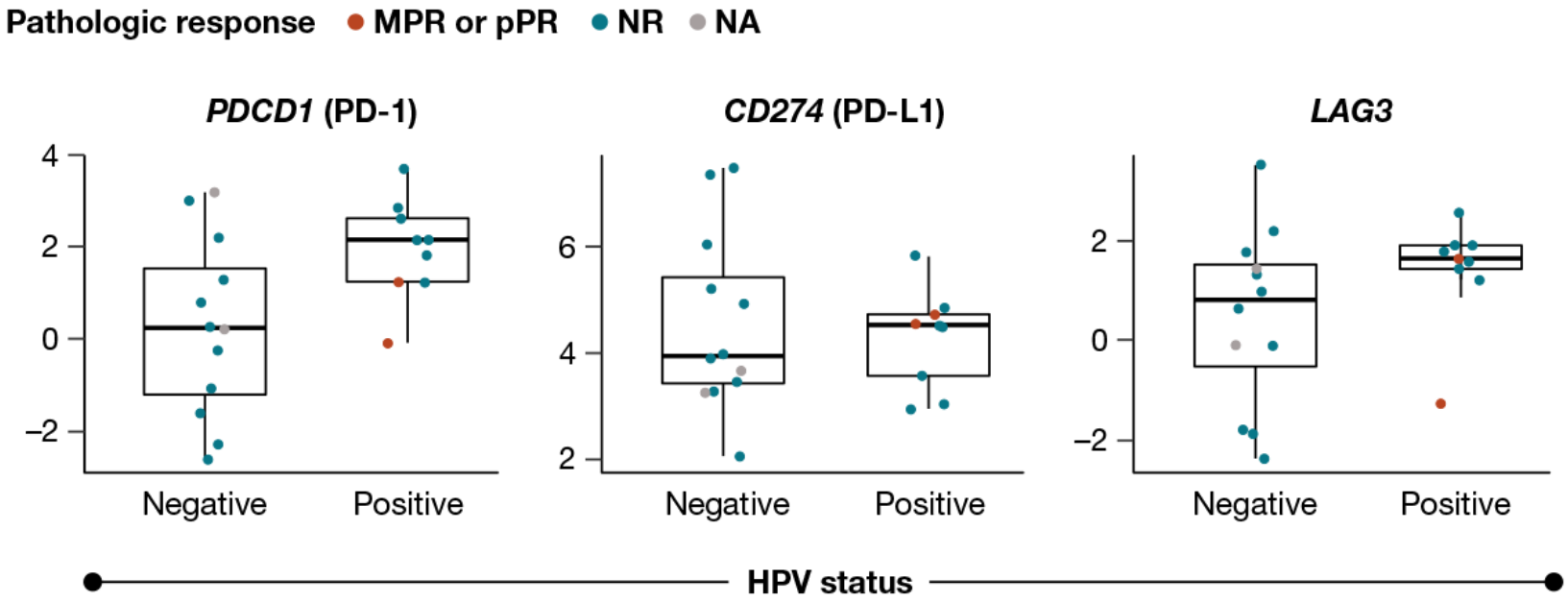
Figure S6 Gene expression profiling (RNAseq) for immune checkpoint molecules in patients with relevant available data (HPV-positive, n=9; HPV-negative, n=12). Expression of the immune checkpoints *PDCD1* (PD-1) and *LAG3* appeared to be higher in HPV-positive versus HPV-negative tumors. Because there were only two pathologic responses among these samples (both in HPV-positive tumors), no conclusions can be drawn about a potential relationship between immune cell signatures and pathologic response. Of the 21 patients with RNAseq data, 19 had surgery and two had neither surgery nor biopsy.



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