

## Supplemental Online Content

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

**eTable 1.** Characteristics of patients with undetectable circulating TTMV-HPV DNA

| Patient         | Clinical T stage <sup>a</sup> | Clinical N stage <sup>a</sup> | Tissue p16 IHC | Tissue HR-HPV RNA ISH | Tissue TTMV-HPV DNA |
|-----------------|-------------------------------|-------------------------------|----------------|-----------------------|---------------------|
| 1               | T1                            | N0                            | positive       | HPV16/18 positive     | TTMV-HPV16 positive |
| 2               | T1                            | N0                            | positive       | HPV16/18 positive     | TTMV-HPV16 positive |
| 3               | T1                            | N1                            | positive       | HPV16/18 positive     | TTMV-HPV16 positive |
| 4               | T4                            | N0                            | positive       | HPV16/18 positive     | TTMV-HPV18 positive |
| 5               | T1                            | N1                            | positive       | HPV16/18 positive     | TTMV-HPV16 positive |
| 6               | T1                            | N0                            | positive       | HPV16/18 positive     | not available       |
| 7               | T1                            | N0                            | positive       | HPV16/18 positive     | not available       |
| 8               | T1                            | N0                            | positive       | HPV16/18 positive     | not available       |
| 9               | T1                            | N2                            | positive       | HPV16/18 positive     | not available       |
| 10              | T2                            | N1                            | positive       | HPV16/18 negative     | TTMV-HPV33 positive |
| 11 <sup>b</sup> | T0                            | N0                            | positive       | not available         | TTMV-HPV16 positive |
| 12              | T2                            | N1                            | positive       | not available         | not available       |

<sup>a</sup>AJCC 8<sup>th</sup> edition clinical staging, adjusted to reflect stage at time of plasma collection.

<sup>b</sup>Patient had diagnostic tonsillectomy, no remaining tumor at time of plasma collection, thus was staged T0N0.

Abbreviations: IHC, immunohistochemistry; ISH, in situ hybridization; TTMV, tumor-tissue-modified virus

**eTable 2.** Radiographic features of extranodal extension compared with circulating TTMV-HPV DNA detectability and score

| Characteristic                   | No. in each category<br>N (% of total) | No. in each category with detectable TTMV-HPV DNA<br>N (% of category) | Effect size <sup>a</sup><br><i>*Proportion difference (95% CI)</i><br>or<br><i>**Kendall's tau (95% CI)</i> | Median TTMV-HPV DNA Score (frag/ml) <sup>b</sup><br>Median (IQR) | Effect size <sup>c</sup><br><i>*Difference in medians (95% CI)</i><br>or<br><i>**η<sup>2</sup> (95% CI)</i> |
|----------------------------------|--|--|---|--|---|
| Total                            | 96                                     |  |   | 365 (74-2688)  |   |
| CT: Perinodal fat stranding      |  |  | 0.04<br>(-0.07, 0.15)*  |  | 497<br>(-41, 1536)*   |
| No                               | 48 (50)                                | 43 (90)  |   | 248 (38-1904)  |   |
| Yes                              | 48 (50)                                | 45 (94)  |   | 745 (152-3513)   |   |
| CT: Absent perinodal fat plane   |  |  | 0.19<br>(0.005, 0.37)*  |  | 452<br>(14, 1428)*  |
| No                               | 22 (23)                                | 17 (77)  |   | 106 (13-795)   |   |
| Yes                              | 74 (77)                                | 71 (96)  |   | 557 (110-5285)   |   |
| CT: Lobular nodal contours       |  |  | 0.12<br>(0.06, 0.36)*   |  | 505<br>(44, 1287)*  |
| No                               | 31 (32)                                | 24 (77)  |   | 209 (19-1712)  |   |
| Yes                              | 65 (68)                                | 64 (98)  |   | 714 (110-2968)   |   |
| CT: Irregular nodal margin       |  |  | -0.04<br>(-0.17, 0.10)*   |  | -21<br>(-652, 1571)*  |
| No                               | 69 (72)                                | 64 (93)  |   | 375 (75-2407)  |   |
| Yes                              | 27 (28)                                | 24 (89)  |   | 354 (21-5403)  |   |
| CT: Nodal central necrosis       |  |  | 0.16<br>(0.06, 0.26)*   |  | 1333<br>(251, 2717)*  |
| No                               | 50 (52)                                | 42 (84)  |   | 210 (23-881)   |   |
| Yes                              | 46 (48)                                | 46 (100)   |   | 1543 (193-5986)  |   |
| CT: Conglomerate nodes           |  |  | 0.21<br>(0.08, 0.332)*  |  | 786<br>(259, 2364)*   |
| No                               | 39 (41)                                | 31 (79)  |   | 95 (19-795)  |   |
| Yes                              | 57 (59)                                | 57 (100)   |   | 881 (205-6043)   |   |
| CT: Number of nodal ENE features |  |  | 0.026<br>(0.004, 0.047)**   |  | 0.03<br>(0.00, 0.09)**  |
| 0-1                              | 19 (20)                                | 15 (79)  |   | 58 (13-386)  |   |
| 2-3                              | 27 (28)                                | 23 (85)  |   | 248 (68-2410)  |   |
| >3                               | 50 (52)                                | 50 (100)   |   | 823 (162-5404)   |   |

<sup>a</sup>Effect size metrics for comparisons of the proportion of subjects with detectable TTMV-HPV DNA are: Proportion difference for characteristics with 2 categories, labeled with one star (\*); and Kendall's tau for characteristics with more than 2 categories, labeled with 2 stars (\*\*).

<sup>b</sup>TTMV scores are rounded to nearest integer value.

<sup>c</sup>Effect size metrics for comparisons of median TTMV-HPV DNA scores are: Difference in medians for characteristics with 2 categories, labeled with one star (\*); and Eta-squared (η<sup>2</sup>) for characteristics with more than 2 categories, labeled with two stars (\*\*). Abbreviations: TTMV, tumor-tissue-modified virus; IQR, interquartile range; ENE, extranodal extension

**eTable 3.** Sensitivity analysis including only HPV16-positive OPSCCs of clinicopathologic and radiographic characteristics compared with circulating TTMV-HPV16 DNA detectability and score

| Characteristic                | No. in each category<br>N (% of total) | No. in each category with detectable TTMV-HPV16 DNA<br>N (% of category) | Effect size <sup>a</sup><br><i>*Proportion difference (95% CI)</i><br>or<br><i>**Kendall's tau (95% CI)</i> | Median TTMV-HPV16 DNA Score (frag/ml) <sup>b</sup><br>Median (IQR) | Effect size <sup>c</sup><br><i>*Difference in medians (95% CI)</i><br>or<br><i>**η<sup>2</sup> (95% CI)</i> |
|-------------------------------|--|--|---|--|---|
| Total                         | 95                                     | 86 (91)  |   | 386 (68-3895)  |   |
| Clinical characteristics      |  |  |   |  |   |
| Sex                           |  |  |   |  |   |
| Female                        | 11 (12)                                | 9 (82)   | 0.10<br>(-0.14, 0.33)*  | 68 (6-193)   | 574<br>(200, 1545)*   |
| Male                          | 84 (88)                                | 77 (92)  |   | 642 (102-3977)   |   |
| Age (years)                   |  |  |   |  |   |
| <50                           | 10 (11)                                | 10 (100)   | -0.013<br>(-0.038, 0.013)**   | 5695 (211-10292)   | 0.05<br>(0.00, 0.12)**  |
| 50-59                         | 26 (27)                                | 24 (92)  |   | 161 (31-1438)  |   |
| 60-69                         | 41 (43)                                | 37 (90)  |   | 287 (95-2968)  |   |
| 70+                           | 18 (19)                                | 15 (83)  |   | 1895 (354-7861)  |   |
| Clinical T stage <sup>d</sup> |  |  |   |  |   |
| T0                            | 8 (8)                                  | 7 (88)   | 0.028<br>(0.001, 0.056)**   | 1328 (40-8574)   | 0.02<br>(0.00, 0.04)**  |
| T1                            | 32 (34)                                | 24 (75)  |   | 268 (2-4590)   |   |
| T2                            | 33 (35)                                | 33 (100)   |   | 714 (117-3074)   |   |
| T3                            | 9 (9)                                  | 9 (100)  |   | 558 (209-1946)   |   |
| T4                            | 13 (14)                                | 13 (100)   |   | 354 (110-2968)   |   |
| Clinical N stage <sup>d</sup> |  |  |   |  |   |
| N0                            | 10 (11)                                | 4 (40)   | 0.029<br>(0.001, 0.057)**   | 0 (0-21)   | 0.03<br>(0.00, 0.06)**  |
| N1                            | 59 (62)                                | 57 (97)  |   | 558 (93-4451)  |   |
| N2                            | 24 (25)                                | 23 (96)  |   | 1829 (267-4730)  |   |
| N3                            | 2 (2)                                  | 2 (100)  |   | 3339 (22-6656)   |   |
| CT: Primary tumor diameter    |  |  |   |  |   |
| <2cm                          | 44 (52)                                | 37 (84)  | 0.022<br>(-0.005, 0.048)**  | 360 (26-5970)  | 0.04<br>(0.00, 0.11)**  |
| 2-3.9cm                       | 36 (43)                                | 36 (100)   |   | 563 (155-2546)   |   |
| ≥4cm                          | 4 (5)                                  | 4 (100)  |   | 1608 (179-12301)   |   |
| CT: Largest node diameter     |  |  |   |  |   |
| <2cm                          | 20 (24)                                | 14 (70)  | 0.032<br>(0.005, 0.058)**   | 50 (0-267)   | 0.07<br>(0.00, 0.16)**  |
| 2-2.9cm                       | 22 (26)                                | 21 (95)  |   | 1829 (109-7575)  |   |
| 3-3.9cm                       | 24 (29)                                | 24 (100)   |   | 739 (208-6014)   |   |
| ≥4cm                          | 18 (21)                                | 18 (100)   |   | 1525 (344-6656)  |   |
| CT: No. nodal ENE features    |  |  |   |  |   |
| 0-1                           | 16 (19)                                | 13 (81)  | 0.024<br>(0.000, 0.049)**   | 106 (17-590)   | 0.04<br>(0.00, 0.11)**  |
| 2-3                           | 25 (30)                                | 21 (84)  |   | 248 (68-2410)  |   |
| >3                            | 43 (51)                                | 43 (100)   |   | 1438 (248-6656)  |   |
| PET: Primary tumor SUVmax     |  |  |   |  |   |
| <5                            | 14 (15)                                | 11 (79)  | 0.026<br>(0.001, 0.050)**   | 770 (13-2690)  | 0.05<br>(0.00, 0.12)**  |

|                   |         |          |                           |                 |                        |
|-------------------|---------|----------|---------------------------|-----------------|------------------------|
| 5-10              | 22 (24) | 18 (82)  |                           | 360 (10-5285)   |                        |
| 10-15             | 26 (28) | 24 (92)  |                           | 248 (68-935)    |                        |
| >15               | 31 (33) | 31 (100) |                           | 1282 (205-7575) |                        |
| PET: Nodal SUVmax |         |          |                           |                 |                        |
| <5                | 17 (18) | 11 (65)  | 0.036<br>(0.011, 0.061)** | 32 (0-117)      | 0.07<br>(0.00, 0.14)** |
| 5-10              | 23 (25) | 20 (87)  |                           | 1253 (193-5285) |                        |
| 10-15             | 33 (35) | 33 (100) |                           | 343 (93-3895)   |                        |
| >15               | 20 (22) | 20 (100) |                           | 1882 (642-7116) |                        |

<sup>a</sup>Effect size metrics for comparisons of the proportion of subjects with detectable TTMV-HPV DNA are: Proportion difference for characteristics with 2 categories, labeled with one star (\*); and Kendall's tau for characteristics with more than 2 categories, labeled with 2 stars (\*\*).

<sup>b</sup>TTMV scores are rounded to nearest integer value.

<sup>c</sup>Effect size metrics for comparisons of median TTMV-HPV DNA scores are: Difference in medians for characteristics with 2 categories, labeled with one star (\*); and Eta-squared ( $\eta^2$ ) for characteristics with more than 2 categories, labeled with two stars (\*\*).

<sup>d</sup>AJCC 8<sup>th</sup> edition staging.

Abbreviations: TTMV, tumor-tissue-modified virus; IQR, interquartile range; frag/ml, fragments per ml; ENE, extranodal extension

**eFigure. Circulating TTMV-HPV DNA score by HPV genotype.** Horizontal lines indicate medians (dashed) and interquartile ranges (solid).

