

Table S1. Patient Characteristics.

| Patient Characteristics | | |
|---------------------------|------------------|------------|
| Covariate | Status | N (%) |
| HPV | negative | 96(67.1%) |
| | positive | 47(32.9%) |
| p16 | negative | 98(68.5%) |
| | positive | 45(31.5%) |
| Ethnicity | Caucasian | 88(61.5%) |
| | African American | 14(9.8%) |
| | Hispanic | 40(28%) |
| | Asian | 1(0.7%) |
| Tobacco | Never | 56(39.2%) |
| | Active/Former | 87(60.8%) |
| Phimosis | No | 81(57.4%) |
| | Yes | 60(42.6%) |
| Circumcised | No | 101(71.6%) |
| | Yes | 40(28.4%) |
| Clinical T stage (cT) | 1 | 45(31.7%) |
| | 2 | 78(54.9%) |
| | 3 | 16(11.3%) |
| | 4 | 3(2.1%) |
| Clinical N stage (cN) | 0 | 74(51.7%) |
| | 1 | 28(19.6%) |
| | 2 | 12(8.4%) |
| | 3 | 29(20.3%) |
| Pathological T stage (pT) | 1 | 37(26.6%) |
| | 2 | 71(51.1%) |
| | 3 | 26(18.7%) |
| | 4 | 5(3.6%) |
| Pathological N stage (pN) | 0 | 71(51.1%) |
| | 1 | 16(11.5%) |
| | 2 | 12(8.6%) |
| | 3 | 40(28.8%) |
| Histology | usual SCC | 117(81.8%) |
| | basaloid | 15(10.5%) |
| | pap. NOS | 3(2.1%) |
| | sarcomatoid | 1(0.7%) |
| | verrucous | 5(3.5%) |
| | warty | 2(1.4%) |
| Grade | 1 | 27(18.9%) |

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|-------------------------------|------------------------|-----------|
| | 2 | 55(38.5%) |
| | 3 | 61(42.7%) |
| LVI | No | 71(50.7%) |
| | Yes | 69(49.3%) |
| Neoadjuvant Chemotherapy | No | 97(67.8%) |
| | Yes | 46(32.2%) |
| Response to Chemotherapy | CR | 4(8.7%) |
| | PR | 16(34.8%) |
| | SD | 10(21.7%) |
| | PD | 15(32.6%) |
| Greater than 75% staining p16 | No | 98(68.5%) |
| | Yes | 45(31.5%) |
| p16 IHC staining pattern | 0 | 58(40.6%) |
| | 1 | 36(25.2%) |
| | 2 | 17(11.9%) |
| | 3 | 32(22.4%) |
| Survival Status | No Evidence of Disease | 60(42.6%) |
| | Died of Disease | 42(29.8%) |
| | Died | 28(19.9%) |
| | Alive with Disease | 11(7.8%) |

Table S2. Patient characteristics associated with HPV status. Some differences observed but percentages are similar to patient characteristics associated with p16 status. .

| Covariate | Status | HPV = negative | HPV = positive | Fisher's Exact Test P-value (2-Tail) |
|--|------------------|-----------------------|-----------------------|---|
| p16 | Negative | 87(88.8%) | 11(11.2%) | < 0.001 |
| | Positive | 9(20%) | 36(80%) | |
| Age at diagnosis (median, min, max) | | 96 (58.3, 48.0, 68.6) | 47 (59.7, 49.6, 68.5) | 0.58 |
| Number of lymph nodes collected (median, min, max) | | 80 (30.0, 20.0, 41.5) | 37 (27, 20, 37) | 0.54 |
| Total Positive Nodes (median, min, max) | | 80 (1, 0, 2.5) | 38 (1, 0, 2) | 0.89 |
| Ethnicity | Caucasian | 54(61.4%) | 34(38.6%) | 0.050 |
| | African American | 8(57.1%) | 6(42.9%) | |
| | Hispanic | 33(82.5%) | 7(17.5%) | |
| | Asian | 1(100%) | | |
| Tobacco Use | Never | 42(75%) | 14(25%) | 0.144 |
| | Active/Former | 54(62.1%) | 33(37.9%) | |
| Phimosis | No | 49(60.5%) | 32(39.5%) | 0.075 |
| | Yes | 45(75%) | 15(25%) | |
| Circumcised | No | 71(70.3%) | 30(29.7%) | 0.168 |
| | Yes | 23(57.5%) | 17(42.5%) | |
| cT stage | 1 | 33(73.3%) | 12(26.7%) | 0.242 |
| | 2 | 52(66.7%) | 26(33.3%) | |
| | 3 | 8(50%) | 8(50%) | |
| | 4 | 3(100%) | | |
| cN stage | 0 | 48(64.9%) | 26(35.1%) | 0.955 |
| | 1 | 20(71.4%) | 8(28.6%) | |
| | 2 | 8(66.7%) | 4(33.3%) | |
| | 3 | 20(69%) | 9(31%) | |
| pT stage | 1 | 29(78.4%) | 8(21.6%) | 0.382 |
| | 2 | 46(64.8%) | 25(35.2%) | |
| | 3 | 16(61.5%) | 10(38.5%) | |
| | 4 | 3(60%) | 2(40%) | |
| pN stage | 0 | 50(70.4%) | 21(29.6%) | 0.812 |
| | 1 | 12(75%) | 4(25%) | |
| | 2 | 7(58.3%) | 5(41.7%) | |
| | 3 | 27(67.5%) | 13(32.5%) | |
| Grade | 1 | 23(85.2%) | 4(14.8%) | < 0.001 |
| | 2 | 43(78.2%) | 12(21.8%) | |
| | 3 | 30(49.2%) | 31(50.8%) | |
| Histology | usual SCC | 83(70.9%) | 34(29.1%) | < 0.001 |

| | | | | |
|----------------------|--------------|-----------|-----------|---------|
| | basaloid | 3(20%) | 12(80%) | |
| | pap. NOS | 3(100%) | | |
| | sarcomatoid | 1(100%) | | |
| | verrucous | 5(100%) | | |
| | warty | 1(50%) | 1(50%) | |
| IHC 75% staining | Less than | 87(88.8%) | 11(11.2%) | < 0.001 |
| | Greater than | 9(20%) | 36(80%) | |
| IHC Staining Pattern | 0 | 53(91.4%) | 5(8.6%) | < 0.001 |
| | 1 | 31(86.1%) | 5(13.9%) | |
| | 2 | 8(47.1%) | 9(52.9%) | |
| | 3 | 4(12.5%) | 28(87.5%) | |

Table S3. Patient characteristics associated with p16 status. Some differences observed but percentages are similar to patient characteristics associated with HPV status. .

| Covariate | Status | p16 = negative | p16 = positive | Fisher's Exact Test P-value (2-Tail) |
|--|------------------|-----------------------|-----------------------|--------------------------------------|
| HPV | negative | 87(90.6%) | 9(9.4%) | < 0.001 |
| | positive | 11(23.4%) | 36(76.6%) | |
| Age at diagnosis (median, min, max) | | 98 (59.5, 45.1, 68.8) | 45 (58.2, 49.8, 68.3) | 0.811 |
| Number of lymph nodes collected (median, min, max) | | 81 (30.0, 20.0, 41.0) | 36 (27.0, 18.0, 39.0) | 0.580 |
| Total Positive Nodes (median, min, max) | | 81 (1, 0, 3) | 37 (1, 0, 2) | 0.522 |
| Ethnicity | Caucasian | 57(64.8%) | 31(35.2%) | 0.050 |
| | African American | 7(50%) | 7(50%) | |
| | Hispanic | 33(82.5%) | 7(17.5%) | |
| | Asian | 1(100%) | | |
| Tobacco Use | Never | 44(78.6%) | 12(21.4%) | 0.044 |
| | Active/Former | 54(62.1%) | 33(37.9%) | |
| Phimosis | No | 48(59.3%) | 33(40.7%) | 0.011 |
| | Yes | 48(80%) | 12(20%) | |
| Circumcised | No | 72(71.3%) | 29(28.7%) | 0.231 |
| | Yes | 24(60%) | 16(40%) | |
| cT stage | 1 | 32(71.1%) | 13(28.9%) | 0.131 |
| | 2 | 55(70.5%) | 23(29.5%) | |
| | 3 | 7(43.8%) | 9(56.3%) | |
| | 4 | 3(100%) | | |
| cN stage | 0 | 50(67.6%) | 24(32.4%) | 0.946 |
| | 1 | 20(71.4%) | 8(28.6%) | |
| | 2 | 9(75%) | 3(25%) | |
| | 3 | 19(65.5%) | 10(34.5%) | |
| pT stage | 1 | 27(73%) | 10(27%) | 0.271 |
| | 2 | 47(66.2%) | 24(33.8%) | |
| | 3 | 15(57.7%) | 11(42.3%) | |
| | 4 | 5(100%) | | |
| pN stage | 0 | 49(69%) | 22(31%) | 0.721 |
| | 1 | 11(68.8%) | 5(31.3%) | |
| | 2 | 7(58.3%) | 5(41.7%) | |
| | 3 | 30(75%) | 10(25%) | |
| Grade | 1 | 23(85.2%) | 4(14.8%) | 0.002 |
| | 2 | 43(78.2%) | 12(21.8%) | |
| | 3 | 32(52.5%) | 29(47.5%) | |

| | | | | |
|----------------------|--------------|-----------|-----------|---------|
| Histology | usual SCC | 87(74.4%) | 30(25.6%) | < 0.001 |
| | basaloid | 1(6.7%) | 14(93.3%) | |
| | pap. NOS | 3(100%) | | |
| | sarcomatoid | 1(100%) | | |
| | verrucous | 5(100%) | | |
| | warty | 1(50%) | 1(50%) | |
| IHC 75% staining | Less than | 98(100%) | | < 0.001 |
| | Greater than | | 45(100%) | |
| IHC Staining Pattern | 0 | 58(100%) | | < 0.001 |
| | 1 | 36(100%) | | |
| | 2 | 4(23.5%) | 13(76.5%) | |
| | 3 | | 32(100%) | |

Table S4. Samples with discordance between HPV and p16 status. The columns are from right to left – sample ID, HPV status, p16 status determined by HS analysis, percentage of p16 staining, p16 staining pattern, and the HPV analysis used to determine HPV status.

| Sample ID | HPV Status | p16 Status | % Staining | Staining pattern | Cobas or RNAscope |
|-----------|------------|------------|------------|------------------|-------------------|
| 24 | Negative | Positive | > 75% | 2 | Cobas |
| 29 | Negative | Positive | >75% | 2 | RNAscope |
| 46 | Negative | Positive | >75% | 3 | RNAscope |
| 65 | Negative | Positive | >75% | 2 | Cobas |
| 93 | Negative | Positive | >75% | 2 | Cobas |
| 126 | Negative | Positive | >75% | 3 | RNAscope |
| 149 | Negative | Positive | >75% | 3 | RNAscope |
| 74 | Negative | Positive | >75% | 2 | RNAscope |
| 119 | Negative | Positive | >75% | 3 | RNAscope |
| 137 | Positive | Negative | < 75% | 2 | RNAscope |
| 140 | Positive | Negative | < 75% | 1 | RNAscope |
| 21 | Positive | Negative | < 75% | 1 | Cobas |
| 61 | Positive | Negative | < 75% | 0 | Cobas |
| 19 | Positive | Negative | < 75% | 1 | Cobas |
| 20 | Positive | Negative | < 75% | 1 | Cobas |
| 25 | Positive | Negative | < 75% | 0 | Cobas |
| 35 | Positive | Negative | < 75% | 1 | Cobas |
| 99 | Positive | Negative | < 75% | 0 | Cobas |
| 101 | Positive | Negative | < 75% | 0 | Cobas |
| 112 | Positive | Negative | < 75% | 0 | Cobas |

Table S5. Samples that were found to have a p16 staining pattern of 2. We have separated out the samples that p16 and HPV status matched (Sample ID 1 through 23) and samples that p16 and HPV did not match (Sample ID 24 through 137).

| Sample Number | Sample ID | HPV Status | p16 Status | % Staining | Staining Pattern |
|---------------|-----------|------------|------------|------------|------------------|
| | | | | | |
| 1 | 1 | Positive | Positive | >75% | 2 |
| 2 | 30 | Positive | Positive | >75% | 2 |
| 3 | 58 | Positive | Positive | >75% | 2 |
| 4 | 68 | Positive | Positive | >75% | 2 |
| 5 | 88 | Positive | Positive | >75% | 2 |
| 6 | 105 | Positive | Positive | >75% | 2 |
| 7 | 111 | Positive | Positive | >75% | 2 |
| 8 | 148 | Positive | Positive | >75% | 2 |
| 9 | 143 | Negative | Negative | < 75% | 2 |
| 10 | 151 | Negative | Negative | < 75% | 2 |
| 11 | 23 | Negative | Negative | < 75% | 2 |
| | | | | | |
| 1 | 24 | Negative | Positive | >75% | 2 |
| 2 | 29 | Negative | Positive | >75% | 2 |
| 3 | 65 | Negative | Positive | >75% | 2 |
| 4 | 74 | Negative | Positive | >75% | 2 |
| 5 | 93 | Negative | Positive | >75% | 2 |
| 6 | 137 | Positive | Negative | < 75% | 2 |