Published in final edited form as:

Oral Oncol. 2017 April ; 67: 77–82. doi:10.1016/j.oraloncology.2017.02.004.

Human papillomavirus (HPV) 16 antibodies at diagnosis of HPV-related oropharyngeal cancer and antibody trajectories after treatment

Yuehan Zhang^{a,1}, Tim Waterboer^{b,1}, Robert I. Haddad^c, Brett A. Miles^d, Alicia Wentz^a, Neil D. Gross^e, Carole Fakhry^f, Harry Quon^g, Jochen H. Lorch^c, Christine G. Gourin^f, Daniel Clayburgh^h, Krzysztof J. Misiukiewiczⁱ, Jeremy D. Richmon^f, Peter E. Andersen^h, Marshall R. Posnerⁱ, and Gypsyamber D'Souza^{a,*}

^aDepartment of Epidemiology, Johns Hopkins Bloomberg School of Public Health, 615 N Wolfe St, Baltimore, MD 21205, USA

^bGerman Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

^cDepartment of Adult Oncology, Dana-Farber Cancer Institute, 450 Brookline Ave, Boston, MA 02215, USA

^dDepartment of Otolaryngology, Icahn School of Medicine at Mount Sinai Medical Center, 1 Gustave L. Levy Pl, New York, NY 10029, USA

^eDivision of Surgery, Department of Head and Neck Surgery, University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA

^fDepartment of Otolaryngology–Head and Neck Surgery, Johns Hopkins University School of Medicine, 733 N Broadway, Baltimore, MD 21205, USA

^gDepartment of Radiation Oncology, Johns Hopkins University School of Medicine, 733 N Broadway, Baltimore, MD 21205, USA

^hDepartment of Otolaryngology–Head and Neck Surgery, Oregon Health and Science University, 3181 SW Sam Jackson Park Rd, Portland, OH 97239, USA

*Corresponding author. gdsouza2@jhu.edu (G. D'Souza).

¹These authors contributed equally to this paper.

Conflict of interest

The following authors have no conflicts related to this work: YZ AW CF TW

- GD received previous research support from Merck Inc.
- NG has received travel expense reimbursement from Intuitive Surgical, Inc, and Medrobotics, Inc.
- RIH has been compensated as a consultant for Celgene, Merck, and Eisai and has received institutional research funding from Merck, Bristol-Myers Squibb, VentiRx, and Boehringer-Ingelheim.
- JHL has been compensated as a consultant for Eisai and has received institutional research funding from Novartis.
- BAM has received institutional research funding from Advaxis.
- PEA has received honoraria from and been paid to participate in a speakers' bureau for Ethicon and travel expense reimbursement from Medrobotics.

¹Tisch Cancer Institute, Head and Neck Oncology Center, Icahn School of Medicine at Mount Sinai Medical Center, 1 Gustave L. Levy Pl, New York, NY 10029, USA

Abstract

Objectives—Despite the fact that HPV-driven oropharyngeal cancer (HPV-OPC) has relatively low recurrence rates, intensive post-therapy monitoring remains the standard of care. Post-treatment biomarkers are needed to risk stratify HPV-OPC patients for more individualized surveillance intensity and which remain at higher recurrence risk.

Materials and Methods—115 HPV-OPC patients (ascertained by p16 immunohistochemistry and/or in-situ hybridization) from a multicenter prospective case study (HOTSPOT) had blood collected at diagnosis, and 64 of these also had blood collected at post-treatment follow-up visits for up to two years. Samples were centrally tested for antibodies to the L1, E1, E2, E4, E6, and E7 proteins of HPV16.

Results—At diagnosis, most HPV-OPC cases were seropositive to HPV16 E6 (85%). In post therapeutic samples, HPV16 antibody level decreased slowly over time, but only 3 (of 51 cases seropositive at enrollment) dropped low enough to be classified as seronegative. At 3 years after diagnosis, cumulative risk of recurrence was 10.2% and 0% in HPV16 E6 seropositive and E6 seronegative HPV-OPC cases, respectively ($p = 0.18$). Risk of recurrence was increased, although not statistically significant, in those with higher HPV16 E6 antibody levels at diagnosis (per log antibody level, hazard ratio [HR] = 1.81, 95%CI = 0.47–6.92).

Conclusion—This study confirms the high seroprevalence of HPV oncogenic antibodies at diagnosis of HPV-OPC. HPV16 E6 antibody levels decrease after treatment, but most cases remain seropositive for up to two years. HPV16 E6 antibody levels at diagnosis did not appear to be a strong predictor of recurrence.

Keywords

Antibodies; HPV; E6; OPSCC; Seroprevalence

Introduction

Infection with human papillomavirus (HPV) is the primary cause of oropharyngeal squamous cell cancer (OPC) in the United States [1]. Following treatment, HPV-driven oropharyngeal cancer (HPV-OPC) has good survival, with a 5-year survival rate of 82% [2–4]. While recurrence risk remains low among HPV-OPC [5–7], current standard of care surveillance includes intensive posttherapy monitoring that could include physical exam, fiberoptic examinations, and radiologic imaging procedures. These procedures are associated with significant time, resources, and additional costs. Post-treatment biomarkers are needed to risk stratify which patients are candidates for reduced surveillance intensity and which patients remain at higher recurrence risk.

Recent research demonstrates that HPV16 serum antibodies are present for many years before diagnosis of HPV-OPC [8], likely the result of unidentified oropharyngeal precursor lesions. We expect these pre- premalignant or early cancers arise from long-term

oropharyngeal HPV16 infection, although this natural history has not yet been fully elucidated [9]. In contrast, HPV16 antibodies are rare in healthy controls (<1%) [10]. At the time of diagnosis, HPV16 E6 antibodies are detected in most HPV-OPC cases [11,12]. Importantly, HPV16 E6 serology is not a good marker for predicting risk of cervical cancer (another HPV-related cancer) [13], increasing its potential for predicting HPV-OPC specifically when detected.

In this study, we sought to determine the kinetics of HPV16 antibodies after diagnosis and treatment of HPV-OPC, and whether antibody sero-reversion or changes in antibody levels would be useful for post-treatment risk stratification. To evaluate this, serum samples from HPV-OPC in the “HPV Oral Transmission Study in Partners Over Time” (HOTSPOT) study [5], a case series of HPV-OPC across four United States sites, were tested for HPV serum antibodies.

Materials and methods

Patients were enrolled in the HOTSPOT study, a multicenter prospective study of 166 incident HPV-OPC cases enrolled between October 2009 and May 2013 in head and neck cancer clinics at four study sites: Johns Hopkins Hospital (JHH), Mount Sinai Medical System (MSMS), Dana Farber Cancer Institute (DFCI), and Oregon Health and Science University (OHSU). A subset of these participants (n = 115, 69%) had a blood sample collected around diagnosis. Participants also had blood collected post-therapy, when possible, at follow-up visits targeted during routine clinical follow-up around 9, 12, 18 and 24 months after diagnosis. A detailed computer assisted self -interview (CASI) risk factor survey, tumor sample, and oral rinse and gargle sample, were also collected around diagnosis [14]. Medical record abstraction was performed every six months for recurrence and survival.

Of 166 HPV-OPC patients enrolled, 115 (69%) had a blood sample collected at diagnosis, and 64 (39%) had at least one post-treatment blood sample collected. Analysis for this project was restricted to subjects with blood samples, with trajectories in antibody levels explored among the 64 subjects with 2 or more blood samples collected. HPV-OPC cases were classified as HPV-related based upon p16 immunohistochemistry and/or HPV16 DNA in-situ hybridization (ISH) [14]. Confirmation of HPV-related malignancy utilized centralized ISH testing for 34 cases and local p16 (n = 79) or ISH (n = 2) testing in the remaining cases. Most of the 115 HPV-OPC cases included were known to be p16 positive (106 of 106 tested) and HPV16 ISH positive (75 of 79 tested), with 67 confirmed to be positive for both p16 and HPV16 ISH.

Serum samples were sent to the German Cancer Research Center (DKFZ, Heidelberg, Germany) for HPV serologic testing using multiplex assays [15]. Antigens were affinity-purified, bacterially expressed fusion proteins with N-terminal Glutathione *S*-transferase. Samples were analyzed for antibodies to the HPV major capsid protein (L1), the early oncoproteins (E6, E7), and other early proteins (E1, E2, E4) of the following carcinogenic mucosal types: HPV16 and HPV18 (L1, E1, E2, E4, E6, and E7); HPV31, HPV33, HPV45 (L1, E6, and E7); and of the non-carcinogenic mucosal types HPV6 and HPV11 (L1, E6,

and E7), plus the major capsid protein (VP1) of BK polyomavirus. Mean fluorescence intensity (MFI) values were dichotomized as antibody positive or negative, using standardized cutoff values [16]. Based on previous work optimizing the cut-point for HPV16 E6, the main HPV genotype and protein of interest as it relates to HPV-OPC, we applied a more specific cutoff of 1000 MFI for positivity [8]. Oral rinse samples were tested for HPV16 DNA by PCR, as previously described [14].

Analytic methods

Individual seroprevalence of each HPV antibody was calculated at diagnosis and each of the post-treatment follow-up visits. Characteristics of HPV-OPC cases who were HPV16 E6 seropositive and seronegative at the time of diagnosis were compared using Fisher's exact test for categorical and non-parametric equality-of-medians test for continuous variables. Risk of recurrence was modeled using Kaplan Meier curves, overall and by HPV16 E6 antibody level at diagnosis. Spaghetti plots were used to graph the HPV16 E6 (and E7) antibody levels for each individual who was seropositive at diagnosis and had post-therapy serum samples. The mean levels of the HPV16 E6 were estimated at each visit. A line indicating the predicted trajectory of HPV16 E6 (and E7) antibody levels based on a linear regression model was added to the graph. We used generalized estimating equations (GEE) methods with a working exchangeable correlation structure to estimate the change in mean HPV16 E6 antibody levels over time.

Results

Characteristics of the 115 HPV-OPC cases with serum collected at diagnosis and tested for HPV serum antibodies are described in Table 1. Most (85%; 98/115) HPV-OPC cases were HPV16 E6 seropositive at diagnosis. Median age of patients was 56 years, 90% were male, 56% were never smokers, and 95% were American Joint Committee on Cancer (AJCC) stage III or IV. Comparing HPV-OPC cases that were seropositive versus seronegative for HPV16 E6 antibodies at diagnosis, characteristics were similar for stage, oropharyngeal subsite, age, gender, and risk behaviors, but HPV16 E6 seropositive cases were more likely to be Caucasian and to have a higher HPV16 DNA copy number detected in oral exfoliated cells (Table 1).

HPV seroprevalence at diagnosis

The HPV seroprevalence among HPV-OPC cases at diagnosis varied considerably by HPV type antigen (Table 2). The most common early antibodies detected were HPV16 E6 antibodies, with a seroprevalence of 85%. Antibodies to HPV16 E1, E2, and E7 also had a high seroprevalence (70%, Table 2). Two-thirds of cases (66%) were positive for both HPV16 E6 and E7 antibodies at diagnosis, and 95% had antibodies to at least one of the HPV16 E1, E2 and/or E6 antigens detected.

Seroprevalence of most other HPV types was lower, including low prevalence of E6 antibodies to non-oncogenic HPV types (HPV6 and 11) and other oncogenic HPV types like HPV18, 31, and 45 (Table 2). E6 seroprevalence was relatively high for HPV33 (67%) and

HPV58 (47%), likely due to cross-reactivity with HPV16. Among HPV16 E6 seronegative cases, seroprevalence to other HPV type antibodies was low (Table 2).

Association of HPV antibody levels and risk of recurrence

Median follow-up among cases was 2.2 years (IQR = 2.0–3.2). There were 10 recurrences observed among 115 HPV-OPC cases during this follow-up; among those who recurred, the median time to recurrence was 1.3 years after diagnosis (range = 0.6–3.1). At 3 years after diagnosis, cumulative risk of recurrence was 10.2% among cases HPV16 E6 seropositive at diagnosis, compared to 0% among cases HPV16 E6 seronegative at diagnosis (Fig. 1, $p = 0.18$).

All 10 cases who recurred were seropositive for HPV16 E6 at diagnosis, but many cases who did not recur were also HPV16 E6 seropositive (100% vs 84%, $p = 0.19$, Supplemental Table 1). In univariate analysis, risk of recurrence appeared to be increased in those with higher HPV16 E6 level at diagnosis (per log antibody level, HR = 1.81, 95%CI = 0.47–6.92), however this difference was not statistically significant (Table 3). After adjusting for the AJCC cancer stage, the impact of HPV16 E6 appeared stronger, but remained non-statistically significant (aHR = 2.00, 95%CI = 0.43–9.43). Findings were similar when examining HPV16 E6 levels one year after diagnosis (HR = 1.78, 95% CI = 0.33–9.61).

HPV antibody level trajectories after diagnosis

We also explored trajectories in HPV antibodies among HPV-OPC cases over time after diagnosis. There were 64 HPV-OPC cases that had at least one post-treatment blood sample collected, in addition to the blood sample at cancer diagnosis. This included one, two, and three or more post-treatment blood samples in 44%, 23%, and 33% of the 64 cases, respectively. Cases with and without post-treatment blood collected were similar in terms of age, gender, race, tumor HPV16 status, cancer stage and sub-site, and risk behaviors; however, cases with post-treatment blood collection were more likely to be from certain study sites and had longer follow-up in study (2.9 vs 2.0 years, $p = 0.003$, Supplemental Table 2). Only 3 (of 51, 5.9%) of patients seropositive for HPV16 E6 at enrollment became seronegative during the study (Fig. 2a).

As described in Table 2, in post-therapeutic HPV-OPC samples at 1, 1.5 and 2 years after diagnosis, the majority of cases remained HPV16 seropositive for E1 (67–83%), E2 (71–77%), E6 (75–79%), and E7 (67–75%). Among those subjects that were HPV16 E6 positive at diagnosis, HPV16 E6 seropositivity at the subsequent 12-month visit remained very high (97%). Similarly, among subjects that were HPV16 E6 positive at 12 and 18 months, 100% and 95% remained seropositive at the subsequent 18 and 24 month visits, respectively. Results were qualitatively similar when restricted to those with known HPV16 ISH-positive tumor samples (Supplemental Table 3).

Among HPV-OPC cases seropositive to HPV16 E6 at diagnosis, the mean antibody levels detected after treatment decreased significantly over time ($p < 0.001$; Fig. 2a). Similar decreases in antibodies after treatment were observed for cases that were seropositive at diagnosis to HPV16 E7 ($p = 0.001$; Fig. 2 b), E1 ($p < 0.001$), E2 ($p = 0.04$) and E4 ($p = 0.01$). The decrease in HPV16 E6 antibody level was similar among patients who did and

did not recur ($\beta_{\text{MFI}} = -1574 \text{ MFI/year}$ vs -1018 MFI/year , $p_I = 0.15$). At 1 and 2 years after diagnosis the mean HPV16 E6 level decreased from 8211 MFI to 6623 MFI and 5559 MFI, respectively ($\beta = -1090 \text{ MFI/year}$, $p < 0.001$); however levels remained well above the seropositive threshold of 1000 MFI. HPV16 E7 levels also decreased, from a mean of 7234 MFI at diagnosis to 6034 MFI, and 5951 MFI, at 1 and 2 years after diagnosis ($\beta = 909 \text{ MFI/year}$, $p = 0.001$). To assess if the observed declines in antibody levels were driven by HPV or nonspecific changes in immunoglobulins, the trajectory of BK virus levels after treatment was evaluated. In contrast to observed trends for HPV, BK virus levels remained stable over time ($\beta = 77.8 \text{ MFI per year}$, $p = 0.63$).

Discussion

This study confirms a high seroprevalence of HPV oncogenic antibodies at diagnosis with HPV-OPC and that most cases remain seropositive for up to 2 years after treatment. Among HPV-OPC cases, there was some suggestion that HPV16 E6 seropositivity at diagnosis might be associated with higher recurrence risk, but after therapy, antibody levels were not predictive of recurrence. High E6 seroprevalence in HPV-OPC cases after therapy suggests these antibodies are immune markers of previous disease response (i.e. early immune response to malignant transformation), but antibody generation may not be dynamic enough after tumor eradication to be used as biomarkers of risk stratification for recurrence among cases.

Only a few studies have previously evaluated antibody stability following treatment for an HPV-driven head and neck cancer. Findings of this study are consistent with previous studies in showing that HPV16 E6 seroprevalence remains high after therapy. Several previous studies reported that HPV16 seropositivity remained high among cases after therapy (Supplemental Table 4), including: 98.5% of cases positive for HPV16 E6 and/or E7 at one-year after completion of treatment in a study by Koslabova and colleagues [17], 95% of HPV16 E6 positive cases at two-years after diagnosis in a smaller study by Rubenstein and colleagues [18].

The decrease in HPV16 E6 level observed in this study, is also consistent with previous research (Supplemental Table 4). Significant decreases in mean antibody levels over time were reported for HPV16 E6 and E7 serum antibodies in two studies [17,19] and these studies suggested the reduction in antibody level was associated with decreased risk of recurrence [17,19]. Higher E6 antibody titer at diagnosis was associated with significantly increased risk of recurrence in one of these studies, although there were only 6 recurrences in that study [19]. This prospective multiinstitutional study found a similar association with recurrence (Fig. 1; Table 3), although not statistically significant. These findings are in contrast with a recent report from MD Anderson which noted lower median antibody levels among 8 patients with recurrence than 23 patients without recurrence [20]; (Supplemental Table 4).

HPV16 E6 seroprevalence had the highest sensitivity (i.e. least false negatives) for HPV-OPC disease of the markers considered. E7 seroprevalence was lower among cases, consistent with previous research which showed E7 also has poorer specificity (i.e. has false

positives) [8,21]. While most (85%) of HPV-OPC were HPV16 E6 seropositive, the combined marker of any E1, E2 or E6 seroprevalence identified an additional 10% of cases, for a total sensitivity of 95% of HPV-OPC cases. One type, HPV31, had low E6 but high E7 seroprevalence, which may be due to HPV31 E6 protein sequence variation [22].

We hypothesized *a priori* that an OPC patient's HPV16 E6 antibody levels would detectably decrease in the two years post-treatment because the antigen load would be rather low and not be presented in an excessively pro-immunogenic context, e.g. virus-like display. While we observed a statistically significant decline in the average of HPV16 E6 antibody levels over time, the magnitude of the change was not as large as expected, and most cases remained well above the cut-off for seropositivity even two years after diagnosis. At present, the observed persistence of the HPV16 E6 antibody response could be explained in four ways: (1) continued antigen expression, perhaps from persistent disease post-treatment or second related malignancy, (2) persistence of antigen, especially on follicular T-helper cells in the lymph nodes, (3) long lived plasma cells, or (4) antigenic boost after localized definitive therapy (XRT +/-Chemo). Our previous research showed HPV16 DNA in oral exfoliated cells is common at diagnosis but rarely detectable after therapy, including a lack of detectable HPV virus in some cases with persistent/recurrent disease [5]. Given the low rates of recurrence post-treatment for HPV-driven OPC, it seems unlikely that residual disease would explain continued detection of antibodies post treatment. We know of no experimental evidence to date that might distinguish between these, and believe that sophisticated proteomics would be needed to determine if the E6 antibody species remains the same over time or if new species are constantly produced.

This study had several strengths and limitations. The study included incident HPV-OPC cases from multiple U.S. academic centers, followed prospectively, with serum collection at defined intervals and tested centrally. All cases were diagnosed in the last 5 years (between 2009 and 2012), representing the current clinical landscape. However, our results only included HPV antibodies up to 2 years after diagnosis and seropositivity might decrease after longer intervals. Further, patients remain at risk of recurrence for at least 5 years after treatment [23]. This study had limited samples size and consequently a limited number of recurrent cases as would be expected in these patients, so we may be underpowered to explore associations with recurrence. Although study design outlined specific time intervals for serum collection, there was variation in the timing of blood collection. This is however one of the largest studies to evaluate the HPV16 seropositivity and kinetic profiles of these antibodies after treatment. Not all cases had tumor HPV16 testing and therefore cases were determined to be HPV-related using either p16 and/or HPV16 ISH. Since p16 does not have perfect specificity for HPV-OPC it is possible that some cases were misclassified as HPV-OPC.

Conclusions

Current monitoring of HPV-OPC post-treatment includes a variety of frequent clinical and radiological examinations that are based on standard oncological surveillance protocols rather than being specific for HPV related disease, and does not currently include serologic or virologic testing. This research aimed to explore trends of HPV16 antibodies after

treatment to inform whether seroreversion was common and whether seropositivity was predictive of disease recurrence. Our results do not support the utility of HPV16 E6 antibodies in the short term monitoring for disease recurrence. Longer-term studies would be needed to evaluate if this marker might have predictive utility for recurrence within a longer interval.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The work was supported by the Johns Hopkins Richard Gelb Cancer Prevention Award, and the Oral Cancer Foundation.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.oraloncology.2017.02.004>.

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Fig. 1.

Risk of recurrence among 115 HPV-OPC cases in follow-up, by HPV16 E6 seropositivity at diagnosis. Survival (% recurrence free) for seronegative (n = 17) and seropositive (n = 98) at 2 years was 100.0% and 89.8%, respectively, and at 4 years was 100.0% and 86.0%, respectively.

Fig. 2.

Trend in HPV16 level over time since diagnosis by individual, among HPV-OPC seropositive at diagnosis for HPV16 E6 (left, $n = 51$; $\beta = -1090$ MFI/year) and HPV16 E7 (right, $n = 45$; $\beta = -909$ MFI/year). Bold line shows linear predicted antibody levels average across individuals. Dotted line shows cutoff for seropositivity (HPV16 E6: 1000 MFI; HPV16 E7: 548 MFI). Red line shows antibody levels among individuals with cancer recurrence during the study follow up. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

Table 1

Description of 115 HPV-positive oropharyngeal cancer cases by HPV16 E6 seropositivity at diagnosis.

	HPV+ OPC Cases		
	HPV16 E6 seropositive (N = 98)	HPV16 E6 seronegative (N = 17)	P value ^a
<i>Related biomarkers</i>			
Tumor HPV16 ISH positive (among 79 tested)	63 (95%)	12 (92%)	0.52
Tumor p16 positive (among 106 tested)	89 (100%)	17 (100%)	–
Oral rinse: HPV16 DNA copynumber: median (IQR)	4 (0–178)	0 (0–0)	0.02
<i>AJCC TNM Cancer Stage</i>			0.19
I	0 (0%)	0 (0%)	
II	4 (5%)	1 (6%)	
III	12 (14%)	5 (29%)	
IV (a, b, and c)	72 (82%)	11 (65%)	
Cancer Sub-site			0.92
Tonsil	45 (46%)	7 (41%)	
Base of Tongue	44 (45%)	9 (53%)	
Oropharynx NOS	9 (9%)	1 (6%)	
<i>Treatment</i>			0.10
Surgery only	1 (1%)	0 (0%)	
Surgery & radiation (±chemotherapy) ^b	27 (33%)	1 (7%)	
Chemotherapy and/or radiation	54 (66%)	14 (93%)	
<i>Study Site</i>			0.52
Dana Farber Cancer Institute	36 (37%)	6 (35%)	
Johns Hopkins Hospital	30 (31%)	8 (47%)	
Oregon Health and Science University	7 (7%)	0 (0%)	
Mount Sinai Medical System	25 (26%)	3 (18%)	
<i>Patient demographics</i>			
Age in years: median (IQR)	56 (50–60)	57 (53–64)	0.73
Male gender	90 (92%)	14 (82%)	0.21
Caucasian non-Hispanic	93 (95%)	12 (71%)	0.01
Married/Living as Married	82 (85%)	12 (71%)	0.16
Family income <\$100,000	41 (48%)	9 (53%)	0.79
Never drinker	17 (18%)	3 (18%)	1.00
Never smoker	55 (56%)	9 (53%)	1.00
20 pack years tobacco use	16 (16%)	4 (27%)	0.47
<i>Number of blood samples collected post-treatment</i>			0.17
0	47 (48%)	4 (24%)	
1	21 (21%)	7 (41%)	
2	12 (12%)	3 (18%)	
3 or 4	18 (18%)	3 (18%)	
Years from diagnosis to last blood sample: median (IQR)	0.8 (0–1.5)	1.2 (0.5–1.6)	0.28

	HPV+ OPC Cases		
	HPV16 E6 seropositive (N = 98)	HPV16 E6 seronegative (N = 17)	P value^a
Years of follow up: median (IQR)	2.1 (2.0–3.1)	2.9 (1.9–3.4)	0.57

^aFisher's exact test for categorical and non-parametric equality-of-medians test for continuous variables.

^bIncludes 3 patients who had surgery and radiation but not chemotherapy. Of the 25 patients who received surgery & chemo & radiation, 24 were HPV16 E6 seropositive and 1 was HPV16 E6 seronegative.

HPV seropositivity among all HPV-OPC cases (n = 115) and those HPV-OPC cases with post-treatment blood samples (n = 64), by timepoint of blood collection and HPV type and antigen.

Table 2

Months after diagnosis	All HPV-OPC 0 months N = 115	HPV-OPC with >1 post-tx sample				
		0 months N = 42	12 months N = 31	18 months N = 24	24 months N = 24	
		%				
Median time from diagnosis (years)	N	0	1.1	1.5	2.0	
<i>HPV16 Seropositivity</i>						
HPV16 E6	98	85%	79%	77%	75%	
HPV16 E7	83	72%	67%	71%	75%	
HPV16 E1	80	70%	67%	81%	83%	
HPV16 E2	89	77%	71%	77%	75%	
HPV16 E4	52	45%	33%	58%	42%	
HPV16 (E6&E7)	76	66%	64%	61%	67%	
HPV16 (E1&E2&E6)	66	57%	60%	61%	67%	
HPV16 (E1 or E2 or E6)	109	95%	83%	94%	88%	
HPV16 L1	70	61%	64%	61%	46%	
<i>E6 Seropositivity for other HPV types tested</i>						
HPV6 E6	0	0%	0%	0%	0%	
HPV11 E6	4	3%	5%	10%	4%	
HPV18 E6	4	3%	10%	6%	0%	
HPV31 E6	4	3%	0%	0%	4%	
HPV33 E6	77	67%	57%	52%	42%	
HPV45 E6	15	13%	7%	6%	4%	
HPV58 E6	54	47%	40%	39%	33%	
<i>E7 Seropositivity for other HPV types tested</i>						
HPV6 E7	1	1%	0%	0%	0%	
HPV11 E7	8	7%	0%	0%	0%	
HPV18 E7	9	8%	10%	3%	4%	
HPV31 E7	66	57%	52%	65%	63%	
HPV33 E7	72	63%	48%	68%	54%	

Months after diagnosis	All HPV-OPC		HPV-OPC with >1 post-tx sample			
	0 months N = 115		0 months N = 42	12 months N = 31	18 months N = 24	24 months N = 24
	N	%				
Median time from diagnosis (years)	0		0	1.1	1.5	2.0
HPV45 E7	9	8%	9%	10%	6%	0%
HPV58 E7	48	42%	44%	33%	39%	38%
<i>L1 Seropositivity for other HPV types tested</i>						
HPV6 L1	31	27%	28%	19%	29%	21%
HPV11 L1	28	24%	27%	21%	32%	21%
HPV18 L1	22	19%	25%	10%	19%	8%
HPV31 L1	32	28%	28%	19%	39%	13%
HPV33 L1	23	20%	20%	17%	26%	4%
HPV45 L1	21	18%	20%	14%	19%	8%
HPV58 L1	31	27%	27%	21%	29%	8%
Among subjects HPV 16 E6 seropositive at diagnosis	N = 98		N = 51	N = 35	N = 25	N = 20
HPV16 E6	98	100%	100%	94%	96%	90%
HPV33 E6	73	75%	76%	63%	56%	45%
HPV58 E6	52	53%	63%	49%	44%	40%
Among subjects HPV 16 E6 seronegative at diagnosis	N = 17		N = 13	N=7	N=6	N=4
HPV16 E6	0	0%	0%	0%	0%	0%
HPV33 E6 ^a	4	24%	23%	29%	33%	25%
HPV58 E6 ^a	2	12%	8%	0%	17%	0%

^a At baseline, four OPC cases were seropositive for HPV33 E6, 2 of which were also seropositive for HPV58 E6. Two of these 4 cases with seropositivity for other oncogenic HPV types had tumors tested for HPV16 ISH and both were HPV16-positive, suggesting these cases were caused by HPV16 not HPV 33 or 58. All 4 cases had p16-positive tumors.

Table 3

Risk of recurrence among 115 HPV-OPC cases (10 of who recurred), by level of HPV16 E6 antibody level at diagnosis.

HPV16 E6	HR (95% CI)
Per log increase	1.81 (0.47, 6.92)
Per 1000 MFI increase	1.13 (0.91, 1.40)
By tertile of MFI ^a	
Lowest	1.00
Middle	0.67 (0.11, 4.00)
Highest	1.90 (0.45, 7.96)

HR: hazard ratio.

95% CI: 95% confidence interval.

^aHPV16E6 levels at each tertile group at cancer diagnosis (range): Lowest: 1–6165 MFI, Middle: 6166–8682 MFI, Highest: 8683 MFI.