

Clinical analysis of HPV-positive oropharyngeal squamous cell cancer according to HPV genotype

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HIGHLIGHT

- Among 174 HPV genotype-specific cases, HPV-16 accounted for 74.7% and non-HPV-16 comprised 25.3%.
- Both HPV-16 and non-HPV-16 groups showed marked male predominance.
- Among non-HPV-16 OPSCC cases, HPV-33 (6.3%), HPV-35 (4.6%), HPV-58 (3.4%), and HPV-18 (3.4%) were the most prevalent genotypes.
- HPV-35 genotype was associated with poorer overall survival compared with HPV-16.
- Given the substantial proportion of non-HPV-16 genotypes and the marked male predominance observed in our cohort, our findings may have implications for future HPV prevention strategies.

ABSTRACT

Objective: Oropharyngeal squamous cell carcinoma (OPSCC) has undergone a major epidemiologic transition, with high-risk human papillomavirus (HPV) emerging as the dominant etiologic factor. Although HPV-16 accounts for most HPV-positive OPSCC worldwide, a substantial minority of tumors harbor non-HPV-16 genotypes, the clinical and prognostic significance of which remains uncertain, particularly in Asian populations. This study aimed to investigate HPV genotype distribution and survival outcomes in a single-institution cohort of HPV DNA-positive OPSCC regardless of p16 and to assess the prognostic significance of HPV genotype.

Methods: We retrospectively reviewed patients treated with curative intent for HPV-positive OPSCC at Seoul National University Bundang Hospital between 2003 and 2023. HPV status was determined using p16 immunohistochemistry and HPV DNA genotyping. Patients were classified as having HPV-16 or non-HPV-16 genotype. Overall survival (OS) and disease-free survival (DFS) were analyzed using Kaplan–Meier method and compared using the log-rank test.

Results: Of 254 eligible patients, 174 with HPV DNA-positive tumors and available HPV genotyping results were included in the final analysis. HPV-16 accounted for 74.7% of tumors, while non-HPV-16 genotypes comprised 25.3%, most commonly HPV-33, HPV-35, HPV-58, and HPV-18. A marked male predominance was observed across all genotypes. The clinical stage distribution did not differ significantly between the HPV-16 and non-HPV-16 groups overall; however, HPV-33-positive tumors showed higher nodal stage compared with HPV-16. OS and DFS were similar between the HPV-16 and pooled non-HPV-16 groups. In genotype-specific analyses, DFS did not differ significantly across groups, whereas HPV-35 was associated with poorer OS compared with HPV-16.

Conclusions: In this cohort, non-HPV-16 genotypes accounted for approximately one-quarter of HPV-positive OPSCC cases and demonstrated clinicopathologic heterogeneity with selected genotype-specific prognostic differences. Given the substantial proportion of non-HPV-16 genotypes and the marked male predominance observed in our cohort, our findings may have implications for future HPV prevention strategies.

Keywords: oropharyngeal cancer, human papilloma virus, genotype, survival, vaccination

Level of Evidence: 4

VISUAL ABSTRACT

Clinical Analysis of HPV-positive Oropharyngeal Squamous Cell Cancer According to HPV type

Background

Increasing prevalence of HPV+ OPSCC

- HPV-16 most prevalent (nearly 90%)
- Non-HPV-16 clinically non-negligible yet, lacks study



Study Cohort

Retrospective review of HPV-associated OPSCC patients
Seoul National University Bundang Hospital, 2003~2023
HPV data (HPV DNA test, p16 IHC)



HPV Genotype

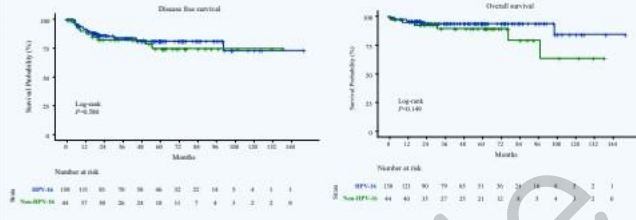
HPV-16 accounted for 74.7%
Non-HPV-16 comprised 25.3%

■ HPV-16 ■ HPV-58
 ■ HPV-33 ■ HPV-18
 ■ HPV-35 ■ Other hrHPV
 ■ HPV_NOS

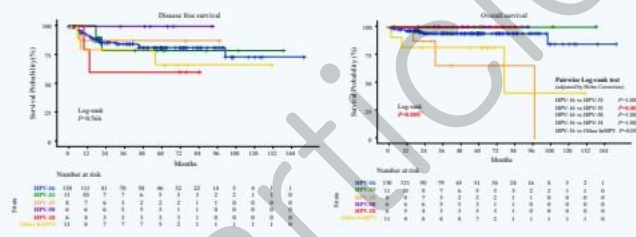


Outcomes

HPV-16 vs. Non-HPV-16



Subgroup Analysis



INTRODUCTION

Oropharyngeal squamous cell carcinoma (OPSCC) has undergone a significant epidemiologic shift in recent decades, largely attributable to the increasing prevalence of high-risk human papillomavirus (HPV) infection as a causative factor[1]. HPV-positive OPSCC is now recognized as a distinct disease entity, typically associated with younger age, fewer comorbidities, and improved prognosis compared with HPV-negative OPSCC[2]. Among high-risk HPV types, HPV-16 is the most prevalent, accounting for the majority of HPV-positive OPSCC worldwide[3].

However, non-HPV-16 high-risk genotypes are increasingly being detected in OPSCC, and their clinical significance remained incompletely defined. Several studies, including population-based analyses and multicenter cohort studies, have suggested that patients with non-HPV-16 genotypes may experience inferior survival compared with those harboring HPV-16, even after adjustment for clinical covariates[4-8]. However, the evidence remains inconsistent[9-11], and data from Asian populations are particularly limited[12-14].

Few studies have specifically examined clinicopathologic features and oncologic outcomes of OPSCC according to HPV genotype, particularly in Asian cohorts[13, 14]. Given this gap, further investigation is warranted to clarify the prognostic relevance of genotype variation in patients with HPV-positive OPSCC.

This study aimed to investigate HPV genotype distribution and survival outcomes in a single-institution cohort of HPV DNA-positive OPSCC regardless of p16 and to assess the prognostic significance of HPV genotype.

MATERIALS AND METHODS

Study Population and Ethical Considerations

Patients treated with curative intent for OPSCC at our institute between 2003 and 2023 were retrospectively reviewed. Patients with incomplete follow-up, missing HPV status, or incomplete treatment data were excluded. HPV status was assessed using p16 IHC and HPV DNA testing. Clinical data, including electronic medical records, imaging studies (computed tomography, magnetic resonance imaging, positron emission tomography, and ultrasonography), and pathology reports, were comprehensively reviewed.

A retrospective review of electronic medical records was conducted after approval was obtained from the Institutional Review Board of our institute (IRB No. B-2305-831-102). The study was conducted in accordance with the Declaration of Helsinki, and the requirement for individual informed consent was waived because of the retrospective nature of the study.

Evaluation for HPV status

For all patients, tissue biopsies were obtained from primary tumors or metastatic cervical lymph nodes for diagnostic evaluation. HPV-positive OPSCC was defined as OPSCC showing positivity on either an HPV DNA test or p16 IHC, with p16 IHC used as a surrogate marker.

HPV DNA testing and genotyping were performed using the PANA RealTyper™ HPV Kit (PANAGENE, Korea), a real-time polymerase chain reaction–based system using peptide nucleic acid (PNA) probes, according to the manufacturer’s instructions. The assay detects a total of 40 HPV genotypes, of which 22 are individually specified, including 20 high-risk types (HPV-16, -18, -26, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66, -68, -69, -70, -73, and -82) and 2 low-risk types (HPV-6 and -11). The remaining 18 types (HPV-30, -32, -34, -40, -42, -43, -44, -54, -55, -61, -62, -67, -74, -81, -83, -84, -87, and -90) are collectively reported as HPV not otherwise specified” (HPV-NOS, HPV genotypes not otherwise specified) (Supplementary Table 1).

p16 IHC, a surrogate marker for HPV infection, was performed on formalin-fixed paraffin-embedded tissues using the CINtec® anti-p16 monoclonal antibody (clone E6H4, Ventana Medical Systems) on the Benchmark Ultra automated platform. Both cytoplasmic and nuclear staining were independently evaluated by head and neck pathologists. Specimens with diffuse (>75% of tumor cells), moderate to high intensity staining were considered p16-positive.

In OPSCC, tumors positive for HPV DNA regardless of p16 were defined as HPV DNA-positive OPSCC, and tumors positive for both HPV DNA and p16 were defined as HPV DNA-positive/p16-positive OPSCC. Based on HPV genotyping, tumors in which HPV-16 was detected were classified into the HPV-16 group, whereas tumors in which genotypes other than HPV-16 were detected were classified into the non-HPV-16 group.

Outcome assessment

Overall survival (OS) was defined as the time from diagnosis to death from any cause. Disease-free survival (DFS) was defined as the time from diagnosis to the first documented local, regional, or distant recurrence after completion of treatment. To differentiate true recurrences from residual disease, a 6-month threshold was applied: lesions detected within 6 months after treatment completion that required additional therapy were considered part of the initial disease course.

Statistical analysis

Patients were initially stratified into the HPV-16 and non-HPV-16 groups, with the latter further subclassified according to predominant genotypes. Between-group comparisons were performed using the Mann–Whitney U test for continuous variables and the Chi-square or Fisher’s exact tests for categorical variables, as appropriate. For comparisons involving more than two groups, the Kruskal–Wallis H test was used.

Survival outcomes were analyzed using the Kaplan–Meier method, with differences between groups were assessed using the log-rank test. When more than two groups were compared, pairwise log-rank tests with Holm’s correction were additionally performed if the overall result was statistically significant (P -value < 0.05). Univariable and multivariable Cox proportional hazards regression analyses were performed to identify prognostic factors for survival outcomes. Firth-penalized Cox regression was applied to account for sparse data and low event counts within certain covariate categories.

Missing data were handled using complete-case analysis, with cases containing missing values excluded from the relevant statistical analyses. No imputation was performed.

Statistical analyses were conducted with SPSS (version 25.0; IBM Corp), R (version 4.3.1; R Foundation for Statistical Computing), and GraphPad Prism (version 8.0; GraphPad Software). A P -value < 0.05 was considered statistically significant.

RESULTS

Clinical characteristics of study population

A total of 389 patients received definitive treatment for OPSCC at our institution between 2003 and 2023. After excluding patients with incomplete treatment or follow up data (n=35) and those with missing HPV DNA status (n=100), 254 patients remained eligible for study inclusion. Patients were stratified according to HPV DNA status: 177 were HPV DNA-positive and 77 were HPV DNA-negative. Among HPV-positive OPSCC cases, three patients lacked genotype-specific data and were excluded. Finally, 174 patients with HPV DNA-positive OPSCC and available HPV genotyping results met the study criteria and were included in the final analysis (Fig. 1).

HPV DNA-positive OPSCC cases were classified into two groups: those harboring HPV-16 (n=130) and those harboring non-HPV-16 genotypes (n=44). HPV-16 accounted for 74.7% (130/174) of all HPV-genotyped cases, consistent with previous reports demonstrating its predominance in HPV-positive OPSCC.

Among non-HPV-16 cases, the relatively prevalent high-risk genotypes HPV-18, HPV-33, HPV-35, and HPV-58 were analyzed separately, whereas the remaining high-risk genotypes (HPV-26, -31, -39, -45, -51, -52, -53, -56, -59, -66, -68, -69, -70, -73, and -82) were grouped as “other high-risk HPV genotypes” (other hrHPV). The 18 types (HPV-30, -32, -34, -40, -42, -43, -44, -54, -55, -61, -62, -67, -74, -81, -83, -84, -87, and -90) are collectively reported as HPV-NOS. (Supplementary Table 1)

Non-HPV-16 genotypes, comprising 25.3% (44/174) of all HPV-genotyped cases, were distributed as follows: HPV-33, 25.0% (11/44), HPV-35, 18.2% (8/44), HPV-58, 13.6% (6/44), HPV-18, 13.6% (6/44), other hrHPV 25.0% (11/44), and HPV-NOS 4.6% (2/44) (Fig. 2). Coinfection with multiple HPV genotypes was rare, occurring in 1.7% of cases (3/174).

Clinical characteristics of study population according to HPV status

The patient characteristics according to HPV status (HPV-16 and non-HPV-16 groups) are summarized in Table 1. Overall, patients in the HPV-16 group were diagnosed at a younger age than those in the non-HPV-16 group ($P=0.019$). Smoking status also differed significantly between the two groups ($P=0.049$), with a higher proportion of ex-smokers in the non-HPV-16 group (59.1% vs. 39.2%). A significant difference was observed in AJCC 7th edition nodal stage distribution, with non-HPV-16 cases showing more advanced nodal stage than HPV-16 cases ($P=0.040$), whereas no significant difference was found for AJCC 8th edition nodal stage ($P=0.130$). No significant differences were observed between the groups with respect to sex, ECOG (Eastern

Cooperative Oncology Group) performance status, alcohol consumption, primary tumor subsite, treatment modality, AJCC 8th edition cT, cN, and cTNM stage, or AJCC 7th edition cT and cTNM stage.

Genotype-specific analyses were performed across six subgroups (HPV-16, HPV-33, HPV-35, HPV-58, HPV-18, and other hrHPV) to further characterize non-HPV-16 OPSCC. According to the AJCC 8th edition staging system, overall stage distribution did not differ significantly across genotypes (Supplementary Table 2).

However, pairwise comparisons with HPV-16 demonstrated that HPV-33 was associated with a significantly higher cN stage ($P=0.009$) (Table 2). No significant differences were observed for HPV-35, HPV-58, HPV-18, or other hrHPV genotypes.

According to the AJCC 7th edition, cT, cN, and cTNM stages did not differ significantly across genotypes (Supplementary Table 3). In pairwise comparisons with HPV-16, HPV-33 showed a higher cN stage ($P=0.012$), whereas no significant differences were observed for the other genotypes, consistent with the findings based on the AJCC 8th edition (Table 3).

Survival Outcomes according to HPV Status

To assess treatment outcomes, OS and DFS were evaluated. Initial Kaplan–Meier analyses comparing the HPV-16 and non-HPV-16 groups demonstrated no significant differences in DFS ($P=0.539$) or OS ($P=0.149$) (Fig. 3). In Firth-penalized Cox regression analysis of DFS according to HPV genotype (HPV-16 vs. non-HPV-16), HPV genotype was not associated with DFS in either univariable or multivariable analysis (Supplementary Table 4). Also, Firth-penalized Cox regression analysis of OS according to HPV genotype (HPV-16 vs. non-HPV-16) was conducted (Supplementary Table 5). HPV genotype was not significantly associated with OS in either univariable or multivariable analysis.

Genotype-specific analyses were subsequently performed across six genotype subgroups: HPV-16, HPV-33, HPV-35, HPV-58, HPV-18, and other hrHPV. Detailed data on follow-up duration, deaths, and recurrence patterns according to HPV genotype subgroups are provided in Supplementary Table 6. DFS did not differ significantly across genotypes ($P=0.566$) and pairwise comparisons with HPV-16 similarly showed no significant differences (Fig. 4A). In contrast, OS differed significantly according to HPV genotype ($P=0.005$) (Fig. 4B). Pairwise log-rank tests with Holm correction revealed significantly poorer OS among patients with HPV-35 compared with those with HPV-16 ($P=0.009$). Patients with other hrHPV genotypes showed a trend

toward poorer OS relative to those with HPV-16, although this did not reach statistical significance ($P=0.058$). No significant differences in OS were observed among patients with HPV-33, HPV-58, or HPV-18 compared with those with HPV-16.

In Firth-penalized Cox regression analysis of DFS among six genotype subgroups, no individual HPV genotype showed a significant association with DFS compared with HPV-16 (Table 4). In multivariable analysis, primary site classified as others and AJCC 8th edition stage III remained independent predictors of worse DFS. Also, in Firth-penalized Cox regression analysis of OS in six genotype subgroups, HPV-35 was significantly associated with worse OS compared with HPV-16 in both univariable and multivariable analyses. Other hrHPV genotypes showed a significant association with worse OS in univariable analysis, but this did not persist after adjustment. AJCC 8th edition stage III and stage IV disease were also independent predictors of worse OS in multivariable analysis (Table 5).

Sensitivity analysis in HPV DNA-positive/p16-positive OPSCC

Among the 174 HPV DNA-positive OPSCC cases, 160 (92.0%) were p16-positive and classified as HPV DNA-positive/p16-positive OPSCC, including 121/130 (93.1%) in the HPV-16 group and 39/44 (88.6%) in the non-HPV-16 group. Of the remaining cases, HPV DNA-positive/p16-negative OPSCC was identified in 1/130 (0.8%) and 4/44 (9.1%) cases, respectively, while p16 results were missing in 8/130 (6.2%) and 1/44 (2.3%). After excluding missing p16 data, the proportion of HPV DNA-positive/p16-positive OPSCC remained significantly higher in the HPV-16 group than in the non-HPV-16 group (121/122 [99.2%] vs. 39/43 [90.7%], Fisher's exact test, $P=0.017$).

Given that HPV DNA-positive/p16-negative OPSCC is associated with a worse prognosis, a sensitivity analysis was performed in HPV DNA-positive/p16-positive group ($n=160$), excluding HPV DNA-positive/p16-negative cases, to confirm the robustness of the above findings. DFS and OS was compared between HPV-16/p16-positive and non-HPV-16/p16-positive groups. Kaplan–Meier survival analysis demonstrated no significant differences in DFS ($P=0.762$) or OS ($P=0.233$) between HPV-16/p16-positive and non-HPV-16/p16-positive groups (Supplementary Fig. 1). HPV genotype (HPV-16/p16-positive vs. non-HPV-16/p16-positive) was not associated with DFS in either univariable or multivariable analysis (Supplementary Table 7). In multivariable analysis, primary site classified as others and AJCC 8th edition stage III remained independent predictors of worse DFS. Also, HPV genotype group (HPV-16/p16-positive vs. non-HPV-16/p16-positive) was

not associated with OS in either univariable or multivariable analysis. In multivariable analysis, AJCC 8th edition stage II, stage III, and stage IV disease remained independent predictors of worse OS (Supplementary Table 8). The results of the sensitivity analysis in the HPV DNA-positive/p16-positive subgroup were consistent with those of the overall HPV DNA-positive OPSCC cohort.

Sensitivity Analysis according to Different Genotype Classification

Additional sensitivity analyses were performed using several alternative HPV genotype classifications. Specifically, DFS and OS were analyzed according to four classifications schemes: (1) phylogenetic species classification, (2) 4-valent vaccine coverage, (3) 9-valent vaccine coverage, and (4) a three group vaccine coverage classification (Supplementary Figs. 2–5). In the three vaccine-covered group classification, the groups were divided as the 4-valent vaccine-covered genotype group (HPV-16 and -18), the 9-valent vaccine-covered genotype group excluding the 4-valent vaccine-covered genotypes (HPV-31, -33, -45, -52, and -58), and the non-covered group. In phylogenetic species classification, there was no significant difference in DFS and OS between alpha-9 and alpha-7 groups. Under the 4-valent vaccine coverage classification, no significant differences in DFS or OS were observed between the vaccine-covered genotypes and the other genotypes. Under the 9-valent vaccine coverage classification, there was no significant difference in DFS between the vaccine-covered genotypes and the other genotypes; however, OS was significantly worse in genotypes not covered by the 9-valent vaccine. In the three-group vaccine coverage classification, OS differed significantly among the three groups ($P=0.038$). In pairwise comparisons with Holm correction, the 4-valent vaccine-covered group showed significantly better OS than the non-covered group ($P=0.036$).

DISCUSSION

HPV-positive OPSCC has increased markedly over recent decades, in contrast to declining incidence at other tobacco-related head and neck subsites, and represents a clinicopathologically distinct entity characterized by rising incidence, male predominance, younger age at diagnosis, and lower cumulative tobacco exposure compared with HPV-negative disease[2, 3]. Prognosis is generally superior, which has prompted interest in

treatment de-intensification strategies, and the AJCC 8th edition provides a distinct staging framework for HPV-positive OPSCC[2, 15].

Genotype-level heterogeneity within HPV-positive OPSCC remains incompletely defined. Meta-analyses and large multi-country datasets consistently demonstrate that HPV-16 overwhelmingly predominates among HPV-positive OPSCC cases, whereas non-HPV-16 types collectively account for a smaller yet clinically meaningful proportion[1, 16].

In North America, single-center and regional cohorts demonstrate a consistent pattern with HPV-16 predominating (100% and 88% in two series), with HPV-35 (5.1%) and HPV-33 (3.7%) representing the most frequent non-HPV-16 types[10, 17]. French cohorts reflect a broader European pattern, characterized by a high prevalence of HPV-16 (88.7%–96.4%) and lower frequencies of non-HPV-16 genotypes[18, 19]. In a tertiary-center series encompassing multiple head and neck subsites in France, HPV-16 was identified in 93.8% of HPV-positive OPSCC cases, consistent with findings from OPSCC-specific studies[20]. In Northern Europe, a Danish population-based cohort of patients with HPV DNA-positive/p16-positive OPSCC identified HPV-16 in 86.1% of cases, followed by HPV-33 (7.4%) and HPV-35 (3.4%)[5]. Norwegian cohort study reported HPV-33 (9.4%) as the most frequent non-HPV-16 genotype in a population dominated by HPV-16 (85.0%)[21].

Compared to Western cohorts, East Asian series report a broader distribution of non-HPV-16 genotypes[13, 14, 22-24]. In Hong Kong, a territory-wide analysis showed that HPV-16 accounted for 79.3% of HPV-positive OPSCC, whereas HPV-58 (8.3%) and HPV-26 (5.0%) were the most common non-HPV-16 genotypes[22]. A Korean hospital-based study reported HPV-16 as the dominant genotype (84.3%), followed by HPV-35 (6.9%) and HPV-33 (4.4%)[13]. In a previous multicenter Korean tonsillar cancer cohort, both HPV-16 and HPV-18 were identified at equal frequencies (43.9%), highlighting substantial genotype heterogeneity within the region[14]. Japanese multicenter studies similarly confirmed HPV-16 predominance (88.6% and 90.0%) among HPV-positive OPSCC cases, with relatively infrequent detection of non-HPV-16 genotypes[23, 24].

Taken together, the available evidence indicates that HPV-16 accounts for approximately 85.0%–96.4% of HPV-positive OPSCC cases in Western populations and 43.9%–84.3% in East Asia. Although HPV-16 remains the dominant genotype across regions, the proportion and distribution of non-HPV-16 genotypes vary by geography and cohort characteristics. HPV-33 and HPV-35 are more frequently reported in Western studies, whereas HPV-58 and HPV-18 are more common in East Asian series[1, 5, 13, 14, 16, 22].

In our genotyped cohort (n = 174), HPV-16 accounted for 74.7% of cases, whereas non-HPV-16 genotypes

comprised 25.3%, with HPV-33 (6.3%), HPV-35 (4.6%), HPV-58 (3.4%), and HPV-18 (3.4%) being the most prevalent. This proportion of non-HPV-16 genotypes (~25%) more closely resembles that reported in Hong Kong and Korean cohorts than that observed in Western populations. Notably, the non-HPV-16 composition of our cohort reflects both the HPV-33/HPV-35 predominance characteristic of Western series and the enrichment of HPV-58/HPV-18 reported in East Asia. Given that our cohort represents a contemporary Korean population, this intermediate pattern has practical implications for the design of diagnostic genotyping panels and for modeling the potential impact of HPV vaccination strategies in the region.

In our genotyped HPV DNA-positive OPSCC cohort, patients with non-HPV-16 genotypes showed similar sex distribution, ECOG performance status, primary tumor site distribution, and treatment modalities compared with those with HPV-16, whereas patients with HPV-16 were younger and had more favorable smoking status. Most of clinical stage distribution did not differ significantly between the HPV-16 and non-HPV-16 groups according to either the AJCC 7th or 8th edition. According to the AJCC 7th edition, non-HPV-16 group tended to have more advanced nodal stage than HPV-16 group, whereas this difference was not observed under the AJCC 8th edition. Previous institutional and registry-based studies reported comparable baseline T, N, and overall stage distributions across HPV genotypes[5, 11]. In another single-center study, patients with non-HPV-16 genotypes (HPV-18, 33, or 35) were slightly older and presented with higher cT categories than those with HPV-16 OPSCC[6].

In genotype-specific subgroup analyses, statistically significant differences in cN stages were observed between HPV-16 and HPV-33 under both the AJCC 8th and 7th edition, suggesting a potentially distinct nodal presentation for HPV-33-positive OPSCC. However, the distinct nodal stage distribution observed in HPV-33-positive OPSCC was not associated with inferior survival in our cohort. In a Korean multicenter tonsillar cohort, HPV-18 was associated with older age and showed a trend toward higher cT stage compared with HPV-16, although this difference was not statistically significant[14].

In our cohort, OS and DFS did not differ significantly between HPV-16 and non-HPV-16 groups. These findings are consistent with those from large registry and single-institution cohorts reporting no significant survival differences between HPV-16 and non-HPV-16 genotypes[6, 10, 11, 16]. In a Danish registry of HPV-positive OPSCC (n=769), no differences in OS or DFS were observed between patients with HPV-16 and those with high-risk non-HPV-16 genotypes[5]. Similarly, in a single-institution U.S. cohort of approximately 200 patients, 3-year OS, DFS, and locoregional control did not differ significantly between HPV-16 and non-HPV-

16 genotypes[11]. A North American series likewise reported no clear differences in overall or recurrence-free survival between the HPV-16 and non-HPV-16 groups.[6]

In contrast to our findings, some studies have reported genotype-specific survival differences, with non-HPV-16 genotypes demonstrating inferior OS and/or DFS compared with HPV-16[4, 8]. In the Carolina Head and Neck Cancer Epidemiology (CHANCE) study, non-HPV-16 genotypes were associated with poorer OS than HPV-16 (HR: 1.92; 95% CI: 1.03, 3.60)[8]. An analysis of The Cancer Genome Atlas (TCGA) head and neck squamous cell carcinoma cohort similarly demonstrated inferior 3-year OS among patients with non-HPV-16 genotypes compared with HPV-16 (49% vs 88% for HPV-16; $P=0.003$), although HPV genotype was not an independent prognostic factor after multivariable adjustment[4]. Furthermore, a recent meta-analysis reported significantly lower pooled 5-year OS for patients with non-HPV-16 genotypes than for those with HPV-16 ($P=0.008$), with a trend toward lower pooled 5-year RFS ($P=0.063$)[25].

In our genotype-specific analyses, HPV-35 was associated with significantly worse OS than HPV-16, whereas other hrHPV genotypes showed a trend toward worse OS that did not reach statistical significance. HPV-33, HPV-18 and HPV-58 did not differ significantly from HPV-16. In contrast, DFS did not differ significantly across genotypes, and no pairwise comparisons with HPV-16 reached statistical significance. These findings suggest that observed survival differences are driven by specific non-HPV-16 subtypes rather than by non-HPV-16 status overall. Prior studies have reported heterogeneous genotype-specific survival patterns. A study using analyses from TCGA associated HPV-33 with inferior survival and distinct genomic and immune profiles[7]. In a Korean multicenter tonsil cancer cohort, HPV-18 was associated with significantly worse outcomes than HPV-16, with lower DFS ($P=0.020$) and poorer OS ($P=0.013$)[14]. In a Danish cohort, genotypes other than HPV-33 and HPV-35 had significantly reduced OS compared with HPV-16 (HR: 2.33, $P=0.027$)[5].

In our cohort, HPV DNA-positive/p16-negative discordance was rare among HPV-16 cases but was more frequent in non-HPV-16 genotypes. These findings are concordant with a recent meta-analysis demonstrating that patients with non-HPV-16 OPSCC were 0.4 times as likely to be p16-positive as those with HPV-16 OPSCC ($P=0.020$)[25]. Previous studies have shown that patients with HPV DNA-positive/p16-negative OPSCC have significantly worse outcomes than those with HPV DNA-positive/p16-positive tumors[26]. Taken together, the higher frequency of HPV DNA-positive/p16-negative tumors in non-HPV-16 OPSCC may partially explain the modest survival decrement observed in this subgroup; however, current evidence is insufficient to establish a causal relationship[25]. Biologically, p16 discordance may reflect differences in viral

oncogene activity or host cell cycle regulation, suggesting that not all HPV-positive tumors are driven by equivalent E6/E7-mediated pathways, particularly among non-HPV-16 subtypes. These findings highlight the limitations of relying on p16 IHC alone as a surrogate marker for HPV-positive OPSCC, particularly in non-HPV-16 tumors and support the complementary use of HPV genotyping in this context.

The increase in HPV DNA testing and p16 IHC at our institution from the early 2010s appears to reflect a broader global shift in the recognition of HPV-positive OPSCC (Supplementary Fig. 6). Although p16 was formally incorporated into staging later in the AJCC 8th edition, clinical practice likely changed earlier in response to emerging evidence. In particular, the landmark study by Ang et al. in 2010 showed the favorable survival of HPV-positive OPSCC and helped drive the early adoption of HPV-related diagnostic testing at many leading institutions, even before formal guideline standardization[27]. In this context, the temporal trend in our cohort likely reflects early integration of these findings into routine practice.

This study has several limitations. First, its retrospective single-institution design may have introduced selection and information bias. Second, although this cohort represents one of the largest Asian series with HPV genotyping, the number of non-HPV-16 cases was modest, particularly within individual genotype subgroups. In particular, the HPV-35, HPV-58, and HPV-18 subgroups each comprised fewer than ten patients; therefore, survival estimates for these groups should be interpreted with caution and considered mainly exploratory because they are statistically unstable and vulnerable to random variation. In addition, because the study period spanned nearly two decades, temporal changes in diagnostic approaches, treatment strategies, and supportive care may have influenced clinical outcomes despite the use of standardized institutional management protocols. Finally, because this cohort reflects a Korean population, the findings may not be generalizable to other populations.

Uterine cervical cancer progresses through screen-detectable precursors of cervical intraepithelial neoplasia (CIN), enabling population-level interception through primary high-risk HPV testing with cytology or colposcopy triage based on FIGO staging[28]. In contrast, HPV-positive OPSCC lacks a validated population-based screening paradigm because the relevant epithelium resides deep within the tonsillar crypts, no preinvasive analog of CIN has been identified, and oral or oropharyngeal HPV infection in the general population is typically transient, resulting in a very low positive predictive value for cancer[2, 3, 15, 29]. Consequently, OPSCC most often presents with clinically apparent disease rather than screen-detected lesions and is frequently characterized by small primary tumors with prominent nodal metastases[2, 15].

In addition, the point prevalence of oral HPV infection has been reported to range from approximately 15% to 31% over follow-up[30]. Seventeen HPV genotypes have been identified (6, 11, 16, 18, 31, 33, 39, 43, 45, 51, 53, 56, 58, 59, 66, 70, and 82), and new incident infections continue to occur over time, supporting the inference that the oral mucosa may serve as an important reservoir and route of HPV transmission[30]. Beyond sexual intercourse, close oral contact, including deep kissing and oral sex, is also likely to facilitate HPV transmission. Smoking has been shown to independently increase the risk of oral HPV infection. Moreover, oral HPV infection often diverges from anogenital infection, supporting the concept of an independent oral niche and providing a plausible explanation for OPSCC occurring in individuals without a high-risk sexual history[30,31].

OPSCC is characterized by difficulty in early detection, marked male predominance, distinct oral transmission dynamics, and diverse latent HPV carriage within the oral and oropharyngeal mucosa[2, 3, 15, 29-31]. Overall, the proportion of non-HPV-16 genotypes in our cohort (25.3%) was comparable to that reported in Asian cohorts, whereas the relative predominance of HPV-33 and HPV-35 resembled patterns observed in Western populations.[1, 5, 13, 14, 16, 22]. Unlike uterine cervical cancer, which predominantly affects women and benefits from established population-based screening programs, HPV-positive OPSCC exhibits a strong male predominance across genotypes, underscoring the importance of gender-neutral prevention strategies. Currently, the National Immunization Program in South Korea provides the 4-valent HPV vaccine to adolescent girls and selected young women[32]. In contrast, HPV vaccination strategies in the United States, parts of Europe, and Australia have increasingly moved toward gender-neutral vaccination, often using the 9-valent vaccine. Given the substantial proportion of non-HPV-16 genotypes and the marked male predominance observed in our cohort, our findings may have implications for future HPV prevention strategies.

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Figure legends

Fig. 1. Flow chart of patient selection

HPV, human papilloma virus; OPSCC, oropharyngeal squamous cell carcinoma; HPV DNA-positive OPSCC: tumors positive for HPV DNA regardless of p16

Fig. 2. Distribution of HPV Genotypes in HPV DNA-positive Oropharyngeal Squamous Cell Carcinoma.

HPV, human papilloma virus; OPSCC, oropharyngeal squamous cell carcinoma; Other high-risk HPV, high-risk HPV genotypes except HPV-16, -33, -35, -58, and -18; HPV-NOS, HPV genotypes not otherwise specified,

Fig. 3. DFS and OS between HPV-16 and non-HPV-16 groups in HPV DNA-positive OPSCC (n = 174)

HPV-16 and non-HPV-16 groups demonstrated no significant differences in DFS ($P=0.539$) or OS ($P=0.149$).

DFS, disease-free survival; OS, overall survival; HPV, human papilloma virus; OPSCC, oropharyngeal squamous cell carcinoma;

Fig. 4. Subgroup analysis of DFS and OS according to HPV genotype in HPV DNA-positive OPSCC

(A) DFS were comparable across genotypes ($P=0.566$); (B) OS differed significantly according to HPV genotype ($P=0.005$), with HPV-35 exhibiting significantly worse OS compared to HPV-16 (Pairwise Log-rank test adjusted by Holm correction, $P=0.009$)

DFS, disease-free survival; OS, overall survival; HPV, human papilloma virus; OPSCC, oropharyngeal squamous cell carcinoma; Other high-risk HPV, high-risk HPV genotypes except HPV-16, -33, -35, -58, and -18

Supp. Fig. 1. DFS and OS between HPV-16 and non-HPV-16 groups in HPV DNA-positive/p16-positive OPSCC (n = 160)

In the sensitivity analysis restricted to HPV DNA-positive/p16-positive cases, no significant differences in DFS ($P=0.762$) or OS ($P=0.233$) were observed between HPV-16 and non-HPV-16 groups, consistent with the overall cohort.

DFS, disease-free survival; OS, overall survival; HPV, human papilloma virus; OPSCC, oropharyngeal squamous cell carcinoma;

Supp. Fig. 2. DFS and OS in HPV DNA-positive OPSCC according to phylogenetic species classification: Alpha-9 (HPV-16-related) group, Alpha-7 (HPV-18-related) group, and others.

Alpha-9 (HPV-16-related) group, HPV-16, -31, -33, -35, -52, and -58 and Alpha-7 (HPV-18-related) group includes HPV-18, -39, -45, -59, and -68. All remaining genotypes were classified as others. There were no significant differences in DFS and OS between Alpha-9 and Alpha-7 groups

DFS, disease-free survival; OS, overall survival; HPV, human papilloma virus; OPSCC, oropharyngeal squamous cell carcinoma;

Supp. Fig. 3. DFS and OS in HPV DNA-positive OPSCC according to 4-valent vaccine coverage: the 4-valent vaccine-covered group and others.

The 4-valent vaccine-covered group includes HPV-16 and 18 and all remaining genotypes were classified as others. No significant differences in DFS or OS were observed between the 4-valent vaccine-covered genotypes and the other genotypes.

DFS, disease-free survival; OS, overall survival; HPV, human papilloma virus; OPSCC, oropharyngeal squamous cell carcinoma;

Supp. Fig. 4. DFS and OS in HPV DNA-positive OPSCC according to 9-valent vaccine coverage: the 9-valent vaccine-covered group and others.

The 9-valent vaccine-covered group includes HPV-16, -18, -31, -33, -45, -52, and -58 and all remaining

genotypes were classified as others. Though DFS did not differ between the 9-valent vaccine-covered genotypes and the other genotypes, OS was significantly worse in genotypes not covered by the 9-valent vaccine ($P=0.012$).

DFS, disease-free survival; OS, overall survival; HPV, human papilloma virus; OPSCC, oropharyngeal squamous cell carcinoma;

Supp. Fig. 5. DFS and OS in HPV DNA-positive OPSCC according to three genotype groups: 4-valent vaccine covered genotypes, 9-valent vaccine covered genotypes excluding HPV-16/18, and non-covered genotypes.

The 4-valent vaccine covered genotypes includes HPV-16 and -18 and the 9-valent vaccine covered genotypes excluding HPV-16/18 includes HPV-31, -33, -45, -52, and -58. All remaining genotypes were classified as non-covered genotypes. In the three-group vaccine coverage classification, OS differed significantly among the three groups ($P=0.038$), with the 4-valent vaccine-covered group showing significantly better OS than the non-covered group (Pairwise Log-rank test adjusted by Holm correction, $P=0.036$).

DFS, disease-free survival; OS, overall survival; HPV, human papilloma virus; OPSCC, oropharyngeal squamous cell carcinoma;

Supp. Fig. 6. Trend of HPV DNA testing and p16 IHC over 20 years

Temporal change of HPV tests in our institution reports increase in HPV DNA testing and p16 IHC from the early 2010s.

HPV, human papilloma virus; IHC, immunohistochemistry.

Table 1. Clinical Characteristics of HPV-Positive OPSCC according to HPV Genotype group (HPV-16 vs. non-HPV-16) (n = 174)

Variable	Overall (n = 174)	HPV-16 (n = 130)	Non-HPV-16 (n = 44)	P-value
Age (yr, mean±SD)	57.8 ± 10.8	56.8 ± 11.2	60.7 ± 8.8	0.019*
Sex , n (%)				0.764
female	16.0 (9.2%)	13.0 (10.0%)	3.0 (6.8%)	
male	158.0 (90.8%)	117.0 (90.0%)	41.0 (93.2%)	
ECOG_PS , n (%)				0.332
0+1	169.0 (97.1%)	125.0 (96.2%)	44.0 (100.0%)	
2+3	5.0 (2.9%)	5.0 (3.8%)	0.0 (0.0%)	
Alcohol , n (%)				0.393
non-drinker	84.0 (48.3%)	60.0 (46.2%)	24.0 (54.5%)	
social drinker	45.0 (25.9%)	33.0 (25.4%)	12.0 (27.3%)	
binge drinker (>soju 1bottle/week)	45.0 (25.9%)	37.0 (28.5%)	8.0 (18.2%)	
Smoking , n (%)				0.049*
non-smoker	72.0 (41.4%)	57.0 (43.8%)	15.0 (34.1%)	
ex-smoker	77.0 (44.3%)	51.0 (39.2%)	26.0 (59.1%)	
current smoker	25.0 (14.4%)	22.0 (16.9%)	3.0 (6.8%)	
Primary site , n (%)				0.673
tonsil	152.0 (87.4%)	114.0 (87.7%)	38.0 (86.4%)	
tongue base	20.0 (11.5%)	15.0 (11.5%)	5.0 (11.4%)	
others (soft palate, PPW)	2.0 (1.1%)	1.0 (0.8%)	1.0 (2.3%)	
Treatment summary , n (%)				0.927
Surgery only	31.0 (17.8%)	24.0 (18.5%)	7.0 (15.9%)	
Surgery+RT	22.0 (12.6%)	15.0 (11.5%)	7.0 (15.9%)	
Surgery+CCRT	28.0 (16.1%)	20.0 (15.4%)	8.0 (18.2%)	
RT±Salvage surgery	15.0 (8.6%)	11.0 (8.5%)	4.0 (9.1%)	
CT±Salvage surgery	1.0 (0.6%)	1.0 (0.8%)	0.0 (0.0%)	
CCRT±Salvage surgery	77.0 (44.3%)	59.0 (45.4%)	18.0 (40.9%)	
AJCC8_cT , n (%)				0.257
T1	43.0 (24.7%)	32.0 (24.6%)	11.0 (25.0%)	
T2	88.0 (50.6%)	70.0 (53.8%)	18.0 (40.9%)	
T3	9.0 (5.2%)	7.0 (5.4%)	2.0 (4.5%)	
T4	34.0 (19.5%)	21.0 (16.2%)	13.0 (29.5%)	
AJCC8_cN , n (%)				0.13
N0	16.0 (9.2%)	10.0 (7.7%)	6.0 (13.6%)	
N1	116.0 (66.7%)	92.0 (70.8%)	24.0 (54.5%)	
N2	25.0 (14.4%)	15.0 (11.5%)	10.0 (22.7%)	
N3	17.0 (9.8%)	13.0 (10.0%)	4.0 (9.1%)	
AJCC8_cTNM , n (%)				0.466
Stage I	106.0 (60.9%)	82.0 (63.1%)	24.0 (54.5%)	
Stage II	20.0 (11.5%)	15.0 (11.5%)	5.0 (11.4%)	
Stage III	45.0 (25.9%)	30.0 (23.1%)	15.0 (34.1%)	
Stage IV	3.0 (1.7%)	3.0 (2.3%)	0.0 (0.0%)	
AJCC7_cT , n (%)				0.247
T1	43.0 (24.7%)	32.0 (24.6%)	11.0 (25.0%)	
T2	88.0 (50.6%)	70.0 (53.8%)	18.0 (40.9%)	
T3	9.0 (5.2%)	7.0 (5.4%)	2.0 (4.5%)	
T4a	32.0 (18.4%)	19.0 (14.6%)	13.0 (29.5%)	
T4b	2.0 (1.1%)	2.0 (1.5%)	0.0 (0.0%)	
AJCC7_cN , n (%)				0.04*
N0	16.0 (9.2%)	10.0 (7.7%)	6.0 (13.6%)	
N1	25.0 (14.4%)	16.0 (12.3%)	9.0 (20.5%)	

N2a	19.0 (10.9%)	18.0 (13.8%)	1.0 (2.3%)	
N2b	72.0 (41.4%)	58.0 (44.6%)	14.0 (31.8%)	
N2c	25.0 (14.4%)	15.0 (11.5%)	10.0 (22.7%)	
N3	17.0 (9.8%)	13.0 (10.0%)	4.0 (9.1%)	
AJCC7_cTNM, n (%)				0.483
Stage I	2.0 (1.1%)	2.0 (1.5%)	0.0 (0.0%)	
Stage II	18.0 (10.3%)	11.0 (8.5%)	7.0 (15.9%)	
Stage III	13.0 (7.5%)	8.0 (6.2%)	5.0 (11.4%)	
Stage IVA	120.0 (69.0%)	92.0 (70.8%)	28.0 (63.6%)	
Stage IVB	18.0 (10.3%)	14.0 (10.8%)	4.0 (9.1%)	
Stage IVC	3.0 (1.7%)	3.0 (2.3%)	0.0 (0.0%)	

ECOG, Eastern cooperative oncology group performance status; PPW, posterior pharyngeal wall; RT, radiotherapy; CT, chemotherapy; CCRT, concomitant chemoradiotherapy;

Between-group comparisons were performed using the Wilcoxon rank sum (Mann–Whitney U) test for continuous variables and the Pearson's Chi-squared test or Fisher's exact tests for categorical variables, as appropriate.

*Statistically significant ($P < 0.05$)

Table 2. Pairwise comparison of clinical staging according to HPV genotype subgroups by AJCC 8th

Edition

AJCC 8th Edition	cT stage	cN stage	cTNM stage
HPV-16 vs. HPV-33	0.199	0.009*	0.062
HPV-16 vs. HPV-35	0.249	0.077	0.630
HPV-16 vs. HPV-58	0.726	0.645	0.858
HPV-16 vs. HPV-18	1	0.136	1
HPV-16 vs. Other hrHPV	0.256	0.394	0.781

Other hrHPV, Other high-risk type HPV

Fisher's exact test was used to compare the distributions of cT, cN, and cTNM stages between groups.

*Statistically significant ($P<0.05$)

Table 3. Pairwise comparison of clinical staging according to HPV genotype subgroups by AJCC 7th

Edition

AJCC 7th Edition	cT stage	cN stage	cTNM stage
HPV-16 vs. HPV-33	0.265	0.012*	0.227
HPV-16 vs. HPV-35	0.293	0.230	0.922
HPV-16 vs. HPV-58	0.680	0.868	0.759
HPV-16 vs. HPV-18	1	0.253	0.216
HPV-16 vs. Other hrHPV	0.306	0.104	0.535

Other hrHPV, Other high-risk type HPV

Fisher's exact test was used to compare the distributions of cT, cN, and cTNM stages between groups.

*Statistically significant ($P<0.05$)

Table 4. Firth-Penalized Cox Regression Analysis of DFS among six HPV genotype subgroups (HPV-16, HPV-33, HPV-35, HPV-58, HPV-18, and other hrHPV) in HPV DNA-positive OPSCC

Characteristic	Univariable		Multivariable	
	HR (95% CI)	P-value	HR (95% CI)	P-value
HPV genotype				
HPV-16	Reference		Reference	
HPV-33	1.25 (0.32–4.96)	0.751	1.31 (0.29–5.91)	0.723
HPV-35	1.19 (0.21–6.79)	0.846	1.67 (0.25–11.02)	0.597
HPV-58	0.42 (0.02–7.96)	0.561	0.27 (0.01–6.16)	0.412
HPV-18	3.12 (0.78–12.42)	0.106	3.57 (0.74–17.14)	0.112
other hrHPV	2.01 (0.61–6.59)	0.25	1.13 (0.28–4.54)	0.868
Age	1.01 (0.98–1.05)	0.491		
Sex				
female	Reference			
male	6.56 (0.38–112.83)	0.195		
ECOG_PS				
0+1	Reference		Reference	
2+3	4.74 (1.22–18.46)	0.025	1.90 (0.35–10.26)	0.453
Alcohol				
non-drinker	Reference		Reference	
social drinker	2.83 (1.19–6.73)	0.019	2.56 (0.94–6.93)	0.065
binge drinker (>soju 1bottle/week)	1.79 (0.69–4.62)	0.23	1.48 (0.46–4.77)	0.512
Smoking				
non-smoker	Reference			
ex-smoker	1.78 (0.78–4.03)	0.169		
current smoker	1.74 (0.55–5.52)	0.346		
Primary site				
tonsil	Reference		Reference	
tongue base	1.04 (0.33–3.28)	0.947	0.90 (0.26–3.14)	0.871
others (soft palate, PPW)	16.01 (3.98–64.46)	<0.001	14.18 (2.02–99.56)	0.008*
Treatment_summary				
Surgery only	Reference		Reference	
Surgery+RT	1.38 (0.35–5.46)	0.645	1.47 (0.33–6.60)	0.615
Surgery+CCRT	1.10 (0.30–4.10)	0.882	0.85 (0.21–3.54)	0.827
RT±Salvage surgery	3.84 (1.03–14.29)	0.045	2.79 (0.60–13.01)	0.192
CT±Salvage surgery	3.47 (0.16–76.04)	0.429	2.41 (0.02–292.19)	0.719
CCRT±Salvage surgery	0.94 (0.30–2.95)	0.912	0.65 (0.19–2.24)	0.495
AJCC8_cTNM				
Stage I	Reference		Reference	
Stage II	1.25 (0.37–4.21)	0.72	1.66 (0.43–6.33)	0.459
Stage III	3.03 (1.37–6.69)	0.006	3.87 (1.42–10.55)	0.008*
Stage IV	2.37 (0.13–44.78)	0.566	3.00 (0.09–99.26)	0.538

Other hrHPV, Other high-risk type HPV; ECOG, Eastern cooperative oncology group performance status; PPW, posterior pharyngeal wall; RT, radiotherapy; CT, chemotherapy; CCRT, concomitant chemoradiotherapy;

Firth's penalized Cox regression analysis were performed to adjust for key clinical confounders. Variables with

a P -value ≤ 0.1 in the univariable Cox regression were included as covariates in the multivariable Cox regression model.

*Statistically significant ($P < 0.05$)

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Table 5. Firth-Penalized Cox Regression Analysis of OS among six HPV genotype subgroups (HPV-16, HPV-33, HPV-35, HPV-58, HPV-18, and other hrHPV) in HPV DNA-positive OPSCC (N = 174)

Characteristic	Univariable		Multivariable	
	HR (95% CI)	P-value	HR (95% CI)	P-value
HPV genotype				
HPV-16	Reference		Reference	
HPV-33	0.58 (0.02–13.40)	0.732	0.44 (0.01–14.57)	0.647
HPV-35	7.13 (1.78–28.60)	0.006	6.56 (1.01–42.72)	0.049*
HPV-58	1.33 (0.06–31.79)	0.86	1.57 (0.05–54.66)	0.803
HPV-18	1.59 (0.07–37.81)	0.775	3.12 (0.09–106.48)	0.528
other hrHPV	4.96 (1.26–19.57)	0.022	5.42 (0.89–33.08)	0.067
Age	1.03 (0.98–1.08)	0.262		
Sex				
female	Reference			
male	0.93 (0.16–5.34)	0.936		
ECOG_PS				
0+1	Reference			
2+3	1.66 (0.08–32.41)	0.739		
Alcohol				
non-drinker	Reference			
social drinker	0.61 (0.14–2.68)	0.509		
binge drinker (>soju 1bottle/week)	1.01 (0.30–3.38)	0.983		
Smoking				
non-smoker	Reference			
ex-smoker	1.70 (0.50–5.84)	0.396		
current smoker	2.90 (0.66–12.67)	0.158		
Primary site				
tonsil	Reference		Reference	
tongue base	0.88 (0.15–5.22)	0.891	1.24 (0.17–8.98)	0.831
others (soft palate, PPW)	21.14 (3.04–147.22)	0.002	10.46 (0.50–219.31)	0.13
Treatment_summary				
Surgery only	Reference		Reference	
Surgery+RT	2.34 (0.25–21.81)	0.454	4.31 (0.36–51.69)	0.249
Surgery+CCRT	0.81 (0.07–9.85)	0.868	0.41 (0.02–6.92)	0.538
RT±Salvage surgery	12.37 (1.74–88.25)	0.012	4.89 (0.41–58.49)	0.21
CT±Salvage surgery	14.51 (0.40–525.71)	0.144	1.20 (0.01–133.29)	0.941
CCRT±Salvage surgery	1.13 (0.15–8.66)	0.907	0.21 (0.02–2.48)	0.214
AJCC8_cTNM				
Stage I	Reference		Reference	
Stage II	1.89 (0.26–13.52)	0.527	7.90 (0.70–88.62)	0.094
Stage III	6.62 (1.72–25.50)	0.006	23.14 (3.45–155.34)	0.001*
Stage IV	52.89 (8.57–326.26)	<0.001	35.29 (2.20–566.56)	0.012*

Other hrHPV, Other high-risk type HPV; ECOG, Eastern cooperative oncology group performance status; PPW, posterior pharyngeal wall; RT, radiotherapy; CT, chemotherapy; CCRT, concomitant chemoradiotherapy;

Firth's penalized Cox regression analysis were performed to adjust for key clinical confounders. Variables with

a P -value ≤ 0.1 in the univariable Cox regression were included as covariates in the multivariable Cox regression model.

*Statistically significant ($P < 0.05$)

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SUPPLEMENTARY TABLES

Supplementary Table 1. Classification of detectable HPV genotypes and definition of HPV genotype subgroups

Genotyping Assay	Specific Genotype	Genotype subgroups
High-risk HPV types	16	HPV-16
	18	HPV-18
	33	HPV-33
	35	HPV-35
	58	HPV-58
	26, 31, 39, 45, 51, 52, 53, 56, 59, 66, 68, 69, 70, 73, 82	Other hrHPV
Low-risk HPV types	6, 11	-
other “detectable” types	30, 32, 34, 40, 42, 43, 44, 54, 55, 61, 62, 67, 74, 81, 83, 84, 87, 90	HPV-NOS

Other hrHPV, high-risk HPV genotypes except HPV-16, -33, -35, -58, and -18; HPV-NOS, HPV genotypes not otherwise specified

Supplementary Table 2. Clinical staging by AJCC 8th Edition stratified according to HPV genotype subgroups

AJCC 8 th Edition	HPV-16 (n=130)	HPV-33 (n=11)	HPV-35 (n=8)	HPV-58 (n=6)	HPV-18 (n=6)	Other hrHPV (n=11)	*P-value
cT stage							0.402
T1	32	5	1	1	1	2	
T2	70	3	3	3	4	4	
T3	7	0	1	0	0	1	
T4	21	3	3	2	1	4	
cN stage							0.071
N0	10	1	2	1	2	0	
N1	92	3	3	5	3	8	
N2	15	4	2	0	1	3	
N3	13	3	1	0	0	0	
cTNM stage							0.314
I	82	3	4	4	5	6	
II	15	3	1	0	0	1	
III	30	5	3	2	1	4	
IV	3	0	0	0	0	0	

*Kruskal-Wallis H test.

Other hrHPV, Other high-risk type HPV

Supplementary Table 3. Clinical staging by AJCC 7th Edition stratified according to HPV genotype subgroups (HPV-16, HPV-33, HPV-35, HPV-58, HPV-18, and other hrHPV)

AJCC 7 th Edition	HPV-16 (n=130)	HPV-33 (n=11)	HPV-35 (n=8)	HPV-58 (n=6)	HPV-18 (n=6)	Other hrHPV (n=11)	*P-value
cT stage							0.422
T1	32	5	1	1	1	2	
T2	70	3	3	3	4	4	
T3	7	0	1	0	0	1	
T4a	19	3	3	2	1	4	
T4b	2	0	0	0	0	0	
cN stage							0.286
N0	10	1	2	1	2	0	
N1	16	2	0	1	1	4	
N2a	18	0	0	1	0	0	
N2b	58	1	3	3	2	4	
N2c	15	4	2	0	1	3	
N3	13	3	1	0	0	0	
cTNM stage							0.316
I	2	0	0	0	0	0	
II	11	2	1	1	2	1	
III	8	1	0	0	1	2	
IVA	92	5	6	5	3	8	
IVB	14	3	1	0	0	0	
IVC	3	0	0	0	0	0	

*Kruskal-Wallis H test.

Other hrHPV, Other high-risk type HPV

Supplementary Table 4. Firth-Penalized Cox Regression Analysis of DFS According to HPV Genotype Group (HPV-16 vs. non-HPV-16) in HPV DNA-positive OPSCC (n = 174)

Characteristic	Univariable		Multivariable	
	HR (95% CI)	P-value	HR (95% CI)	P-value
HPV genotype				
HPV-16	Reference		Reference	
Non-HPV-16	1.28 (0.59–2.79)	0.527	1.10 (0.47–2.61)	0.824
Age	1.01 (0.98–1.05)	0.462		
Sex				
female	Reference			
male	6.77 (0.39–116.21)	0.187		
ECOG_PS				
0+1	Reference		Reference	
2+3	4.81 (1.24–18.71)	0.023	1.93 (0.38–9.89)	0.43
Alcohol				
non-drinker	Reference		Reference	
social drinker	3.03 (1.29–7.10)	0.011	2.86 (1.13–7.23)	0.027*
binge drinker (>soju 1 bottle/week)	1.81 (0.70–4.68)	0.219	1.42 (0.45–4.43)	0.55
Smoking				
non-smoker	Reference			
ex-smoker	1.84 (0.82–4.14)	0.139		
current smoker	1.77 (0.56–5.61)	0.331		
Primary site				
tonsil	Reference		Reference	
tongue base	1.01 (0.32–3.18)	0.981	1.02 (0.30–3.44)	0.973
others (soft palate, PPW)	16.18 (4.02–65.08)	<0.001	13.70 (2.22–84.56)	0.005*
Treatment_summary				
Surgery only	Reference		Reference	
Surgery+RT	1.38 (0.35–5.45)	0.643	1.65 (0.37–7.25)	0.508
Surgery+CCRT	1.10 (0.30–4.07)	0.887	1.13 (0.29–4.41)	0.858
RT±Salvage surgery	3.88 (1.04–14.39)	0.043	4.15 (0.93–18.40)	0.061
CT±Salvage surgery	3.60 (0.16–78.56)	0.416	3.60 (0.03–381.41)	0.59
CCRT±Salvage surgery	0.99 (0.32–3.07)	0.99	0.93 (0.29–3.00)	0.899
AJCC8_cTNM				
Stage I	Reference		Reference	
Stage II	1.19 (0.36–3.98)	0.776	1.44 (0.40–5.20)	0.579
Stage III	2.88 (1.32–6.26)	0.008	2.92 (1.16–7.35)	0.023*
Stage IV	2.32 (0.12–43.74)	0.574	2.43 (0.08–72.00)	0.608

ECOG, Eastern cooperative oncology group performance status; PPW, posterior pharyngeal wall; RT, radiotherapy; CT, chemotherapy; CCRT, concomitant chemoradiotherapy;

Firth's penalized Cox regression analysis were performed to adjust for key clinical confounders. Variables with a P -value ≤ 0.1 in the univariable Cox regression were included as covariates in the multivariable Cox regression model.

*Statistically significant ($P < 0.05$)

Supplementary Table 5. Firth-Penalized Cox Regression Analysis of OS According to HPV Genotype Group (HPV-16 vs. non-HPV-16) in HPV DNA-positive OPSCC (n = 174)

Characteristic	Univariable		Multivariable	
	HR (95% CI)	P-value	HR (95% CI)	P-value
HPV genotype				
HPV-16	Reference		Reference	
Non-HPV-16	2.18 (0.76–6.29)	0.148	2.35 (0.63–8.84)	0.206
Age	1.03 (0.98–1.08)	0.266		
Sex				
female	Reference			
male	0.91 (0.16–5.23)	0.916		
ECOG_PS				
0+1	Reference			
2+3	1.68 (0.09–32.81)	0.733		
Alcohol				
non-drinker	Reference			
social drinker	0.57 (0.13–2.53)	0.461		
binge drinker (>soju 1 bottle/week)	1.02 (0.31–3.41)	0.973		
Smoking				
non-smoker	Reference			
ex-smoker	1.63 (0.48–5.57)	0.439		
current smoker	2.89 (0.66–12.63)	0.159		
Primary site				
tonsil	Reference		Reference	
tongue base	0.91 (0.15–5.35)	0.913	1.13 (0.16–7.96)	0.902
others (soft palate, PPW)	21.45 (3.08–149.45)	0.002	22.50 (1.28–396.11)	0.033*
Treatment_summary				
Surgery only	Reference		Reference	
Surgery+RT	2.34 (0.25–21.81)	0.455	3.18 (0.27–37.95)	0.361
Surgery+CCRT	0.81 (0.07–9.88)	0.87	0.61 (0.05–7.78)	0.701
RT±Salvage surgery	12.39 (1.74–88.41)	0.012	7.08 (0.68–73.41)	0.101
CT±Salvage surgery	14.47 (0.40–524.01)	0.145	1.74 (0.02–155.17)	0.81
CCRT±Salvage surgery	1.08 (0.14–8.29)	0.941	0.29 (0.03–2.88)	0.287
AJCC8_cTNM				
Stage I	Reference		Reference	
Stage II	1.96 (0.27–14.06)	0.502	9.33 (0.87–99.85)	0.065
Stage III	6.80 (1.76–26.29)	0.005	24.71 (3.87–157.53)	<0.001*
Stage IV	54.31 (8.78–335.84)	<0.001	27.22 (1.87–396.99)	0.016*

ECOG, Eastern cooperative oncology group performance status; PPW, posterior pharyngeal wall; RT, radiotherapy; CT, chemotherapy; CCRT, concomitant chemoradiotherapy;

Firth's penalized Cox regression analysis were performed to adjust for key clinical confounders. Variables with a P -value ≤ 0.1 in the univariable Cox regression were included as covariates in the multivariable Cox regression model.

*Statistically significant ($P < 0.05$)

Supplementary Table 6. Follow-up Duration and Clinical Events According to HPV Genotype in HPV DNA-Positive OPSCC

Variable	Overall (n=174)	HPV-16 (n=130)	HPV-33 (n=11)	HPV-35 (n=8)	HPV-58 (n=6)	HPV-18 (n=6)	Other hrHPV (n=11)
Median follow-up, months (IQR)	50.28 (21.85- 74.5)	47.88 (21.41- 74.5)	58.17 (29.38- 76.7)	32.02 (28.00- 51.76)	59.40 (51.30- 67.93)	52.32 (24.01- 76.18)	63.67 (39.07- 69.52)
Deaths, n (%)	14 (8.05)	8 (6.15)	0	3 (37.5)	0	0	3 (27.27)
Recurrences, n (%)	30 (17.24)	21 (16.15)	2 (18.18)	1 (12.50)	0	2 (33.33)	3 (27.27)
Local	4	2	0	1	0	0	1
Regional	15	9	2	0	0	1	2
Distant	15	13	1	0	0	1	0

Other hrHPV, Other high-risk type HPV

Supplementary Table 7. Firth-Penalized Cox Regression Analysis of DFS According to HPV Genotype Group (HPV-16/p16-positive vs. non-HPV-16/p16-positive) in HPV DNA-positive/p16-positive OPSCC (n = 160) for sensitivity analysis

Characteristic	Univariable		Multivariable	
	HR (95% CI)	P-value	HR (95% CI)	P-value
HPV genotype				
HPV-16/p16-positive	Reference		Reference	
Non-HPV-16/p16-positive	1.18 (0.52–2.66)	0.695	1.15 (0.46–2.86)	0.758
Age	1.00 (0.97–1.04)	0.869		
Sex				
female	Reference			
male	5.57 (0.32–95.95)	0.237		
ECOG_PS				
0+1	Reference		Reference	
2+3	6.85 (1.73–27.22)	0.006	3.42 (0.72–16.33)	0.123
Alcohol				
non-drinker	Reference		Reference	
social drinker	3.02 (1.25–7.29)	0.014	2.53 (0.95–6.74)	0.064
binge drinker (>soju 1bottle/week)	1.67 (0.61–4.58)	0.319	1.54 (0.48–4.98)	0.468
Smoking				
non-smoker	Reference			
ex-smoker	1.85 (0.79–4.34)	0.155		
current smoker	1.81 (0.56–5.87)	0.323		
Primary site				
tonsil	Reference		Reference	
tongue base	1.20 (0.38–3.81)	0.752	1.19 (0.35–4.06)	0.781
others (soft palate, PPW)	13.96 (2.43–80.05)	0.003	14.84 (1.46–150.41)	0.022*
Treatment_summary				
Surgery only	Reference		Reference	
Surgery+RT	1.46 (0.37–5.79)	0.59	1.79 (0.38–8.44)	0.463
Surgery+CCRT	1.08 (0.29–4.03)	0.905	1.02 (0.26–4.00)	0.983
RT±Salvage surgery	3.70 (0.85–16.18)	0.082	4.80 (0.98–23.59)	0.053
CT±Salvage surgery	3.67 (0.17–81.38)	0.411	4.12 (0.04–467.68)	0.557
CCRT±Salvage surgery	1.10 (0.35–3.41)	0.872	0.92 (0.28–3.06)	0.897
AJCC8_cTNM				
Stage I	Reference		Reference	
Stage II	1.28 (0.38–4.36)	0.693	1.49 (0.40–5.55)	0.548
Stage III	3.59 (1.60–8.06)	0.002	3.41 (1.29–9.01)	0.013*
Stage IV	2.60 (0.14–49.84)	0.526	2.25 (0.07–70.74)	0.644

ECOG, Eastern cooperative oncology group performance status; PPW, posterior pharyngeal wall; RT, radiotherapy; CT, chemotherapy; CCRT, concomitant chemoradiotherapy;

Firth's penalized Cox regression analysis were performed to adjust for key clinical confounders. Variables with a P -value ≤ 0.1 in the univariable Cox regression were included as covariates in the multivariable Cox regression model.

*Statistically significant ($P < 0.05$)

Supplementary Table 8. Firth-Penalized Cox Regression Analysis of OS According to HPV Genotype Group (HPV-16 vs. non-HPV-16) in HPV DNA-positive/p16-positive OPSCC (n = 160) for sensitivity analysis

Characteristic	Univariable		Multivariable	
	HR (95% CI)	P-value	HR (95% CI)	P-value
HPV genotype				
HPV-16	Reference		Reference	
Non-HPV-16	2.23 (0.60–8.31)	0.231	6.28 (0.64–61.48)	0.114
Age	0.99 (0.93–1.05)	0.66		
Sex				
female	Reference			
male	1.74 (0.09–34.96)	0.718		
ECOG_PS				
0+1	Reference			
2+3	3.24 (0.15–70.68)	0.454		
Alcohol				
non-drinker	Reference			
social drinker	0.41 (0.06–2.80)	0.364		
binge drinker (>soju 1 bottle/week)	0.73 (0.15–3.54)	0.693		
Smoking				
non-smoker	Reference			
ex-smoker	1.22 (0.27–5.53)	0.795		
current smoker	2.46 (0.42–14.27)	0.316		
Primary site				
tonsil	Reference		Reference	
tongue base	0.44 (0.02–9.41)	0.6	1.04 (0.04–30.24)	0.98
others (soft palate, PPW)	20.00 (0.62–648.65)	0.091	67.93 (0.40–11591.63)	0.108
Treatment_summary				
Surgery only	Reference		Reference	
Surgery+RT	2.37 (0.23–24.25)	0.467	14.85 (0.67–330.08)	0.088
Surgery+CCRT	0.73 (0.05–9.99)	0.814	0.63 (0.04–9.85)	0.742
RT±Salvage surgery	13.90 (1.44–134.47)	0.023	3.51 (0.11–115.84)	0.481
CT±Salvage surgery	17.91 (0.38–833.87)	0.141	0.88 (0.00–216.29)	0.965
CCRT±Salvage surgery	0.64 (0.06–6.59)	0.71	0.06 (0.00–1.42)	0.082
AJCC8_cTNM				
Stage I	Reference		Reference	
Stage II	5.72 (0.47–69.91)	0.172	128.60 (2.65–6235.20)	0.014*
Stage III	14.92 (1.93–115.10)	0.01	260.96 (8.07–8442.89)	0.002*
Stage IV	191.16 (15.80–2313.38)	<0.001	886.50 (4.37–179714.52)	0.012*

ECOG, Eastern cooperative oncology group performance status; PPW, posterior pharyngeal wall; RT, radiotherapy; CT, chemotherapy; CCRT, concomitant chemoradiotherapy;

Firth's penalized Cox regression analysis were performed to adjust for key clinical confounders. Variables with a P -value ≤ 0.1 in the univariable Cox regression were included as covariates in the multivariable Cox regression model.

*Statistically significant ($P < 0.05$)

Fig. 1.

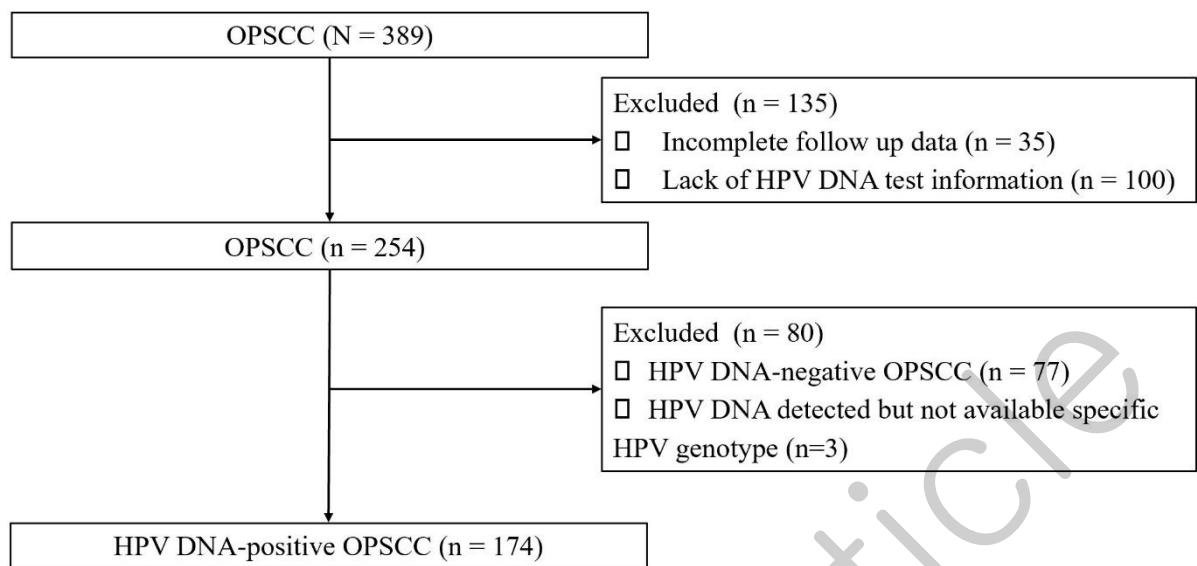


Fig. 2.

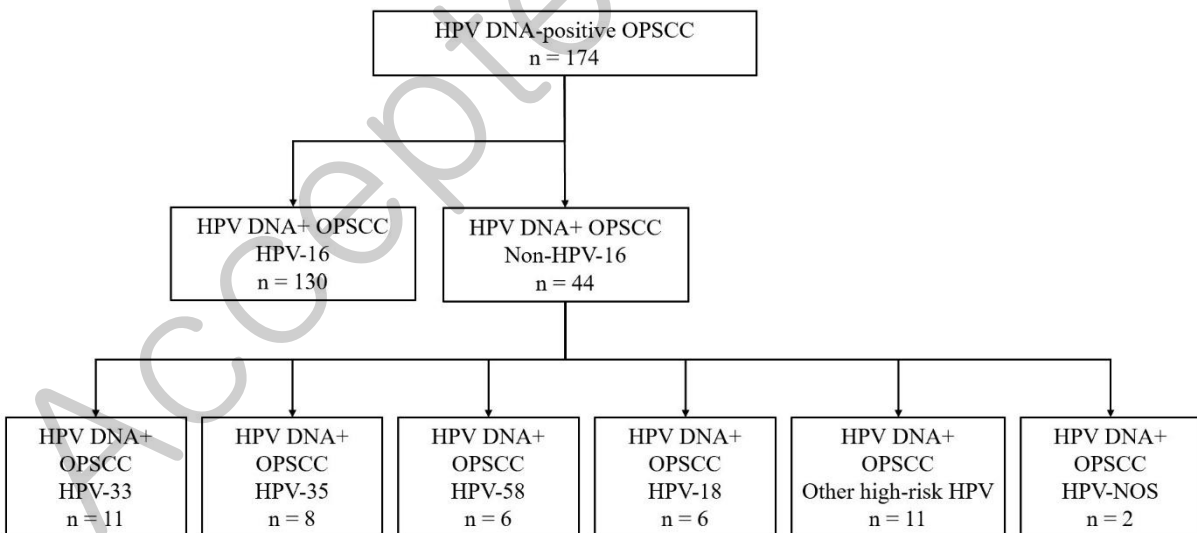


Fig. 3.

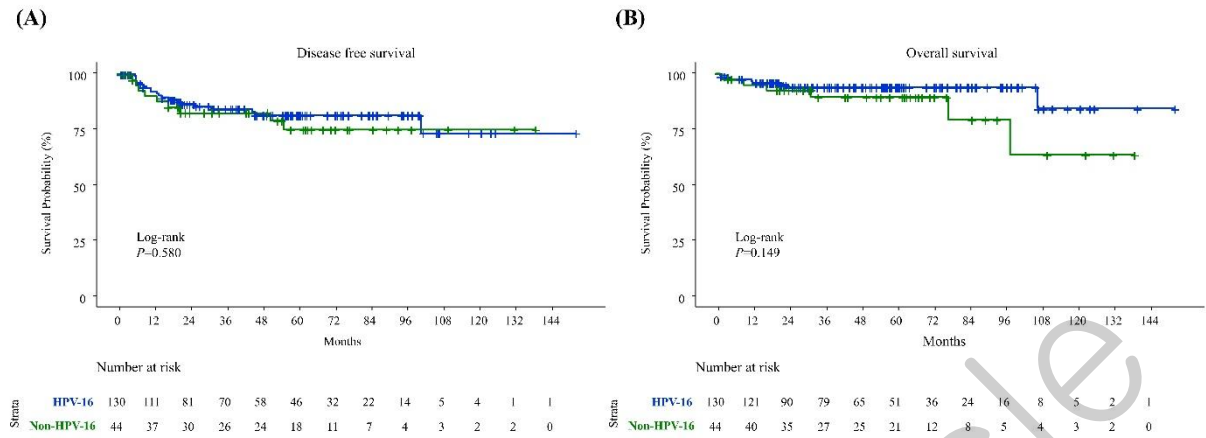
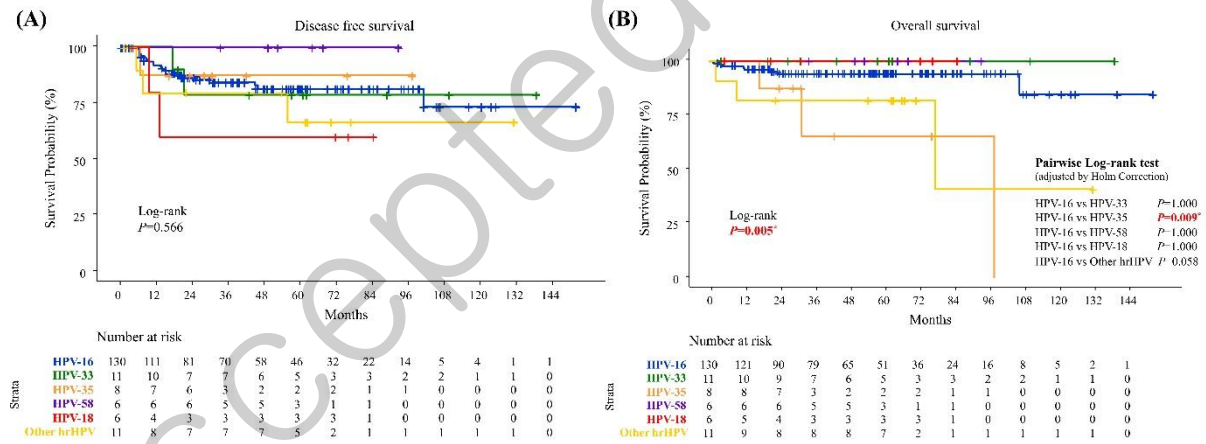
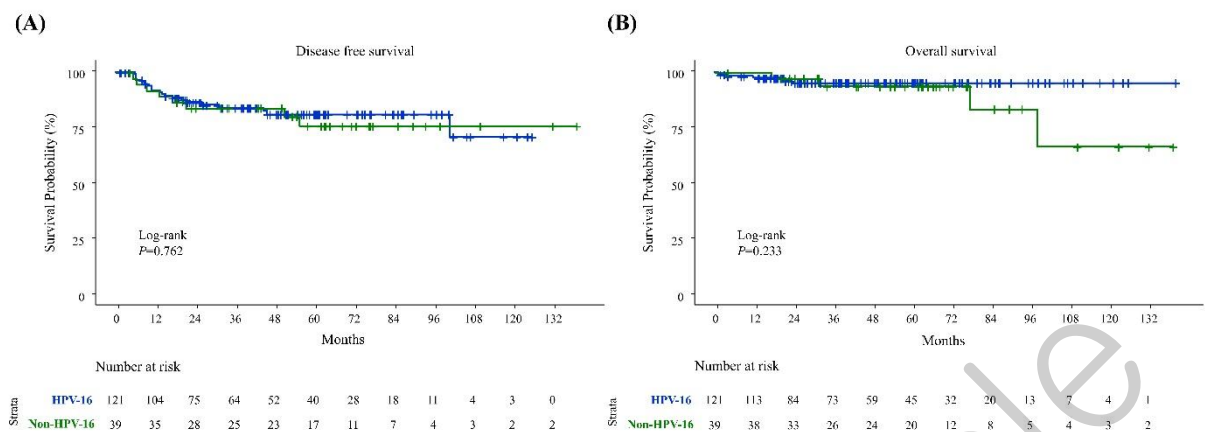


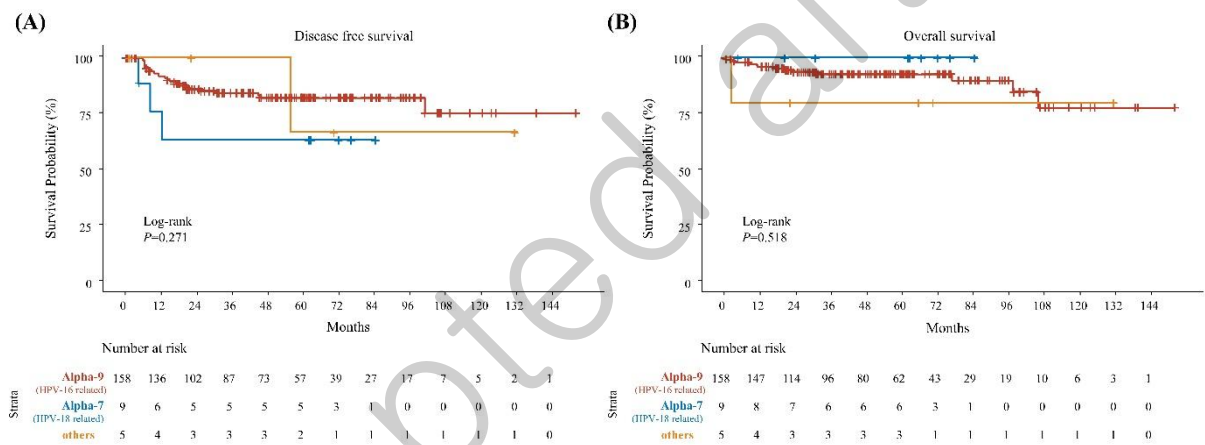
Fig. 4.



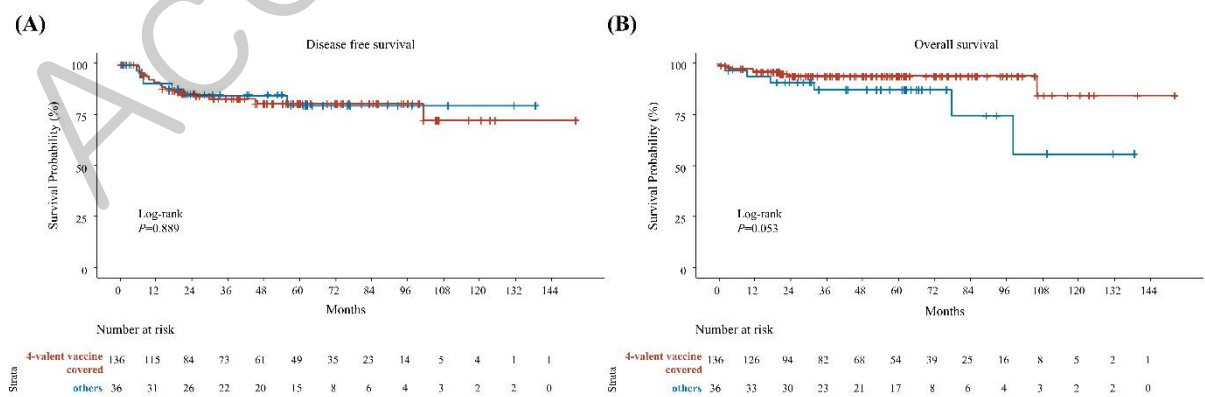
Supp. Fig. 1.



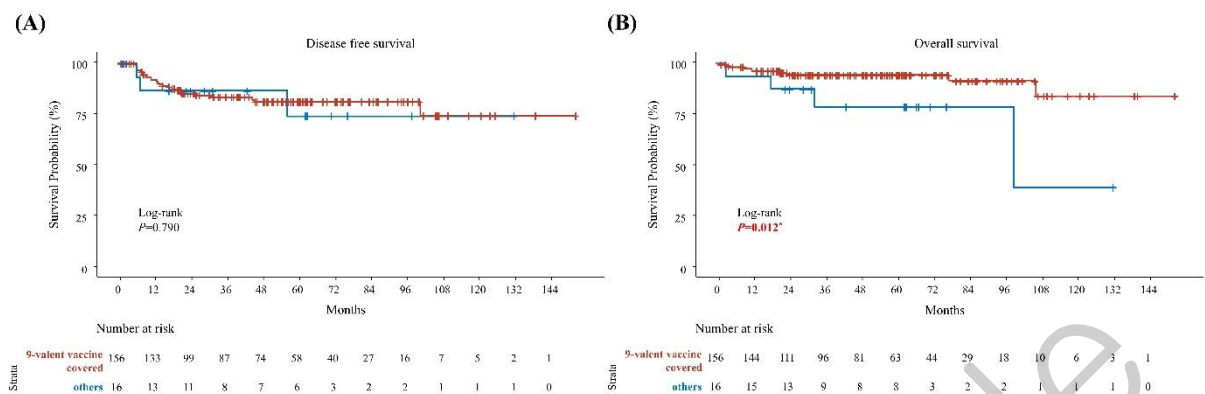
Supp. Fig. 2.



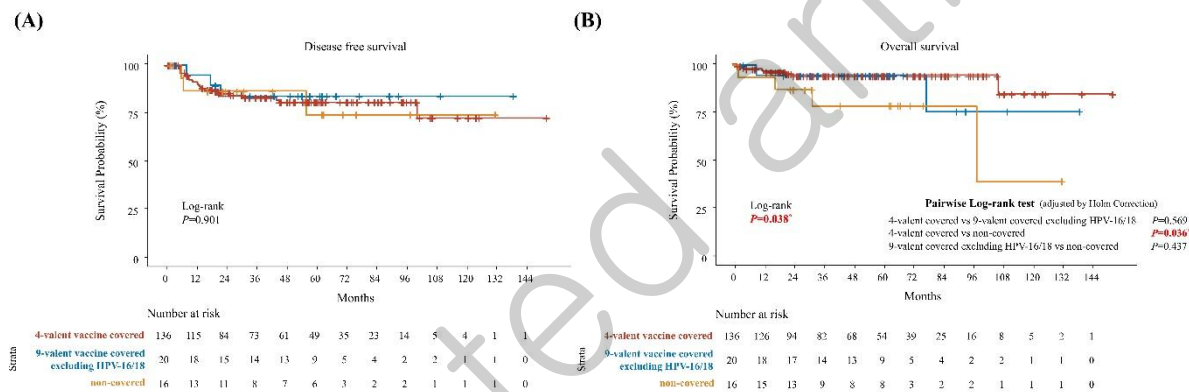
Supp. Fig. 3.



Supp. Fig. 4.



Supp. Fig. 5.



Supp. Fig. 6.

