Effects of Smoking and Drinking on Oropharyngeal Cancer Outcome by HPV Serostatus: A Prospective Cohort Study

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Abstract

Background

The incidence of human papillomavirus (HPV)-driven oropharyngeal cancer (OPC) continues to increase in the US. Patients with HPV-positive [HPV(+)] OPC often have better outcomes than those with HPV-negative [HPV(-)] OPC. To reduce treatment-related morbidity from HPV(+) OPC, "de-escalation" strategies are being evaluated. We investigated the relationship between smoking and alcohol history at diagnosis and OPC prognosis to explore whether this easy-to-obtain information can aid selection of patients that can be successfully treated with less intensive therapies.

Methods

The study population consisted of 371 patients diagnosed with OPC at UPMC otolaryngology clinics [243 HPV(+), 128 HPV(-)]. Information on smoking and alcohol use were collected via interviewer-administered questionnaires; clinical and outcome information was abstracted from medical records. HPV positivity was defined as seropositivity for antibodies against sets of HPV16 or HPV18 antigens. The Kaplan-Meier method and Cox proportional hazards models were used to assess the effects of smoking and alcohol use on overall survival (OS).

Results

Compared to HPV(-) patients, HPV(+) patients were significantly younger ($p=0.005$), more often male ($p<0.0001$), more often never smokers ($p=0.0008$), and smoked fewer pack-years ($p<0.0001$); no significant difference was observed in number of drinks-per-day or drinking status. Grouping by a smoking cutoff of 2 pack-years and controlling for age, sex, race, and stage, those with low smoking exposure had

better OS than those with high exposure for both HPV-stratified groups [HPV(+) p=0.015, HPV(-) p=0.0026]. In contrast, the clinically-used 10 pack-years cutoff was not associated with OS in HPV(+) individuals after adjustment [HPV(+) p=0.11, HPV(-) p=0.0083]. When an alcoholic drinks-per-day measure and 2 pack-years smoking cutoff were included in a single model adjusting for age, sex, race, and stage, drinks-per-day was not significantly associated with OS [HPV(+) p=0.078, HPV(-) p=0.15]. However, the 2 pack-years smoking cutoff remained significantly associated with OS [HPV(+) p=0.026, HPV(-) p=0.0076].

Conclusion

These results suggest that pack-years smoked is associated with OS in HPV(+) and HPV(-) head and neck cancer patients while drinking intensity is not. A 2 pack-years cutoff value may be more appropriate for public health and clinical applications to represent pack-years smoked compared to the commonly used 10 pack-years cutoff.

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Preface

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1.0 Introduction

1.1 Oropharyngeal Cancer Biology and Epidemiology

Oropharyngeal cancers (OPCs) are a subgroup of head and neck cancers that cause significant morbidity and mortality worldwide. This head and neck cancer type affects the region of the throat from the back one-third of the tongue to the epiglottis and typically presents as squamous cell carcinoma (Figure 1). In the US alone in 2021, oral cavity cancers and OPCs are expected to be diagnosed in 54,010 individuals and result in 10,850 deaths[1]. According to the most recent Global Cancer Statistics 2020 report, OPCs alone were diagnosed in an estimated 98,412 individuals worldwide, resulting in approximately 48,143 deaths[2]. Overall, OPCs present an important risk to public health, accounting for 0.5% of all cancer incidence and mortality worldwide in 2020[2].

Survival and morbidity after OPC diagnosis depends heavily on stage of diagnosis. OPCs have a five-year survival rate of approximately 60% for localized cancer and 30% for cancer with distant metastases[3]. Unfortunately, despite the relatively favorable survival rate for localized cancers, OPCs are often discovered late into the disease course due to the lack of easy to identify precancerous lesions and effective screening methods[4]. OPC treatment typically involves chemotherapy and radiation treatment for early cancer stages and surgical interventions for later stages[5]. Overall, these treatments can cause significant morbidity, resulting in severe difficulties in eating, swallowing, and/or speech[6, 7]. After OPC treatment, 25% of patients complain of long-term nerve pain, 50 to 60% of patients report persistent dysphagia, and 60% of patients receiving

radical surgery report speech impairment[8-10]. Treatment-related morbidity continues to reduce patient quality of life even after cancer remission.

Oropharyngeal cancer incidence and mortality risk varies by multiple lifestyle and demographic risk factors. This cancer is primarily one of middle age, with the average age of those affected in the US being 62 years old[1]. OPCs affect males at a rate 4.5 times higher than females, with a worldwide age-adjusted incidence rate of 1.8 per 100,000 for males and 0.4 per 100,000 for females in 2020[2]. Additionally, risk for OPC incidence and mortality is associated with smoking, alcohol consumption, genetics, and human papillomavirus (HPV) infection[11-14].

OPC incidence and mortality rates vary substantially worldwide, consistent with varying prevalence of OPC risk factors (Figure 2). OPC incidence rates are highest in North America and Western Europe, where HPV infection rates are highest and account for over 40% of OPC cases[15, 16]. On the other hand, in developing countries, where tobacco smoking and alcohol consumption are most prevalent, about 75% of head and neck cancers are attributable to these activities[16]. Consistent with other cancers of middle and old age, OPCs are more common in developed countries than in developing countries (Figure 2)[15].

In addition to geographic differences, the distribution of risk factors for OPCs have been shifting over the past several decades. While OPCs have historically been associated mainly with tobacco and alcohol use, in recent decades persistent HPV infection has become an increasingly common risk factor in OPC cases. Potentially associated with the decline in smoking in the late 20th century, the prevalence of HPV-associated OPCs increased from 20% of all OPCs to 70% of all OPCs by 2005[17]. With the transition of the most common OPC risk factors from tobacco and alcohol use to HPV infection, it has become ever-more relevant to identify the epidemiologic

differences between HPV-positive [HPV(+)] and HPV-negative [HPV(-)] oropharyngeal cancers for cancer treatment, prevention, and control.

1.2 Human Papillomavirus Biology and Epidemiology

There are over a hundred subtypes of HPV, with some low-risk strains known to cause warts (most commonly HPV6 and 11) and other high-risk strains known to cause multiple cancers (most commonly HPV16 and 18)[18]. Biologically, HPV is a nonenveloped virus with an icosahedral capsid that infects epithelial cells and can drive molecular changes that cause cancer[19]. The virus contains a genome composed of double-stranded circular DNA that encodes multiple proteins, including the L1 and L2 proteins that compose the viral capsid, E1 and E2 proteins that promote viral genome replication, and E6 and E7 proteins that are the accessory proteins involved in carcinogenesis[19]. The E6 and E7 proteins promote viral replication by causing cells to re-enter the cell cycle and by preventing cell death. In particular, E7 degrades cellular RB proteins to promote S phase cell cycle re-entry while E6 degrades the tumor suppressor protein p53 to prevent apoptosis[20]. Together, the activities of E6 and E7 to trigger cell growth and division are the driving forces behind the warts and cancers associated with HPV[20]. However, variation in the structure and activity of E6 and E7 among HPV strains means that only a few HPV subtypes have a large enough effect on cell replication to pose a cancer risk. Of the high-risk HPV subtypes, HPV16 and 18 cause the large majority of cancers and are of the greatest epidemiologic interest.

Originally found to cause cervical cancer, HPV is the most common sexually transmitted infection in the US and worldwide, playing a causative role in cervical, vulvar, vaginal, penile, anal, and oropharyngeal cancers[18]. The virus is typically passed through skin-to-skin contact, and

the prevalence of oral HPV infection is estimated to be 6.9% in the US adult population[21]. In the head-and-neck region, over 90% of oral HPV infections are sexually acquired[17]. Typically, HPV infections are lytic viral infections that are cleared by the body in less than 2 years[22]. However, approximately 10% of infections can establish long-term latent persistence in cells[22]. While short-term HPV infections typically do not result in cancer, persistent infections with high-risk HPV strains have the potential to cause cancer.

1.3 Role of Human Papillomavirus in Oropharyngeal Cancers

HPV has played an increasingly important role in oropharyngeal cancer. In a 2019 US-based study, HPV DNA was found in over 70% of new OPC cases[17]. HPV16, which has a prevalence of approximately 1% in the general US adult population, is causally associated with OPCs and has been reported to cause 90% of HPV(+) OPCs[4]. In 2021, it is expected that the number of newly incident HPV(+) OPCs will surpass the number of cervical cancer cases, the cancer typically associated with HPV infection, in the US[23].

Biologically, HPV(+) OPCs differ from HPV(-) OPCs in many ways at the genomic and proteomic level. While the tumor mutational burden, or the number of mutations per gene, has been reported to be similar in the two OPC subtypes, the specific genes mutated are distinctly different between HPV(+) and HPV(-) tumors[24]. Mutations in HPV(-) tumors more commonly affect known oncogenes or tumor suppressors, with a few major ones highlighted here[20, 24]. In HPV(-) OPCs, mutations, deletions, or copy number alterations to the CDKNA2A and TP53 tumor suppressor genes are most common and present in approximately 54% and 73.4% of tumors, resulting in decreased levels of p16 and p53 tumor suppressor proteins[20]. In contrast, these two

tumor suppressor proteins are rarely mutated in HPV(+) OPCs, where changes driven by HPV typically result in hallmark increases in p16 and p53 tumor suppressor protein expression[20]. The Epidermal Growth Factor Receptor (EGFR) promotes tumor growth and has been reported to be overexpressed in up to 90% of OPCs[25]. EGFR overexpression has been reported to occur approximately 6.6 times more frequently in HPV(-) OPCs than in HPV(+) OPCs and has been associated with survival in HPV(-) OPCs but not in HPV(+) OPCs[26]. Immune response to HPV(+) tumors is reported to be greater than in HPV(-) tumors as well, with greater immune infiltration in HPV(+) OPCs[27]. In particular, the immune biomarker Programmed Death Ligand 1 (PD-L1), which helps keep immune cells from attacking the body's own cells, is more frequently expressed in HPV(+) OPCs than in HPV(-) OPCs and is associated with improved prognosis[27]. While recommended treatments for OPCs do not differ based on HPV status or biomarker levels, multiple targeted therapies are in development or have been approved for protein targets in the tumor pathway, including a monoclonal antibody against EGFR (cetuximab) and inhibitors of the PD-L1 pathway[25, 28].

HPV(+) OPCs differ epidemiologically from HPV(-) OPCs in many ways, supporting the idea that the mechanisms of carcinogenesis and risks associated with the OPC subtypes differ. Specifically, HPV(+) OPCs tend to occur in younger individuals with less tobacco and alcohol use than HPV(-) OPCs[23]. Consistent with the sexually-transmitted nature of high-risk HPV strains, risk for HPV(+) OPC is associated with oral sex practice and increasing number of sexual partners[17]. Importantly, HPV(+) OPCs tend to be less severe than HPV(-) OPCs, with fewer recurrences and higher survival rates[29, 30]. While only approximately 43% of HPV(-) OPC patients experience progression-free survival at 3 years, 74% of HPV(+) OPC patients experience progression-free survival at 3 years[30]. The reduced risks posed by HPV(+) OPCs were reflected

by the American Joint Committee on Cancer's decision in 2018 to change to the OPC staging manual to separate and downgrade the stage of HPV(+) OPCs compared to HPV(-) OPCs[31]. However, no changes were made to the recommended treatments, as insufficient evidence was available[31]. The lower mortality risk, high morbidity of treatment, and younger age of patients with HPV(+) OPCs have made reduction in treatment intensity, or treatment "de-escalation" for HPV(+) OPCs an attractive area of study. Multiple treatment de-escalation studies have concluded or are ongoing to investigate potential de-escalated treatments that may result in lower treatment morbidity in HPV(+) patients[32]. These studies will be further discussed in later sections (Sections 1.5.3 and 5.2).

1.4 Detection of Human Papillomavirus in Oropharyngeal Cancers

Multiple different methods are used clinically and in research contexts to determine the presence of HPV in tumors. Increased expression of the p16 protein in tumors as detected by immunohistochemistry (IHC) is a commonly used biomarker for HPV infection. This protein is a molecule downstream of the E7 target protein RB[19]. So, E7 activity from an active high-risk HPV infection typically results in a measurable increase in p16 expression[19]. However, this method is somewhat imprecise and subject to subjective decision-making, providing a percent agreement of 80 – 90% with HPV DNA and serology-based methods[33, 34]. The current gold-standards for HPV detection in tumors are RNA or DNA-based polymerase chain reaction and in-situ hybridization methods[34, 35]. However, these DNA-based methods are more difficult to implement and thus are less commonly used clinically than p16 IHC[35].

In contrast, serology-based methods for detecting antibodies against HPV antigens provide relatively simple methods for detecting HPV infection relevant to cancer. In a 2018 study in Europe, serology for antibodies against the oncogenic HPV16 E6 antigen was more strongly associated with OPC survival than p16 or HPV16 DNA markers[33]. Serology methods can also detect presence of HPV prior to the detection of a tumor[36]. However, they are limited by their inability to identify the site of infection or differentiate between current and past infection. Multiplex HPV serology methods have been developed to allow for detection of antibodies against antigens from multiple HPV strains to further increase the efficiency of this method[37]. Overall, there are currently multiple methods utilized to detect HPV infection in OPCs, with p16 IHC being the most commonly clinically applied method.

1.5 Public Health Interventions for HPV(+) Oropharyngeal Cancers

Current public health interventions against OPCs as well as other HPV-associated cancers center on promoting adoption of the HPV vaccine in adolescents and adults. For OPCs specifically, an effective screening method has proven elusive, but investigations into treatment de-escalation methods for lower-risk HPV(+) OPCs are under investigation.

1.5.1 Prevention

Since the US approval of the first HPV vaccine in 2006, vaccination against HPV has been the centerpiece for public health interventions for preventing cancers associated with HPV. Gardasil®4 protected against four HPV strains—the low-risk HPV6 and 11 strains associated with

genital warts and the high-risk HPV16 and 18 strains associated with oropharyngeal, penial, anal, and cervical cancer[18]. Since then, Gardasil®9 was approved in 2014, protecting against additional high-risk HPV strains[18]. HPV vaccination is now recommended for all individuals aged 9 to 26 years, and approved for those up to 45 years old, although the public health benefit of vaccination at a later age is currently uncertain[38]. The vaccine is routinely administered at primary care visits around age 12[18]. Through over a decade of use, the vaccine has been shown to be highly effective and safe, with a nearly 100% efficacy in clinical trials and with no reported increased risk for systemic or serious adverse events[18].

Despite the promise of the HPV vaccine, slow vaccine uptake and a long lead-time for protective effects means that it will be decades until we see the impact of the vaccine as a prevention method. As of 2020, only approximately 56.8% of girls and 51.8% of boys were up-to-date with their HPV vaccine in the US[39]. In addition, the clinical lag-time between HPV infection and development of OPC is approximately 10 to 30 years[23]. So, while promising, the effects of vaccination on reducing HPV(+) OPC incidence will still require decades to manifest. Thus, improving current HPV(+) OPC treatment methods remains critical.

1.5.2 Screening

Screening methods for OPCs are essentially non-existent due to the lack of an identifiable precancerous lesion. In contrast to the effective screening measures for HPV-associated cervical cancers, similar visual screening methods are not available for OPCs because no visually identifiable precancerous lesion is present for the disease[23]. As a result, prevention through detection and therapeutic intervention on a precancerous lesion is not possible for HPV(+) or HPV(-) OPCs. In addition, positivity for HPV alone is not a sufficient predictor of carcinogenesis,

as only a small minority of HPV infections progress to cancer[40]. Overall, these limitations have resulted in the failure to identify useful public health screening methods for prevention or early detection of HPV(+) or HPV(-) OPCs.

By combining measures for multiple OPC risk factors, predictive models have been created to assess one's risk for OPC[41]. However, at this time these models are largely unvalidated and do not explain enough of OPC risk for use as a screening method in the general population[41]. Rather, these models would likely be most useful for identifying a high-risk population for research enrollment and treatment de-escalation studies rather than as an effective screening method[41].

1.5.3 Treatment De-escalation Strategies

Morbidity affecting the skin, oral mucosa, teeth, jaw, throat, and related muscles are associated with the chemotherapy, radiation, and surgical interventions used for OPC treatment[7, 42]. Since HPV(+) OPCs tend to have reduced mortality and recurrence rates compared to HPV(-) OPCs, multiple strategies for reduced treatment intensity have been proposed for these lower-risk HPV(+) cancers. These treatment de-escalation methods include substitution of high-toxicity chemotherapy agents for lower-toxicity agents, and reduction in intensity for radiation therapy or surgery[32, 43-45]. Many clinical trials in this area have reached completion or are on-going, with over 20 active trials listed on clinicaltrials.gov and two recently complete phase 3 trials in Europe[44, 45] (Supplementary Table 1).

While each clinical trial has differing inclusion and stratification criteria, most trials stratify OPC patients by risk groups for multiple risk factors in attempts to identify patient populations who best benefit from de-escalation[32]. These risk factors include high and low tumor stage, nodal involvement, Zubrod lifestyle performance score, and tobacco smoking history[44, 45]. Smoking

history is especially notable, as the mutagenic properties of tobacco smoke are thought to cause genetic changes in the oropharynx that result in increased predisposition to cancer and poorer outcomes[46]. In de-escalation trials, risk stratification for tobacco use is typically categorized by a 10 pack-years cutoff, with those with 10 pack-years of smoking or fewer categorized into a low risk group compared to those with greater than 10 pack-years of smoking[44, 45]. This widely used cutoff stems from the findings of a single 2010 study published in the *New England Journal of Medicine* by Ang et al. that used the cutoff to identify a difference in mortality risk among HPV(+) OPC patients[30].

1.6 Gaps in Literature

In order to identify a target population that would most benefit from de-escalated treatments for OPCs, proper identification of risk factors for use in risk-stratification are necessary[32]. As noted previously, tobacco smoking is associated with increased mortality risk in both HPV(+) and HPV(-) OPCs. So, cumulative exposure to tobacco smoke measured in pack-years is a commonly considered risk factor when identifying a de-escalation treatment population. Many current studies use a 10-pack-years cutoff for stratifying risk in OPCs[44, 45]. This cutoff is based off a somewhat arbitrary cutoff used by Ang et al. in the seminal study identifying tobacco smoking as an important predictor of mortality risk in HPV(+) OPCs[30]. The appropriateness of this cutoff has not been evaluated in subsequent studies to our knowledge, and thus may not be the ideal cutoff value for stratifying OPC mortality risk by tobacco smoking. Additionally, while previous studies have identified alcohol history as an important predictor of OPC risk[12, 41, 47-49], this risk factor is largely un-used when identifying risk groups in OPC de-escalation trials.

In sum, easily obtainable lifestyle measures for tobacco use and drinking intensity are likely important predictors for stratifying mortality risks associated with OPCs. However, the proper cutoff-value for tobacco use in OPC risk stratification requires validation, and the utility of alcohol history as a risk factor for OPC mortality in treatment de-escalation remains to be identified.

1.7 Public Health Significance

HPV(+) OPC is an increasingly prevalent cancer in the US and worldwide. This cancer subtype is typically associated with younger age and lower mortality risk, making the morbid effects of OPC cancer treatments more relevant in identifying the proper treatment method for these patients. Common side-effects of treatments for OPCs include difficulties with speech, swallowing, and eating, often resulting in the requirement of a feeding tube. For younger individuals, this can result in a significant reduction in quality of life than could be avoided with treatment intensity tailored to cancer type and risk. However, the effects of common risk factors of tobacco and alcohol history on mortality associated with HPV(+) OPCs remain to be fine-mapped. Determination of cutoff values for these risk factors can allow for better risk stratification when determining if a de-escalation strategy is appropriate. With a multitude of on-going de-escalation trials, determination of proper risk stratification for these common risk-factors can allow for improved patient selection in treatment de-escalation trials.

2.0 Objectives

2.1 Aim 1: Describe the epidemiologic differences between HPV(+) and HPV(-) OPC patients.

The first objective of this research is to contribute to the existing literature on the differences in patient population characteristics between HPV(+) and HPV(-) OPCs using our study population.

2.2 Aim 2: Investigate the relationships between smoking and alcohol history at diagnosis and survival related to HPV status of OPCs.

The second primary objective of this work is to investigate whether and how a pack-years measure of smoking history and a drinks-per-day measure of alcohol use at diagnosis are predictive of survival for HPV(+) and HPV(-) OPCs. We aim to identify cutoff values for these measures that best stratify mortality risk in HPV(+) OPC patients and that may be useful for patient selection in the context of de-escalation trials. We hypothesize that the previously defined 10 pack-years cutoff is appropriate for risk stratification based on smoking history. Also, we hypothesize that a drinks-per-day measure of alcohol use will significantly predict mortality risk in HPV(+) OPC patients.

3.0 Methods

3.1 Study Population

This study is a retrospective analysis on data collected in a prospective cohort of OPC-diagnosed patients recruited from University of Pittsburgh Medical Center (UPMC) otolaryngology clinics. Between 2005 and 2014, a total of 374 OPC-diagnosed participants were recruited. Epidemiologic and demographic data were collected at diagnosis by face-to-face interview and structured questionnaire. Clinical data, including date of diagnosis, stage, tumor location, etc. were abstracted from medical records. Blood samples were collected at time of study enrollment, processed to extract serum, and stored at -80^{o}C.

Data on participant survival were abstracted from medical records, death records, and other primary resources by a certified cancer registrar. For living participants, the date of last contact was used for censoring in survival analysis. Participants have been followed up to the current day, with a median follow-up time of 4.17 years.

3.2 Multiplex HPV Serology Method and HPV Positivity Definition

HPV serology determination for each participant was performed at the German Cancer Research Center (DKFZ) in Heidelberg, Germany by the research group of Dr. Tim Waterboer using a multiplex serology method developed by the group[37]. The serostatus of each participant was determined for 36 proteins (L1, E6, E7, E1, E2, and E4) across 11 HPV subtypes (HPV6, 11,

16, 17, 31, 33, 35, 45, 52, and 58). In this method, viral fusion proteins bound to spectrally-distinct glutathione bead sets were incubated with participant serum and analyzed by an xMAP flow cytometer-like analyzer. Mean fluorescence intensity (MFI) of each antibody-antigen complex was analyzed, and established cutoff values were used to define positivity for antibodies against each specific HPV antigen. Supplementary Table 2 shows the MFI cutoff values used for each HPV antigen of interest.

Positivity for HPV infection was defined by serological results satisfying one of the two criteria:

1. MFI > 1000 for antibodies against HPV16 E6 antigen
2. Antibody levels above the MFI cutoff for three of the four antigens E1, E2, E6, or E7 for either HPV16 or HPV18.

If either of the above criteria were satisfied, the participant was defined as having HPV(+) OPC, and if neither criteria was met, the participant was defined as having HPV(-) OPC. By this method, we identified 243 HPV(+) cases and 128 HPV(-) cases in our study population, with three participants' results failing quality control.

3.3 Definitions of Covariates

Standardized, interviewer-administered questionnaires were used at study inclusion to assess participant sex, age, smoking status (current, former, or never), pack-years smoked, drinking status (current, former, or never), and drinks-per-day drinking intensity. Current or former smoking status was defined as having ever smoked at least one tobacco product a day for six months or longer. Additionally, pack-years smoked was determined by an interviewer-

administered chart (sample chart, Supplementary Table 3). Number of pack-years smoked was calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person had smoked. Similarly, current or former alcohol consumption was defined as having one or more drinks per month for a year or longer. Drinking intensity information was also determined by an interviewer-administered chart (sample chart, Supplementary Table 4). Drinking intensity in drinks-per-day (drinks/day) was calculated from the number of times per week or month that a participant would have at least one drink multiplied by the average amount of drinks per day of drinking. This per-week or per-month measure was then converted to a per-day value by division. This value was only calculated for participants who reported a continuous drinking period of one year or longer. Otherwise, a drinking intensity of zero was assigned. Most participants reported only one continuous drinking period, but for those with multiple drinking periods, a time-weighted average was calculated.

TNM cancer staging was determined by medical record review, and these stages were categorized into High/Low cancer stage based on the AJCC guidelines for HPV(+) and HPV(-) cancers[31]. Cancers of stage I and II were categorized as low stage while cancers of stage III and IV were categorized as high stage.

3.4 Contal-O'Quigley Method for Smoking and Drinking Cutoff Determination

Using a SAS macro created by Williams et al.[50], we applied the a method first proposed by Contal and O'Quigley[51] to identify optimal cut-points for mortality risk categorization of the continuous pack-years smoked and drinking intensity variables for HPV(+) and HPV(-) OPCs. The Contal-O'Quigley method is a modified log-rank test adjusted for multiple comparisons used

to identify the optimal dichotomization of a continuous variable. This method is most appropriate when a threshold effect is truly present[50]. The cut-point with the lowest p-value by this method identifies the point that dichotomizes the continuous variable with the largest difference between groups.

3.5 Statistical Analysis

Of the 374 study participants originally recruited, three participants were excluded due to missing HPV serology data. The remaining 371 participants were stratified by HPV status and included in all analyses.

Descriptive statistics included median, interquartile range (IQR), and range for continuous variables and frequencies and proportions for categorical variables. Descriptive statistics were included for the variables sex, age, race, smoking status, pack-years smoked, drinking status, drinking intensity, follow-up time, and cancer stage. Comparisons between HPV(+) and HPV(-) groups were performed using chi-square tests or Fisher's exact test for categorical variables and t-tests for continuous variables.

Overall survival (OS) analysis was performed using the Kaplan-Meier method and Cox regression modelling. Kaplan-Meier curves were produced, and log-rank tests were performed to assess the OS differences among patients stratified by HPV status as well as pack-years of smoking. Univariate Cox regression models were created for each HPV group by age, sex, pack-years smoked as a continuous variable, pack-years smoked categorized by 2 and 10 pack-years, drinking intensity as a continuous variable, and drinking intensity categorized by 1 drink/day. Next, multivariate Cox regression models were generated, adjusting for the potential confounding

variables age, sex, and race. Further models adjusting for high/low cancer stage in addition to the previously identified variables were also produced. Finally, a full model including age, sex, race, high/low cancer stage, pack-years of smoking categorized by 2 pack-years, and drinking intensity was produced. Interactions between drinking intensity and pack-years or drinking intensity and pack-years with the included demographic variables were also assessed and determined to have no significant effect on the models.

4.0 Results

In the cohort of 243 participants with HPV(+) OPC and 128 participants with HPV(-) OPC, differences in multiple demographic characteristics were apparent between HPV(+) and HPV(-) OPCs (Table 1). Overall, in our study population, OPCs affected predominantly males in middle age. Due to the population our study recruited from, participants were predominantly white [HPV(+): 98.8%, HPV(-): 95.3%], with no representation of other races except African American [HPV(+): 1.2%, HPV(-): 4.7%]. Comparing HPV(+) OPCs to HPV(-) OPCs, HPV(+) OPCs tended to affect a higher proportion of males (86% male) than HPV(-) OPCs (61.7% male, $p<0.0001$). Additionally, HPV(+) OPCs tended to affect slightly younger individuals, with a median age of 56.1 years old, compared to HPV(-) OPCs, which had a median age of 59.4 ($p=0.005$). While over half of HPV(-) OPCs affected current smokers, never or former smokers made up the majority of HPV(+) OPC cases (Table 1). Consistently, those with HPV(-) OPCs smoked significantly more pack-years (median: 34.1 pack-yrs) compared to those with HPV(+) OPCs (median: 4.3 pack-yrs). In contrast, no significant differences in drinking status and drinking intensity were observed between the two groups. Over half of the patients in either group were current drinkers. Participants with HPV(+) OPCs were followed for a median of 4.43 years while participants with HPV(-) OPCs were followed for a median of 3.59 years. HPV(+) participants predominantly had low-stage OPCs, while HPV(-) participants predominantly had high-stage OPCs ($p<0.0001$, Table 1).

Consistent with categories used in previous studies[30, 44, 45], we dichotomized the continuous pack-years smoked variable by 10 pack-years smoked for survival analysis. In addition, we applied the Contal-O'Quigley method to our continuous pack-years smoked and drinking intensity

variables to identify the optimal categorization of these variables in our dataset. Applying this method to our data, we identified the optimal cut-point for pack-years smoked to be approximately 2 pack-years for both HPV(+) and HPV(-) OPCs (adj. p=0.0086 and adj. p=0.0014, respectively). In contrast, no optimal cut-point was identified for drinking intensity for HPV(+) OPCs, and a somewhat significant cut-point of 0.5 drinks/day was identified for drinking intensity for HPV(-) OPCs (adj. p=0.037). Overall, these results indicate that while a clear threshold for increased mortality risk may be present for smoking at 2 pack-years smoked, such a threshold may not be present for drinking intensity. As a result, for further analysis, a drinking intensity threshold of 1 drink/day, a common cutoff for moderate drinking[52, 53], was used.

By univariate Kaplan-Meier and Cox proportional hazard analysis, participants with HPV(-) OPC faced significantly higher mortality hazard compared to HPV(+) OPCs (HR = 3.03, 95% CI [2.10 – 4.37]; Table 2 & Figure 3). Each 1-year increase in age and presence of high-stage cancer was significantly associated with increased mortality hazard in both groups, but sex and race were not associated with OS (Table 2). Current smoking status was associated with increased mortality hazard in both HPV-stratified groups, but former smoking status was only associated with increased mortality hazard in the HPV(-) group. In either group, a per-pack-year increase in smoking history was not associated with increased mortality hazard, but categorization of smoking by the 10 or 2 pack-years cutoffs was significantly associated with mortality. Categorization by 2 pack-years resulted in slightly higher hazard ratios than categorization by 10 pack-years. Visual inspection of the Kaplan-Meier curves further indicated that categorized pack-years variables univariately predict OS in OPC, with a 2 pack-years cutoff providing slightly improved stratification of mortality hazard compared to a 10 pack-years cutoff (Figure 4). While alcohol consumption was not clearly associated with mortality hazard, there was a slight increase in

mortality hazard with each additional drink-per-day in both HPV(+) and HPV(-) groups (Table 2). However, categorization of drinking by moderate drinking (1 drink/day) only results in a clear increase in mortality hazard in the HPV(-) group (Table 2). Further grouping of participants by low smokers/drinkers, high smokers/low drinkers, low smokers/high drinkers, and high smokers/high drinkers by both the 2 pack-years smoked and 1 drink/day cutoffs indicates that high smokers/low drinkers have similar mortality hazard to high smokers/high drinkers [HPV(+) HR: 2.12 vs 2.61, HPV(-) HR: 7.75 vs 8.00] (Table 2). Overall, this indicates that the increased mortality hazard for high smokers/high drinkers is largely driven by smoking history rather than drinking history as measured by these dichotomous variables. In addition, it indicates that a synergistic interaction between the categorized pack-years smoked and drinking intensity variables is unlikely.

To correct for demographic variation within HPV-stratified groups and the significant association of age with mortality hazard, multivariate Cox regression models for each variable of interest adjusting for age, sex, and race were produced (Table 3). In these adjusted models, much of the data were similar to the unadjusted models, and patients with HPV(-) OPC continued to suffer higher mortality hazard than HPV(+) patients (HR = 2.86, 95% CI [1.95 – 4.20]). Further, pack-years smoked as a continuous variable remained a nonsignificant predictor of mortality while drinking intensity remained a significant predictor of OS. Categorization of drinking intensity remained significantly associated with OS only in the HPV(-) group, but not the HPV(+) group. The patterns for participants grouped by both smoking and drinking also remained consistent for both HPV-stratified groups. Interestingly after adjustment, dichotomization of pack-years by 10 pack-years was no longer a significant predictor of OS in HPV(+) participants while

dichotomization by 2 pack-years remained a significant predictor of OS in HPV(+) participants (Table 3).

Further adjustment in Table 4 to account for differences in high stage (Stage III/IV) compared to low stage (Stage I/II) cancers in addition to age, sex, and race produced similar results to the previous model in Table 3. For HPV(+) participants, while categorization by 10 pack-years was not a significant predictor of OS, categorization by 2 pack-years was a significant predictor of OS. Further, drinking intensity as a continuous measure continued to be a significant predictor of OS in both HPV(+) and HPV(-) groups, albeit with relatively small effect sizes. With adjustment however, a categorized drink intensity variable at 1 drink/day was no longer predictive of mortality hazard in either HPV(+) or HPV(-) groups.

From these results, a full model was developed, including high/low cancer stage, age, sex, race, pack-years smoked categorized by 2 pack-years, and drinking intensity as a continuous variable (Table 5). In this model, cancer stage and age were significant predictors of OS while sex and race were not for HPV(+) and HPV(-) OPCs, consistent with univariate results from Table 1. Additionally, pack-years smoked categorized by 2 pack-years remained a significant predictor of survival in both HPV-stratified groups. However, drinking intensity as a continuous predictor did not significantly predict OS in either group after the additional adjustment for 2 pack-years smoked in this model compared to the model in Table 4.

5.0 Discussion

Consistent with observations made in previous studies[4, 40, 41], HPV(+) individuals in our study population were more often male, younger, and had smoked less than HPV(-) participants. In contrast, while differences in drinking habits have been reported in HPV-stratified groups in previous studies[21, 40], our analysis did not indicate a significant difference in our population. This may be due to a small sample size, however. The far lower prevalence of smoking in the HPV(+) group compared to the HPV(-) group supports the existing molecular evidence that the etiology of HPV(+) OPCs differs from the smoking habits that typically cause HPV(-) OPCs[54-56]. Similarly, the younger age of cancer onset in HPV(+) individuals may reflect differences in the mechanisms of carcinogenesis between HPV(+) and HPV(-) OPCs. The imbalance of OPCs among sexes, especially in HPV(+) OPCs remains to be fully elucidated, but potential explanations include male lifestyle habits and higher rates of HPV transmission from female genitals during oral sex.

Consistent with previous studies, our analysis showed that HPV serostatus and age were associated with OS. However, while racial differences in OS have been previously reported[57], our study lacks a sufficiently diverse population to identify any OS difference among races.

Smoking history measured by pack-years smoked is commonly used in observational studies and clinical trials as an easy-to-obtain measure for risk stratification. With regards to HPV(+) OPCs, many studies utilize a 10 pack-years cutoff for risk stratification based on a seminal paper's use of the cutoff. In the study by Ang et al.[30], the authors found that in a study population of 266 HPV(+) patients, each one pack-year increase in smoking was associated with increased mortality hazard, and the ideal cutoff for mortality risk stratification was 10 pack-years based on a recursive partitioning analysis. In contrast, in our study, we see that a lower pack-years cutoff of

2 pack-years smoked is a more robust predictor of OS than a 10 pack-years cutoff after adjustment for relevant demographics and cancer stage measures. After adjustment for age, sex, race, and cancer stage, the stratification by a 10 pack-years cutoff did not significantly predict mortality hazard in HPV(+) participants while a 2 pack-years cutoff continued to be predictive. Failing to validate the cutoff set by Ang et al.[30], our analysis indicates that a lower pack-years cutoff may stratify mortality risk in HPV(+) OPCs more significantly than a 10 pack-years cutoff. In addition, in HPV(-) individuals, the same cutoff value of 2 pack-years significantly predicts OS, indicating that a cutoff value for risk stratification in HPV(-) participants may also be appropriate. The appropriateness of a lower cutoff value in both HPV(+) and HPV(-) cancers indicates that a much lower cumulative smoking exposure than previously thought may induce the cellular changes and mutations that lead to carcinogenesis and worsening prognosis.

On the other hand, alcohol use is not commonly used as a factor for stratifying mortality risk in HPV(+) OPC de-escalation trials. In our study, we saw that drinking intensity was a significant predictor of OS in HPV(+) and HPV(-) participants on a continuous drinks-per-day basis after adjusting for age, sex, race, and cancer stage. However, the effect size of each additional drink/day was relatively small, and dichotomization of drinking intensity did not significantly predict overall mortality hazard after adjustment. In addition, inclusion of the 2 pack-years smoked cutoff in a model with drinking intensity rendered drinking intensity a nonsignificant predictor of OS, indicating that drinking intensity does not provide additional predictive power past that of smoking history. Consistently, categorization of participants by both 2 pack-years smoked and 1 drink/day indicated that pack-years smoked accounted for most of the mortality hazard increase for participants with high smoking and drinking. In sum, these results indicate that drinking

intensity has relatively little effect on OS independent of smoking history for HPV(+) or HPV(-) OPCs, supporting the exclusion of drinking intensity from risk prediction models.

Overall, this study identified a lower pack-years cutoff that may be more appropriate for mortality risk stratification in HPV(+) and HPV(-) OPCs than was previously identified. Also, the study indicates that drinking intensity is not appropriate for mortality risk stratification.

5.1 Strengths and Limitations

Our study has multiple strengths in its data collection methods and cohort design. Due to the interview-based questionnaire data collection method, we have a high level of data completeness, with no missing demographic data among enrolled participants. Further, the serology-based HPV detection method is non-invasive, convenient, and robust, making it a good candidate for HPV infection testing in the context of OPCs, even when a tumor has not been biopsied[36]. Further, HPV serology is an objective measure of infection compared to the subjective, clinically-utilized p16 IHC staining method. Also, this method allows for differentiation of infection among up to 11 different HPV subtypes[37]. Finally, the design of this cohort study allowed participants to be followed for a median of approximately 4 years, allowing survival analysis methods to be used.

On the other hand, this study suffers from some limitations relating to recall bias, variable definitions, and sample size. Since the measures of pack-years smoked and drinking intensity were calculated based on self-report, recall bias may affect the accuracy of these measures. Additionally, while pack-years is a measure of lifetime smoking exposure, drinking intensity is a measure of rate (drinks/day) rather than of cumulative exposure. As a result, drinking intensity may not accurately

represent lifetime alcohol exposure, which may play an important role in OPC risk and mortality. Finally, the sample size of the study is somewhat limited (HPV(+) n=243, HPV(-) n=128), so the effects of covariates with small effect sizes may have been missed.

A further concern is the decade-long period of study recruitment that makes a time-dependent bias on the data possible. However, given that OPC survival rates have remained largely stagnant over the study period[58], the time-dependent effect on these data is likely minimal.

5.2 Public Health Implications and Future Directions

Smoking and alcohol use are common in the general population and are often used together[59]. Consistent with currently available data[33, 41, 60, 61], our study indicates that smoking increases mortality hazard in both HPV(+) and HPV(-) OPCs. However, data on the effects of alcohol use in HPV(+) OPCs remains mixed[47, 61, 62], and our analysis reflects that, indicating that drinking has limited impact on HPV(+) OPC survival in our cohort. So, larger studies and meta-analyses are needed to reach definitive conclusions. Further, in our analysis, we found no evidence of interaction between tobacco and alcohol use in HPV-stratified OPCs or in the overall cohort, which is consistent with findings from the recent UK-based Head and Neck 5000 study[61]. So, while previous studies consistently show a dose-dependent synergistic interaction between smoking and alcohol use in OPC incidence[41, 63], our data indicates that only smoking is important for mortality risk.

Overall, our study highlights the major role that tobacco smoking plays in increased mortality risk for OPCs, potentially at a much lower threshold than previously thought. This highlights the necessity of public health policies and interventions towards reducing tobacco

smoking to prevent OPCs and other cancers. Decades of research and public health evaluation have afforded us a host of effective interventions. Currently, effective mass-reach approaches include mass media campaigns, taxes and price increases on tobacco products, and smoke-free policies in and near public buildings[64]. For current smokers, individualized cessation methods including self-help groups, counselling, and nicotine replacement therapy are effective, but typically require individual motivated action and personal expense[65]. Continued funding for and application of public health programs such as these remain important for reducing the rates of OPCs affected by smoking.

Further, the increasingly important role that HPV plays in OPCs highlights the need for public health efforts to increase adoption of the HPV vaccine, especially in males who are at increased risk of this cancer. Further investigation into the reasons why males are disproportionately affected by HPV(+) OPCs may also uncover pathways through which promotion of safer sex practices may decrease the rate of HPV(+) OPCs in this population.

Cancer typically has long lag times from carcinogen exposure to tumor detection. Exposure to tobacco, HPV, and alcohol would occur years to decades before development of OPCs, and the amount of time between exposure and tumor detection for HPV(+) and HPV(-) OPCs may be important for preventative and screening measures. However, the median time between exposure to these agents and OPC development does not appear to be well defined. While not included in this analysis, the questionnaire data collected for this study includes time of first exposure data for smoking and alcohol use, and age of first sexual activity, which is associated with HPV infection[66]. As a result, further time-to-event analysis of this data could investigate how the timing of exposure to these factors affect cancer incidence and mortality.

An abundance of clinical trials are ongoing to identify de-escalated treatments that could reduce treatment morbidity to HPV(+) OPC patients while ensuring high cure rates. A critical component of these studies is to identify a low-risk population of HPV(+) OPC patients who can benefit from de-escalated treatments. In our study, we identify a new pack-years cutoff that may better stratify mortality risk by smoking status. Additionally, we identified that drinking intensity, measured by drinks-per-day, is not an additional predictor of mortality risk. Future investigations to replicate these findings in an independent cohort are necessary to confirm that these results do not come from an overfitting of our data. If replicable, a decreased pack-years cutoff can be used in algorithms for selecting low-risk patients for inclusion in de-escalation trials.

Recently, two large stage III clinical trials investigating a treatment de-escalation method for HPV(+) patients concluded. These trials investigated the use of cetuximab, a targeted EGFR inhibitor, compared to cisplatin, a wide-acting chemotherapy agent with a multitude of side effects, as a less toxic alternative therapy in combination with radiation treatment.

Unfortunately, these studies determined that cetuximab treatment resulted in inferior survival outcomes than cisplatin while remaining similarly toxic[44, 45]. Regardless, hopes remain high that an appropriate de-escalation strategy for low-risk HPV(+) OPCs can be identified. Currently, an abundance of clinical trials evaluating reduced intensity radiotherapy, surgery, chemotherapeutic agents, and targeted therapies in the context of HPV(+) OPC are active (Supplementary Table 1). In addition, investigations into risk-stratified interventions for HPV(-) OPCs may prove interesting as well[67].

The results reported in this study add to the epidemiologic literature for HPV(+) and HPV(-) oropharyngeal cancers and may be relevant in improving patient selection in treatment de-escalation clinical trials for this cancer. Our finding that a relatively low level of tobacco smoking

significantly increases HPV(+) OPC mortality has important public health implications for smoking cessation programs and for the development of effective risk-stratification methods that aim to reduce morbidity and mortality from this cancer in the general population.

Appendix A Tables

Table 1: Participant characteristics by HPV serostatus, N=371

	HPV SEROSTATUS				
	Positive (n=243)		Negative (n=128)		
	n	%	n	%	P-value[b]
Sex					**<0.0001**
Male	209	86.0	79	61.7	
Female	34	14.0	49	38.3	
Age, years					**0.005**
Median	56.1		59.4		
IQR[a]	50.4 – 61.8		52.2 – 66.3		
Range	36.5 – 76.2		21.1 – 79.5		
Race					0.069[c]
White	240	98.8	122	95.3	
Black	3	1.2	6	4.7	
Smoking status					**<0.0001**
Never	95	39.1	28	21.9	
Former	84	34.6	30	23.4	
Current	64	26.3	70	54.7	
Pack-years smoked					**<0.0001**
Median	4.3		34.1		
IQR[a]	0 – 33		5.4 – 52.1		
Range	0 – 110.4		0 – 102		
Drinking status					0.332
Never	48	19.8	18	14.1	
Former	63	25.9	32	25	
Current	132	54.3	78	60.9	
Drinking intensity (drinks/day)					0.114
Median	0.86		1.43		
IQR[a]	0.29 – 2.86		0.86 – 4		
Range	0 – 25		0 – 24		
Follow-up time (years)					**0.011**
Median	4.43		3.59		
Range	0.12 – 14.30		0.12-11.94		
Stage					**<0.0001**
Stage I/II (Low Stage)	210	86.4	44	34.4	
Stage III/IV (High Stage)	31	12.8	77	60.2	
Stage Not Available	2	0.8	7	5.47	

[a] IQR: Interquartile Range

[b] t-test for continuous variables, and Chi-square test for categorical variables.

[c] Fisher's exact test

Table 2: Univariate Cox regression survival analysis by HPV serostatus, N=371

	HPV SEROSTATUS			
	Positive (n=243)		Negative (n=128)	
	HR	95% CI	HR	95% CI
Overall mortality hazard	Ref		**3.03**	**2.10 – 4.37**
Stage				
Stage I/II (Low Stage)	Ref		Ref	
Stage III/IV (High Stage)	**2.81**	**1.49 – 5.31**	**2.48**	**1.40 – 4.41**
Stage Not Available	-	-	1.40	0.47 – 4.20
Age, years	**1.042**	**1.008 – 1.078**	**1.034**	**1.009 – 1.060**
Sex				
Male	Ref		Ref	
Female	0.878	0.39 – 1.97	0.716	0.43 – 1.19
Race				
White	Ref		Ref	
Black	3.62	0.88 – 14.9	1.67	0.60 – 4.61
Smoking status				
Never	Ref		Ref	
Former	1.98	0.97 – 4.03	**4.88**	**1.89 – 12.59**
Current	**2.27**	**1.09 – 4.76**	**4.67**	**1.97 – 11.05**
Pack-years smoked	1.009	0.999 – 1.018	1.007	0.999 – 1.015
Pack-years categorized				
< 10	Ref		Ref	
≥ 10	**1.88**	**1.07-3.31**	**3.32**	**1.69-6.54**
< 2	Ref		Ref	
≥ 2	**2.78**	**1.45 – 5.34**	**4.86**	**2.08 – 11.36**
Drinking status				
Never	Ref		Ref	
Former	1.31	0.60 – 2.86	**2.73**	**1.01 – 7.44**
Current	0.82	0.40 – 1.68	2.49	0.99 – 6.29
Drinking intensity (drinks/day)	**1.062**	**1.007 – 1.120**	**1.093**	**1.020 – 1.170**
Drink intensity categorized				
< 1	Ref		Ref	
≥ 1	1.27	0.73 – 2.22	**1.99**	**1.16 – 3.39**
Categorization by pack-years smoked and drinking intensity				
< 2 pack-years smoked & < 1 drink/day	Ref		Ref	
≥ 2 pack-years smoked & < 1 drink/day	2.12	0.97 – 4.64	**7.75**	**2.24 – 26.79**
< 2 pack-years smoked & ≥ 1 drink/day	0.486	0.11 – 2.22	4.38	0.88 – 21.76
≥ 2 pack-years smoked & ≥ 1 drink/day	**2.61**	**1.23 – 5.55**	**8.00**	**2.47 – 25.95**

*Separate model for each covariate shown

Table 3: Cox regression models adjusted for age, sex, and race stratified by HPV serostatus, N=371

	HPV SEROSTATUS			
	Positive (n=243)		Negative (n=128)	
	HR	95% CI	HR	95% CI
Overall mortality hazard	Ref		**2.86**	**1.95 – 4.20**
Pack-years smoked	1.007	0.997 – 1.016	1.004	0.996 – 1.013
Pack-years categorized				
< 10	Ref		Ref	
≥ 10	1.71	0.97 – 3.04	**2.87**	**1.42– 5.79**
< 2	Ref		Ref	
≥ 2	**2.55**	**1.32 – 4.91**	**4.33**	**1.82 – 10.34**
Drinking intensity (drinks/day)	**1.068**	**1.013 – 1.125**	**1.108**	**1.027 – 1.195**
Drink intensity categorized				
< 1	Ref		Ref	
≥ 1	1.36	0.76 – 2.45	**1.85**	**1.04 – 3.31**
Categorization by pack-years smoked and drinking intensity				
< 2 pack-years smoked & < 1 drink/day	Ref		Ref	
≥ 2 pack-years smoked & < 1 drink/day	1.86	0.83 – 4.17	**6.56**	**1.86 – 23.21**
< 2 pack-years smoked & ≥ 1 drink/day	0.50	0.11 – 2.32	4.71	0.92 – 24.10
≥ 2 pack-years smoked & ≥ 1 drink/day	**2.51**	**1.17 – 5.39**	**6.42**	**1.92 – 21.51**

*Separate model for covariate shown

Table 4: Cox regression models adjusted for age, sex, race, and high/low cancer stage stratified by HPV serostatus, N = 371

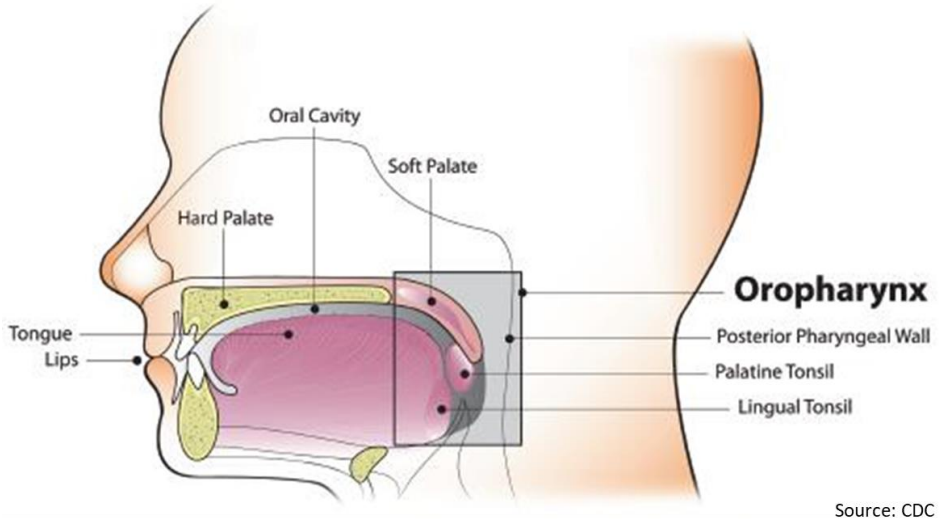
	HPV SEROSTATUS			
	Positive (n=243)		Negative (n=128)	
	HR	95% CI	HR	95% CI
Overall mortality hazard	Ref		**1.92**	**1.24 – 2.98**
Pack-years smoked	1.005	0.996 – 1.015	1.002	0.994 – 1.011
Pack-years categorized				
< 10	Ref		Ref	
≥ 10	1.61	0.90 – 2.89	**2.60**	**1.28 – 5.29**
< 2	Ref		Ref	
≥ 2	**2.29**	**1.17 – 4.46**	**3.85**	**1.60 – 9.27**
Drinking intensity (drinks/day)	**1.061**	**1.006 – 1.120**	**1.106**	**1.020 – 1.198**
Drink intensity categorized				
< 1	Ref		Ref	
≥ 1	1.24	0.68 – 2.27	1.70	0.96 – 3.00
Categorization by pack-years smoked and drinking intensity				
< 2 pack-years smoked & < 1 drink/day	Ref		Ref	
≥ 2 pack-years smoked & < 1 drink/day	1.75	0.78 – 3.92	**6.56**	**1.86 – 23.21**
< 2 pack-years smoked & ≥ 1 drink/day	0.50	0.11 – 2.34	4.71	0.92 – 24.10
≥ 2 pack-years smoked & ≥ 1 drink/day	**2.21**	**1.001 – 4.86**	**6.42**	**1.92 – 21.51**

*Separate model for each covariate shown

Table 5: Full Cox regression model including high/low cancer stage, age, sex, race, pack-years smoked categorized by 2 pack-years, and drinking intensity, N=371

	HPV SEROSTATUS			
	Positive (n=243)		Negative (n=128)	
	HR	95% CI	HR	95% CI
Stage				
Stage I/II (low stage)	Ref		Ref	
Stage III/IV (high stage)	**2.05**	**1.06 – 3.98**	**2.05**	**1.15 – 3.66**
Stage not available	-	-	1.08	0.36 – 3.31
Age, years	**1.033**	**0.997 – 1.07**	**1.031**	**1.003 – 1.06**
Sex				
Male	Ref		Ref	
Female	1.05	0.45 – 2.45	1.25	0.45 – 3.48
Race				
White	Ref		Ref	
Black	3.42	0.81 – 14.5	1.25	0.45 – 3.48
Pack-years categorized				
< 2	Ref		Ref	
≥ 2	**2.15**	**1.10 – 4.23**	**3.40**	**1.38 – 8.33**
Drinking intensity (drinks/day)	1.051	0.994 – 1.111	1.067	0.997 – 1.165

Appendix B Figures



Source: CDC
https://www.cdc.gov/cancer/hpv/basic_info/hpv_oropharyngeal.htm
Use of this image does not imply endorsement by CDC, ATSDR, HHS or the United States Government

Figure 1: Diagram of the oropharynx in relation to the head and neck region[68]

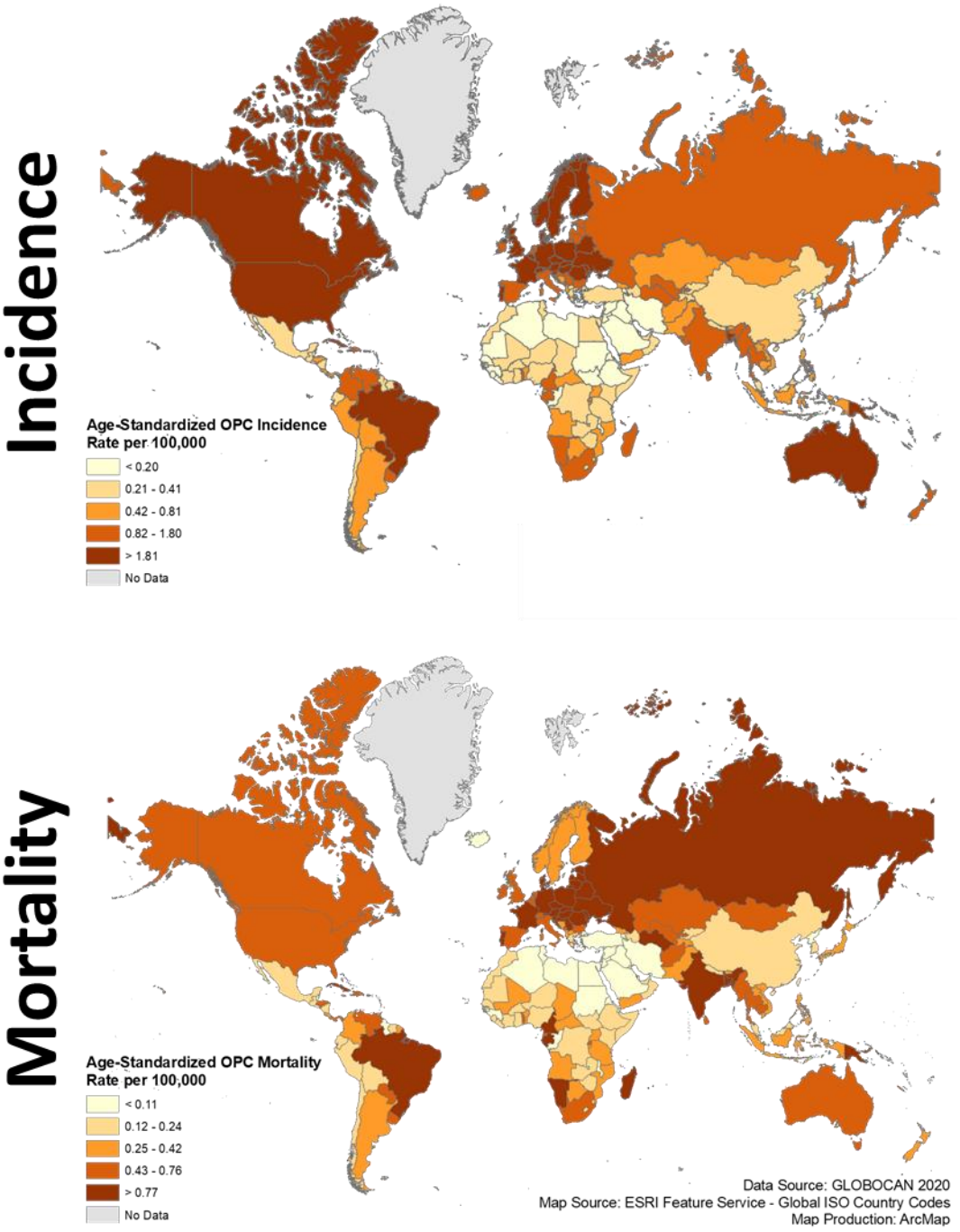


Figure 2: Worldwide oropharyngeal cancer incidence and mortality in 2020[2]

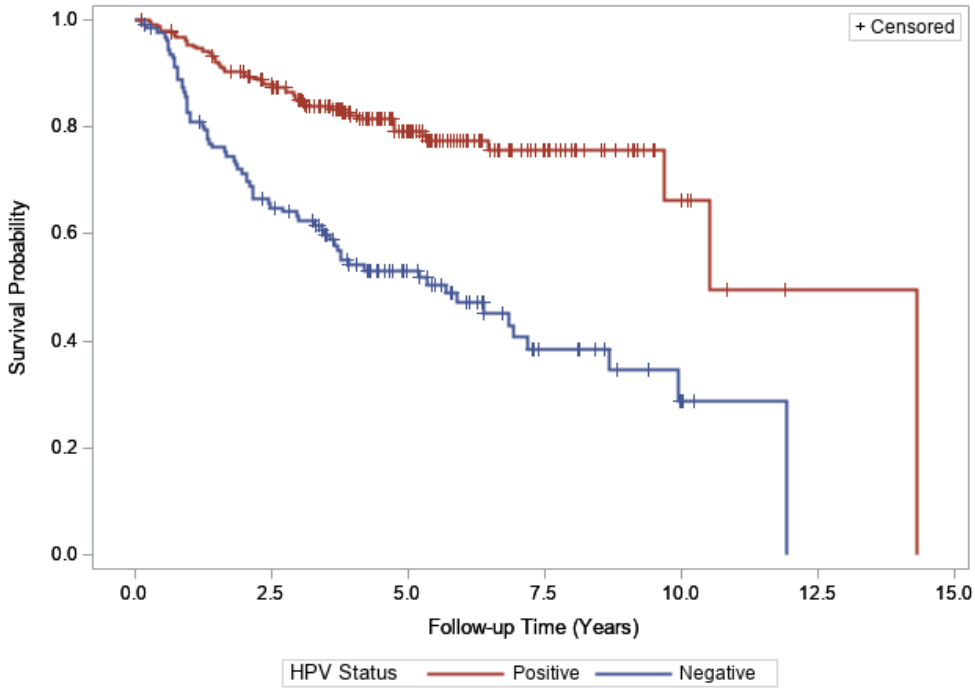


Figure 3: Kaplan-Meier curves of overall survival experience by HPV serostatus

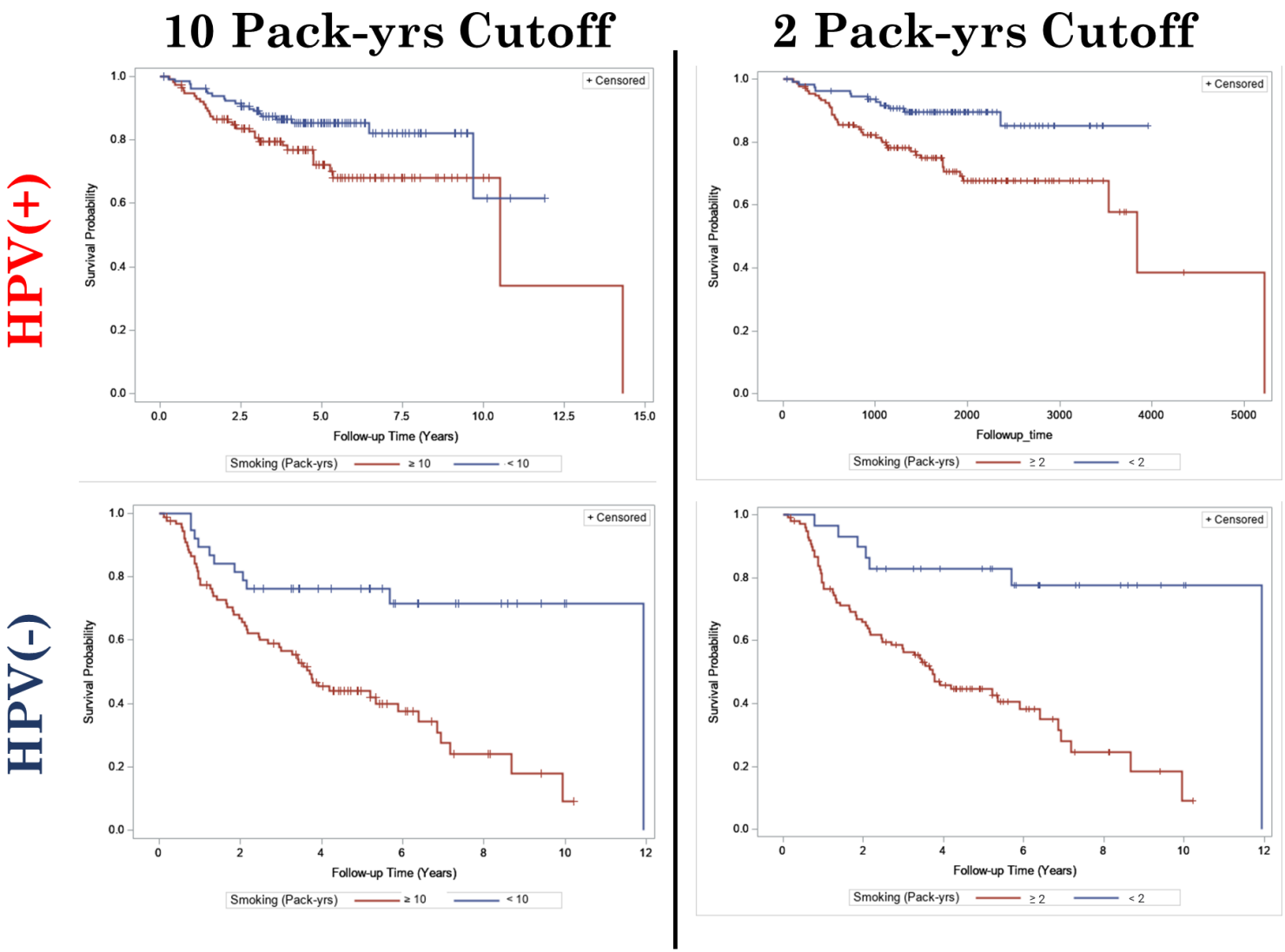


Figure 4: Kaplan-Meier curves illustrating overall survival experience of HPV(+) and HPV(-) OPCs stratified by 2 pack-years and 10 pack-years cutoffs

Appendix C Supplementary Tables

Supplementary Table 1: Active treatment de-escalation studies for HPV(+) oropharyngeal cancers on clinicaltrials.gov (search terms: "de-escalation OR non-inferiority | Oropharyngeal Cancer")

Title	Status	Start Date	Location	NCT #
Radiotherapy Dose De-escalation in HPV-Associated Cancers of the Oropharynx	Recruiting	4/9/2021	United States	NCT04667585
Toripalimab Based Induction Chemotherapy Followed by De-escalation Protocols in HPV-related OPSCC	Recruiting	2/1/2021	China	NCT04867330
Testing Less Intensive Radiation With Chemotherapy to Treat Low-risk Patients With HPV-positive Oropharyngeal Cancer	Recruiting	9/28/2020	United States	NCT04444869
De-escalation Protocols in HPV-related Oropharyngeal Carcinoma in Chinese Populations	Recruiting	7/1/2019	China	NCT04012502
De-Escalation Radiotherapy in Patients With Low-Risk HPV-Related Oropharyngeal Squamous Cell Carcinoma	Recruiting	2/20/2019	Canada	NCT03822897
De-escalation of Adjuvant Radio (Chemo) Therapy for HPV-positive Head-neck Squamous Cell Carcinomas	Recruiting	9/4/2018	Germany	NCT03396718
De-Escalation Protocol Of HPV Mediated Oropharyngeal Squamous Cell Carcinoma	Recruiting	8/6/2018	United States	NCT04638465
Individualized Adaptive De-escalated Radiotherapy for HPV-related Oropharynx Cancer	Recruiting	5/21/2018	United States	NCT03416153
PET-MRI Assessment of Early Tumor Response to Predict Outcomes of HPV-Positive Oropharynx Cancer Patients	Active, not recruiting	5/3/2018	United States	NCT03342378
Primary Radiotherapy Versus Primary Surgery for HPV-Associated Oropharyngeal Cancer	Recruiting	1/26/2018	Canada	NCT03210103
Major De-escalation to 30 Gy for Select Human Papillomavirus Associated Oropharyngeal Carcinoma	Recruiting	10/16/2017	United States	NCT03323463
Adaptive Treatment De-escalation in Favorable Risk HPV-Positive Oropharyngeal Carcinoma	Recruiting	7/10/2017	United States	NCT03215719
Chemotherapy and Locoregional Therapy Trial (Surgery or Radiation) for Patients With Head and Neck Cancer	Active, not recruiting	6/27/2017	United States	NCT03107182

Adaptive Radiotherapy for Head and Neck Cancer	Active, not recruiting	3/15/2017	United States	NCT03096808
Phase II Treatment Stratification Trial Using Neck Dissection-Driven Selection to Improve Quality of Life for Low Risk Patients With HPV+ Oropharyngeal Squamous Cell Cancer	Active, not recruiting	10/31/2016	United States	NCT02784288
Definitive Chemo-Radiotherapy for Regionally Advanced Head and Neck Cancer With or Without Up-front Neck Dissection	Recruiting	10/1/2016	Switzerland	NCT02918955
Post-operative Adjuvant Treatment for HPV-positive Tumours (PATHOS)	Recruiting	10/1/2015	United States and United Kingdom	NCT02215265
Nab-paclitaxel and Carboplatin Followed by Response-Based Local Therapy in Treating Patients With Stage III or IV HPV-Related Oropharyngeal Cancer	Active, not recruiting	9/22/2014	United States	NCT02258659
The Quarterback Trial: Reduced Dose Radiotherapy for HPV+ Oropharynx Cancer	Active, not recruiting	9/1/2012	United States	NCT01706939
Treatment De-Intensification for Squamous Cell Carcinoma of the Oropharynx	Active, not recruiting	1/1/2010	United States	NCT01088802

Supplementary Table 2: MFI cutoffs used to identify positive serology results for HPV antigens of interest

HPV Antigen	MFI Cutoff for Positivity
HPV16 E1	200
HPV16 E2	679
HPV16 E6 (low)	484
HPV16 E6 (high)	1000
HPV16 E7	548
HPV18 E1	200
HPV18 E2	600
HPV18 E6	243
HPV18 E7	789

Supplementary Table 3: Questionaire used to calculate pack-years smoked

CIGARETTE CHART

B2.1 At what age did you first/next start smoking cigarettes?	___ ___ AGE STARTED	___ ___ AGE STARTED	___ ___ AGE STARTED	___ ___ AGE STARTED
B2.2 Did you ever stop smoking them for one year or longer? (IF NO, RECORD REF. AGE IN B2.2a)	1- Yes 2- No* 9- DK (B2.3)	1- Yes 2- No* 9- DK (B2.3)	1- Yes 2- No* 9- DK (B2.3)	1- Yes 2- No* 9- DK (B2.3)
B2.2a How old were you when you first/next stopped?	AGE ___ ___ STOPPED	AGE ___ ___ STOPPED	AGE ___ ___ STOPPED	AGE ___ ___ STOPPED
B2.3 On average, how many cigarettes did you smoke in a day between the ages of (B2.1) and (B2.2a)?	___ ___ CIGARETTES/DAY	___ ___ CIGARETTES/DAY	___ ___ CIGARETTES/DAY	___ ___ CIGARETTES/DAY
B2.4 During this time, did you usually smoke non-filtered or filtered cigarettes?	1- Non-Filter 2- Filter 3- Equal Mix 9- DK	1- Non-Filter 2- Filter 3- Equal Mix 9- DK	1- Non-Filter 2- Filter 3- Equal Mix 9- DK	1- Non-Filter 2- Filter 3- Equal Mix 9- DK
B2.5 Were they <u>usually</u> Menthol or Non-Menthol cigarettes?	1- Menthol 2- Non-Menthol 3- Equal Mix 9- DK	1- Menthol 2- Non-Menthol 3- Equal Mix 9- DK	1- Menthol 2- Non-Menthol 3- Equal Mix 9- DK	1- Menthol 2- Non-Menthol 3- Equal Mix 9- DK
***IF STOPPED SMOKING, ASK: B2.6 Did you ever start regularly smoking cigarettes again after (AGE IN B2.2)?**	1- Yes (B2.1) 2- No (B3) 9- DK (B3)	1- Yes (B2.1) 2- No (B3) 9- DK (B3)	1- Yes (B2.1) 2- No (B3) 9- DK (B3)	1- Yes (B2.1) 2- No (B3) 9- DK (B3)

Supplementary Table 4: Questionaire used to calculate drinking intensity

ALCOHOL CONSUMPTION CHART

C1.1 At what age did you (first/next) start drinking alcohol 1 or more times per month?	___ ___ AGE STARTED	___ ___ AGE STARTED	___ ___ AGE STARTED	___ ___ AGE STARTED
C1.2 Did you ever stop for one year or longer? (IF NO, RECORD REF. AGE IN C1.2a)	1- Yes (C1.2a) 2- No (C1.3) 9- DK (C1.3)	1- Yes (C1.2a) 2- No (C1.3) 9- DK (C1.3	1- Yes (C1.2a) 2- No (C1.3) 9- DK (C1.3	1- Yes (C1.2a) 2- No (C1.3) 9- DK (C1.3
C1.2 How old were you when you (first/next) stopped?	___ ___ AGE STOPPED	___ ___ AGE STOPPED	___ ___ AGE STOPPED	___ ___ AGE STOPPED
C1.3 On average, how many times in a week or month would you have at least on drink, between the ages of ____ and ____?	___ ___ 1- Week 2- Month	___ ___ 1- Week 2- Month	___ ___ 1- Week 2- Month	___ ___ 1- Week 2- Month
C1.4 On a day that you drank, how many glasses would you usually have? (GLASS=CAN=BOTTLE)	___ ___ NUMBER DRANK	___ ___ NUMBER DRANK	___ ___ NUMBER DRANK	___ ___ NUMBER DRANK
C1.5 What would be the highest number that you might have in a day?	___ ___ NUMBER DRANK	___ ___ NUMBER DRANK	___ ___ NUMBER DRANK	___ ___ NUMBER DRANK
***IF STOPPED DRINKING, ASK C1.6 (OTHERWISE GO TO SECTION D):** **C1.6 Did you ever start drinking one or more drinks per month for one year or longer after (C1.2a)?**	1- Yes (C1.1) 2- No (SECT D) 9- DK (SECT D)	1- Yes (C1.1) 2- No (SECT D) 9- DK (SECT D)	1- Yes (C1.1) 2- No (SECT D) 9- DK (SECT D)	1- Yes (C1.1) 2- No (SECT D) 9- DK (SECT D)

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