Survival Benefit of Chemotherapy in Oropharyngeal Cancer Patients Treated with Surgery and Post-Operative Radiation

by

Fawaz Mohammed Makki

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Clinical Epidemiology

School of Public Health
University of Alberta

Abstract

Oropharyngeal squamous cell carcinoma (OPSCC) is the third most common cancer of the head and neck. The incidence of all subtypes of head and neck squamous cell carcinoma has decreased over the past 30 years in Canada and most of the world except for OPSCC. The increasing incidence of OPSCC over the past 10-20 years is driven by Human Papilloma Virus (HPV) type 16 infection. The benefit of chemotherapy in the post-surgical treatment of advanced stage OPSCC is unclear in the current literature especially after the emergence of HPV related OPSCC. This thesis investigated the survival benefit of adding chemotherapy in the primary surgical setting followed by adjuvant radiation therapy in the management of all patients with advanced stage OPSCC. We hypothesized that chemotherapy could have a survival advantage dependent on p16 and tobacco smoking history. Comparative survival analyses were performed between patients who received surgery + radiation therapy (S+RT) and surgery + chemoradiation therapy (S+CRT), stratified according to p16 status and tobacco smoking history. After adjustment for all covariates, smoking status and extracapsular extension were both independent predictors of survival. In our survival analysis for the whole cohort, the addition of chemotherapy was associated with a statistically significant better 5-year overall survival. After stratifying based on their p16 and smoking status, smokers showed a statistically significant better survival benefit from the addition of chemotherapy in post-operative setting. However, further prospective trials that include p16 and smoking status would be recommended to verify this hypothesis.

Preface

This thesis is an original work by Fawaz Makki. Research ethics approval was obtained from the University of Alberta Health Research Ethics Board (No: HREB / Pro00016426, 2015-11-30).

A manuscript version of the thesis is being prepared for publication in peer-reviewed journals. Fawaz Makki was responsible for study design, data analyses, manuscript writing, and manuscript revision for the studies included in this thesis. Dr. Faith Davis & Dr. Vincent Biron provided guidance to the study design, data analyses, manuscript writing, preparation for submission, and manuscript revision. Dr. Ambikaipakan Senthilselvan provided me with great help and guidance with data analysis.

Acknowledgements

I am very grateful to Dr. Faith Davis & Dr. Vincent Biron, my supervisors and mentors. Their assistance and supervision are very appreciated by me. It is a great honor to have been given the chance to work with both of them. Without their guidance and support I believe that my time as a MSc student would not have been nearly as successful as it has been.

I would also like to thank Dr. Ambikaipakan Senthilselvan, my professor in biostatistics and one of the authors in my thesis. His insight and mentorship in biostatistics were instrumental and of great value in developing the final product.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AJCC	American Joint Committee on Cancer	**OPSCC**	Oropharyngeal Squamous Cell Carcinoma
CRT	Chemoradiation Therapy	**OR**	Odds Ratio
CI	Confidence interval	**OS**	Overall Survival
DFS	Disease Free Survival	**OSU**	Ohio State University
DNA	Deoxyribonucleic acid	**PCR**	Polymerase Chain Reaction
DSS	Disease Specific Survival	**Rb**	Retinoblastoma
ECE	Extracapsular Extension	**RT**	Radiation Therapy
ECOG	Eastern Cooperative Oncology Group	**RTOG**	Radiation Therapy Oncology Group
EORTC	European Organization for Research & Treatment of Cancer	**RT-qPCR**	Real-Time quantitative Polymerase Chain Reaction
FFPE	Formalin-Fixed Paraffin-Embedded	**S + CRT**	Surgery + Chemoradiation Therapy
Gy	Gray	**S + RT**	Surgery + Radiation Therapy
HNSCC	Head and Neck Squamous cell carcinoma	**SEER**	Surveillance, Epidemiology, & End Results
HPV	Human Papillomavirus	**T**	Primary tumor size
HR	Hazard ratio	**TLM**	Transoral Laser Microsurgery
IHC	Immunohistochemistry	**TMAs**	Tissue Microarrays
IMRT	Intensity Modulated Radiation Therapy	**TORS**	Transoral Robotic Surgery
ISH	*In Situ* Hybridization	**UA**	University of Alberta
N	Regional lymph nodes	**WHO**	World Health Organization
NCCN	National Comprehensive Cancer Network	**-**	-

Chapter 1: Introduction and Literature Review

1.1 Background

The head and neck is one of the most complex regions of the human body in both its anatomy and physiology. Head and neck squamous cell carcinoma (HNSCC) has major social and functional impacts on patients, affecting functions that are integral to human existence. For example, patients with oral cavity cancer will often have their speech, communication, swallowing, socialization and image significantly altered by the cancer and its treatment. HNSCC is an insidious disease that can have a severe effect on patients' quality of life.

Treatment of HNSCC necessitates a working knowledge of all therapeutic options for delivering optimal care to patients. For patients and their families, quality of life following HNSCC is a pivotal issue, emphasizing that the treatment is more than cure and survival. The head and neck oncology team must appreciate and optimize the longstanding impacts of treatment on their patients.

The etiology of HNSCC involves a complex interplay between host and environmental factors. Studies have shown that alcohol and tobacco exposure are key causative factors for HNSCC in at least 75% of cases.[1-3] The use of both tobacco and alcohol synergistically increase the risk of developing these cancers.[3,4] Infection with human papillomavirus (HPV), especially HPV type 16 (HPV-16), is a risk factor for some types of HNSCC, particularly oropharyngeal squamous cell carcinoma (OPSCC).[5,6] In the United States, the incidence of oropharyngeal cancers caused by HPV infection is increasing, while the incidence of oropharyngeal cancers related to other causes is declining.[5,7] Based on the 2016 Canadian cancer statistics, a total of

3,760 HPV associated cancers were diagnosed in Canada in 2012.[8] Cervical cancers and OPSCC were the most commonly diagnosed accounting for approximately 35% of all HPV-associated cancers in Canada. Sine the 1990s the incidence rate of OPSCC has increased significantly in both males and females. In males, the rate of increased by an average of 3.1% per year from 1997 to 2012.[8] Whereas, in females, the rates increased more slowly at an average annual rate of 1.1%, between 1992 and 2012.[8]

Surgery has been the mainstay of therapy for neoplasms in the head and neck including OPSCC for more than a century during the 1980s. With the introduction of ionizing radiation in the latter half of the 20th century, radiotherapy became an important modality used either independently or in combination with surgery. Although initially chemotherapy was used primarily with palliative intent, it is now used as part of curative approaches when combined with radiation, producing effective treatment responses in patients with HNSCC. Biological or targeted agents also are evolving to become part of standard therapy. Accordingly, understanding and implementing multidisciplinary management strategies are cornerstones for achieving optimal therapeutic outcomes. OPSCC has been the one subsite of HNSCC that has undergone multiple changes in the management guidelines over the past 30 years.[9-11] In patients with advanced stage OPSCC undergoing primary surgery the benefit of postoperative chemotherapy in the current literature is not clear.[12] In 2007, a landmark publication highlighted HPV as one of the etiologies of OPSCC with a significantly and better prognosis than OPSCC that is related to tobacco smoking.[13] Treatment de-escalation has been proposed for those with HPV related advanced stage OPSCC based on the better prognosis and treatment associated witht this disease.[14]

One of the de-escalation approaches eliminates chemotherapy due to its severe toxicity and due to the lack of survival benefit in HPV-OPSCC cohorts.[14,15]

1.2 Study Objectives (Figure 1.1)

The primary objectives of this study were to:

1. Compare patients with advanced stage OPSCC treated with primary surgery followed by radiotherapy (RT) or chemoradiotherapy (CRT) to determine whether the addition of chemotherapy has a significant survival advantage.
2. Evaluate the benefit of chemotherapy on survival dependent on P16 status (surrogate marker for oncogenic HPV) and tobacco smoking history.

We hypothesized that chemotherapy could have a survival advantage dependent on P16 status and tobacco smoking history.

1.3 Head and Neck cancer overview

The majority of head and neck neoplasms arise from the mucosa of the upper aerodigestive tract, including the oral cavity, oropharynx, hypopharynx larynx, nasal cavity, and sinuses, but neoplasms can also originate from the salivary glands, thyroid and parathyroid glands, soft tissue, bone, and skin. The most common malignant neoplasms of the head and neck are HNSCCs.[16,17] Salivary gland malignancies and sarcomas of the soft tissue and bone are relatively infrequent and accounts for 5% & 1% of cancers of the head and neck respectively.[18,19] HNSCC are responsible for >95% of mucosal head and neck cancers, affecting the oral cavity, oropharynx, larynx and hypopharynx, nasopharynx, nasal cavity and paranasal sinuses (Figure 1.2).

HNSCCs are ranked fifth for incidence and cancer-related deaths worldwide,[20] with approximately 500,000 new cases are reported annually.[21] In Canada they are the 13th most common cancer with an estimated 3,600 cases diagnosed in 2011, and responsible for estimated 1,150 deaths.[22] In the US it is the 10th most common cancer, accounting for approximately 3% of all adult malignant neoplasms.[23] From 2013 to 2015, the estimated annual of HNSCC in the US decreased from 53,000 to 40,000 cases, with a decrease in deaths from 11,500 to 7890. [21,24,25] Depending on primary tumor site and overall stage, HNSCC survival varies with 5-year overall survival rates ranging from 89% for early-stage to 27% for advanced-stage disease.[23] Patients with HNSCC are more commonly adult males over 40 years old with almost 50% of patients are over 60 years old.[23]

The majority of HNSCCs are preventable, given the two most important risk factors are tobacco smoking and alcohol consumption. Both tobacco and alcohol have a dose response relationship with the development of HNSCC.[3,26] When consuming both alcohol and tobacco at the same time there is a 10-20 fold increase in carcinogenicity due to the synergistic effect of their interaction.[20] More recently, HPV has been established as an important etiological factor responsible for the increasing incidence of a subset of HNSCC, oropharyngeal squamous cell carcinoma (OPSCC).[27] Other risk factors include genetic predisposition, immunodeficiency, poor oral hygiene and other types of viral infections (Human immunodeficiency virus, Herpes simplex virus, Epstein–Barr virus).[28]

The treatment of HNSCC depends on the site involved and stage according to the TNM staging system developed by the American Joint Committee on Cancer

(AJCC).[29] This staging system is based on the assessment of three elements; the primary tumor size and extent on invasion (T), spread to regional lymph nodes (N), and spread to distant sites (M). T, N, and M stages are combined and translated into an overall stage cancer that can range from stage I – IV (A, B and C). Stages I & II are considered early stage, while stage III & IV are considered advanced stage. Treatment modalities are divided initially as either curative intent or palliative. Palliative therapy is generally used for patients with very advanced stage or distant metastases (IVc or M1) with either no cure or very poor treatment outcomes expected. Treatment with curative intent can be classified based on the stage of the cancer. Early stage (stage I & II) OPSCC patients generally require single modality treatment, which means either a surgical resection or RT. Selection of either surgery or RT depends on the site and size of the primary tumor, its proximity to bone and its depth of infiltration into the underlying soft tissues, availability of treatment options, patient comorbidities and patient preferences. When a patient presents with an advanced stage HNSCC (stage III & IV) combined modality treatments are usually required for curative intent. Combined treatments include: 1) primary surgery followed by adjuvant therapy, which could be either RT or CRT, 2) primary RT or CRT followed by surgery or 3) CRT with salvage surgery for persistent disease. The treatment plans are generally based on a multidisciplinary tumor boards, which include head and neck surgeons, medical oncologists, radiation oncologists, radiologists, pathologists, dentists, prosthodontists, speech language pathologists, nurses, dietitians, and social workers. A multidisciplinary approach provides each patient with a well-organized evaluation and treatment plan towards optimal treatment outcomes.

The assessment of treatment outcomes is based on two main endpoints, survival and quality of life. Survival analyses for HNSCC are generally measured in three ways: 1) overall survival (OS): an event is death from any cause, 2) disease specific survival (DSS): in this variant, the event is death specifically from the cancer, and 3) disease free survival (DFS): an even can be either death or recurrence of the HNSCC. The start point of survival analysis varies, can be from the date of the patient's first visit to a head and neck cancer clinic, a biopsy proven HNSCC, start of treatment, or from the end of treatment. However, when comparing survival estimates it is important to ensure that the starting and ending points are the same.

1.4 Oropharyngeal squamous cell carcinoma (OPSCC) overview

The pharynx is divided into three regions: 1) nasopharynx, 2) oropharynx, and 3) hypopharynx. The oropharynx begins at the anterior aspect of the faucial arch and extends posteriorly to the posterior pharyngeal wall. It includes four subsites: 1) the soft palate 2) palatine tonsils, 3) posterior pharyngeal wall, and 4) the tongue base (posterior third of the tongue) (Figure 1.3). The oropharynx has a major role in swallowing and speech. Cancer involving any of the oropharyngeal subsites can put the patient at great risk of aspiration and/or severe dysphagia requiring a gastrostomy tube as a supplemental or alternative method of feeding. It can also affect the patients' ability to communicate and socialize by altering the voice resonance, pronunciation and articulation.

The most common cancer of the oropharynx is squamous cell carcinoma (90%), followed by Hodgkin lymphoma (8%), and minor salivary gland tumors (2%).[30] Of the four oropharyngeal subsites, squamous cell carcinoma mostly commonly affects the

palatine tonsils and base of tongue. This is attributed to the prolonged contact of the mucosal surface with saliva which contains the carcinogens from tobacco and alcohol compared to the other two subsites (soft palate and posterior pharyngeal wall).[30] The base of tongue used to be the most commonly affected subsite, however with increase in HPV-related OPSCC the palatine tonsils being are not the most affected subsite.[20]

OPSCC is the third most common cancer of the head and neck after the larynx and oral cavity.[30] Due to the significant decrease in the rates of tobacco smoking the incidence of HNSCC has decreased over the past 30 years in Canada and most of the world except for OPSCC.[31-33] According to Surveillance, Epidemiology, and End Results (SEER) data, the incidence of OPSCC has significantly increased over the past 20 years for both males and females (Figure 1.4). The increasing incidence of OPSCC over the past 10-20 years is driven by HPV-16 infection.[5,7,34] In the United States, the prevalence rate of HPV related OPSCC is greater than 60% and rates continue to increase; the incidence almost doubled (70% in the United States and 80% in Canada between 1983 and 2002).[5,35-37] In more recent statistics the incidence of OPSCC among white men and women increased in the United States by almost four-fold between 2000 and 2009.[27] This shift in epidemiologic trends is likely due to increased oral sexual activity, but other unknown factors may also contribute to the increase in HPV-OPSCC.[38] This has changed the demographics of patients with OPSCC to be mainly young (40-60 years old), nonsmoking white males.[35,36,39-41] These patients also tend to be married and well educated with a higher socioeconomic status compared to those who are HPV negative.[42]

The etiology of non-HPV related OPSCCs is similar to other HNSCCs with

tobacco smoking being one of the most important risk factor. Other risk factors include: poor oral hygiene, occupational exposure to organic chemicals, coal and wood dust, genetic and immunologic predisposition.[28]

1.5 Smoking and OPSCC

Worldwide, there are about 1.2 billion smokers and hundreds of millions of smokeless tobacco users.[43,44] Over 1 million cancer deaths annually worldwide are related to tobacco smoking.[44] In the United States alone, tobacco smoking contributes to 33% of all cancer deaths, while in all the developed countries it is around 21%. In cases with OPSCC almost 90% of patients have history of tobacco smoking.[30] In more recent reports of OPSCC epidemiology, about 60% of the HPV positive OPSCC are either current or former smokers, compared to never smokers.[45]

Cigarettes are the main type of tobacco product consumed in the world. There are a variety of carcinogens in cigarette smoke. The most important carcinogens based on their potency are: polycyclic aromatic hydrocarbons, N-nitrosamines, aromatic amines, 1,3-butadiene, benzene, and aldehydes.[46,47] Among drinkers who also smoke, alcohol creates the perfect medium for these carcinogens to dissolve and get absorbed into the mucosal surface much better than the slightly alkaline saliva. This biologic change is thought to explain the synergistic interaction between alcohol and cigarette smoking, resulting multiplicative increase in the risk of developing any of the HNSCC cancers in general and OPSCC in particular.[20,30] Second, alcohol can potentiate the metabolic activation of tobacco and the capacity to solubilize and enhance the penetration of carcinogens into the oropharyngeal tissues.[3] Other types of smoked

tobacco include pipes and cigars. These products are associated with an increased risk of oral (oral cavity and oropharynx) cancer compared to other head and neck subsites, attributed to the type of tobacco used in them.[48] The two major types of tobacco in cigarettes are the black and the blond tobacco, with the former containing more carcinogens.[49] Users of the black tobacco cigarettes have a three-fold relative risk of oral cavity and pharyngeal cancer when compared to blond tobacco cigarette users.[50] For those who stop smoking for one to nine years their risk of developing oral cavity or OPSCC drops by 30%, and almost 50% if they stopped for more than 9 years.[51] With regards to smoking status and the development of OPSCC, a cutoff point of 10 pack-years (number of packs per day * number of years of smoking), has been identified as one of the strongest predictors of poor survival.[36,52] However, 10 pack-years is not a fixed criterion as other research groups such as the Radiation Therapy Oncology Group has suggested the use of 20 pack-year as a cutoff point.[53,54]

A substantial amount of tobacco is consumed worldwide in the form of smokeless tobacco products, including chewing tobacco, betel quid, bidi, chutta, khaini and toombak. These tobaccos mainly cause oral cavity cancers, but has been associated with some OPSCC cases, mainly the base of tongue subsite.[30] It also has been shown that marijuana increases the risk of HNSCCs including the oropharynx.[55,56]

1.6 Human papillomavirus (HPV) overview

Human papillomavirus (HPV) is a small deoxyribonucleic acid (DNA) virus that can be transmitted through any kind of sexual contact including vaginal, anal, and oral sex.[57] It has an affinity towards squamous epithelial cells where it proliferates and

causes an infection. There are over 140 different HPV serotypes that can be associated with specific clinical lesions, however only certain serotypes are associated with lesions at high risk of malignant transformation. HPV 6 and 11 are associated with benign lesions located in the oral cavity and oropharynx such as the common wart, known as squamous papillomas.[58] HPV 16 and 18 are associated with premalignant and malignant lesions of the head and neck with HPV 16 infection being responsible for the overwhelming majority of HPV-OPSCCs.[58-60] Over 90% of HPV related OPSCC are caused by HPV type 16.[61] Additional HPV types associated with HNSCC include types (18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68).[36]

Oral HPV infection is one of the most common sexually transmitted infections.[62] Positive oral HPV infection can be determined by the presence of viral DNA in oral rinses using PCR testing.[63] The National Health and Nutrition Examination Survey provide the most recent estimate of the prevalence of oral HPV infection to date, showing that HPV-16 infection is the most common oral infection and is present in 1% of the U.S. population.[6,59] However the prevalence of any type of oral HPV infection was 7%, that represents 15 million people in the United States in 2009/2010.[6] The prevalence of oral HPV infection is three- to five-fold higher in men than in women with an adjusted prevalence ratio of 2.3 (95% CI, 1.66 to 3.26).[6] The age distribution of oral HPV infection is bimodal with an initial peak at age 30-34 years and a second peak between 55 to 64 years of age.[59] Oral HPV infection is a sexual transmitted disease that can be acquired through oral, vaginal, and anal sex.[6,59,64-66] The prevalence of oral HPV infection is higher overall in men, and increases with more sexual partners but plateaus for men at 15 partners and women at 5 partners.[27,67]

Several factors may explain the higher prevalence in men compared to women: 1) men have more sexual partners than women, 2) higher transmission rates for HPV from female to male than vice versa, and 3) due to the how common the genital HPV infection, the seroconversion rates are much higher among women compared to men, which gives women some immunity against subsequent HPV infection.[67]

The natural history of oral HPV is usually self-limiting and benign with most infections clearing between 7 – 12 months, however a few cases persist longer.[68,69] Several factors influence clearance vs. persistence of oral HPV, including local immunity status, tobacco status, the use of systemic immunosuppression, and sexual behaviors.[68-70]

1.6.1 HPV and OPSCC

It is only in the early 1980s that HPV was studied for its potential role as an etiological risk factor for HNSCC.[71] From a molecular perspective, several papers were published providing evidence supporting the role of HPV in the pathogenesis of of HNSCC, particularly in OPSCC.[72-74] Between 1998 and 2004 multiple case control studies investigated the causal association between HPV infection and OPSCC.[13] However, none of these studies showed clear association between high risk sexual behaviors, and HPV infection and the development of OPCSCC.[75] In 2007, a landmark paper published in the New England Journal of medicine conducted a hospital-based case–control study of 100 patients with OPSCC and 200 control patients without cancer to evaluate the associations between HPV infection and OPSCC.[75] HPV type 16 infection was significantly associated with OPSCC [odds ratio (OR): 14.6, 95% CI: 6.3 to 36.6].[75] High risk sexual behaviors were as well

significantly associated with OPSCC, a total of ≥ 26 vaginal sex partners was significantly associated with OPSCC [OR: 3.1, 95% CI: 1.5 to 6.5], and a total of ≥ 6 oral sex partners was significantly associated with OPSCC [OR: 3.4; 95% CI: 1.3 to 8.8].[75]

In 2007, HPV was acknowledged by the international agency for research against cancer as a risk factor for HNSCC in addition to smoking and alcohol.[13] Most HPV-associated HNSCCs arise in the oropharynx with the palatine tonsils being the most commonly affected subsite.[20] HPVs have also been detected in other head and neck sites, however to a lesser degree compared to the oropharynx [(6- 20% in oral cavity), (20-30% in larynx, nasopharynx, sinuses)]. Their role is still unclear and controversial outside the oropharynx.[76-78]

In the United States, the total prevalence of HPV related OPSCC is greater than 60% of all OPSCCs, and continues to rise.[5,36,37,72,79] Despite the overall decrease in the incidence of HNSCC related to the significant drop in the rates of tobacco smoking, the rates of HPV related OPSCC has increased by 70% in the United States and 80% in Canada between 1983 and 2002.[5,35,80] In more recent statistics from the United States, the incidence of OPSCC among white men and women increased by almost four-fold between 2000 and 2009.[27] According to SEER data, it has been predicted that the incidence of HPV-OPSCC in the United States will soon be greater than cervical cancer.[5] The rise in HPV-OPSCC is not consistent across different geographic locations, presumable due to differences in sexual behaviors and tobacco consumption.[35]

The demographics of patients with HPV-OPSCC are different from those who are

HPV negative. HPV-OPSCC patients tend to be male younger, nonsmokers, and nondrinker, compared to HPV negative OPSCC.[41,81] They also tend to have a higher socioeconomic status, higher education, be married, and have fewer comorbidities.[14,42,82,83] With regards to race, a significantly greater proportion of HPV positive OPSCC are diagnosed in whites than in blacks or other races, accounting for 92-97% of all HPV positive OPSCC.[36,84,85]

The clinical characteristics of HPV positive versus negative OPSCC are also different. HPV-OPSCCs tend to present as a small primary tumors (early T-stage) with more regional metastases (higher N-stage).[14,42,86,87] Almost 10% of all HNSCC present with neck nodal metastasis and an unknown primary site.[88] When the primary site is identified it is usually in the oropharynx and 90% are HPV positive.[87,89-91] On histopathology, HPV-OPSCCs are often basaloid (variant of squamous cell carcinoma), poorly differentiated, and non-keratinized.[92-94]

1.6.2 Diagnosis of HPV associated OPSCC

Determining HPV positivity is of critical importance in the diagnosis and management of OPSCC, as it has implications for prognosis and treatment.[36,95-97] Patients with HPV-OPSCC have significantly better clinical outcomes when compared to patients with HPV-negative- OPSCC.[36,72,84] As previously mentioned, 10% of all HNSCC presents with neck nodal metastasis and an unknown primary. Having the ability to detect the HPV status from nodal metastasis strongly points to the oropharynx as the primary site, which adds great value in the management of the patient either by narrowing the radiation therapy field or to target surgically.[88,98,99]

Knowing the HPV status also helps to determine if a patient is a candidate for clinical trials investigating new treatment regimens for HPV positive OPSCC (de-intensification, new chemotherapy, new radiotherapy regimen and immunotherapy).[100,101]

HPV genome comprises early and late genes that encode early proteins E1-E7 and late proteins L1-L2. The two most important proteins that are responsible for the onset and persistence of the malignant process are E6 & E7 oncogenic proteins. Both E6 & E7 proteins cause multiple genetic and metabolic effects within the cell, the most important of which is their interaction with two intracellular tumor suppressor proteins, p53 and retinoblastoma (Rb).[102] Following the binding of E6 & E7 to p53 & Rb proteins, respectively, both tumor suppressor proteins (p53 & Rb) get degraded.[102] This results in downstream overexpression of p16 tumor suppressor protein. The gold standard for determining HPV status in OPSCC is demonstration of oncogenic HPV DNA in fresh tissue using real-time quantitative polymerase chain reaction (RT-qPCR).[103-105] It has also shown that antibodies against both HPV E6 & E7 oncogenic proteins are strongly associated with the diagnosis of OPSCC. Patients with new OPSCC were seropositive for HPV16 E6 for almost 10 years prior to their diagnosis, and healthy individuals who were seropositive for HPV 16 E6 were strongly associated with future diagnosis of OPSCC (odds ratio [OR], 274; 95% CI, 110 to 681).[106,107] PCR for HPV DNA testing is highly sensitive for the presence of HPV, however two factors may affects its reliability to detect HPV related OPSCC: 1) can not distinguish HPV infections that are truly causative of HPV-SCC (transcriptionally active) from those that are clinically insignificant (so-called “passenger” HPV), 2) The

very high sensitivity of PCR based techniques may allow for the possibility of cross-contamination of HPV DNA from another specimen to yield a false–positive result.[108,109] An alternative measurement, PCR testing for E6/E7 messenger RNA is more specific in that it indicates the presence of a transcriptionally active HPV.[108,110,111] Other techniques that can help detect HPV status includes DNA and RNA in situ hybridization (ISH)[61,112-114]

Due to the high cost and specialized equipment required for HPV DNA PCR, most centers have adopted p16 immunohistochemistry (IHC) as the preferred method of oncogenic HPV detection, which has become the clinical standard.[115-119] P16 is a tumor suppressor gene that is used as a surrogate marker for HPV positive OPSCC. Retinoblastoma protein (Rb) is a tumor suppressor protein that down regulates p16 tumor suppressor protein, however in HPV positive OPSCC, the E7 oncoprotein degrades Rb protein which leads to loss of feedback inhibition and overexpression of p16 tumor suppressor protein which can be detected by routine IHC.[120-122] In HPV negative OPSCC the p16 expression is not upregulated.[123] The main advantages using p16 IHC are: 1) Inexpensive, 2) easy to perform and interpret, 3) highly sensitive for transcriptionally active oncogenic HPV (almost 100%), and 4) very low inter-observer variability when the proper criteria are used.[108,120,124] Despite those advantages, p16 IHC testing has some criteria\limitations which include: 1) for the test to be positive at least 70% of the specimen has to stain positive for P16, if between 50 to 70% of specimen tests positive then additional testing might be required to confirm HPV positivity (HPV DNA PCR, or DNA ISH),[115] 2) while its sensitivity reaches almost

100% its specificity ranges between 85-95%,[108,120,124] 3) lastly, p16 IHC positivity is only reliable and validated for OPSCC.[125]

Overall there is some controversy or lack of uniformity in what is used between different centers to be the ideal testing methods used for checking the status of HPV in OPSCC with the hope that in the near future there will be clear guidelines for HPV testing in OPSCC.[61,108,124,126] Based on the 2013 NCCN guidelines; the acceptable testing methods to detect HPV positive OPSCC are either by the use of IHC for analysis of p16 expression or HPV in ISH for detection of oncogenic HPV DNA in tumor cell nuclei.[127]

1.6.3 Prevention of HPV related OPSCC (vaccines and their benefits)

To date there are no available screening tests available for early detection of HPV related OPSCC. The only preventive intervention available is HPV immunization; however the evidence showing benefit is based on populations of patients with anogenital papillomas or cervical cancer with little data regarding the effectiveness of vaccines for prevention of HPV-OPSCC. However it is thought that HPV vaccination might have the same preventive effect on HPV-OPSCC and this could hopefully halt the progressive increase in its incidence in the future.[128,129]

To be able to get the population immunized against HPV we need to make sure that there is awareness among the high risk population for HNSCC and HPV-OPSCC. Based on an online survey that was administered in January of 2013 in the United States to 2126 randomly selected adults, only 34% reported that they were aware about head and neck cancer and 0.8% were aware that HPV played a role in the causation of head and neck cancer.[23] When questions were asked specifically about

throat cancer and it association with HPV, 12.8% reported awareness.[23] Before starting an immunization program for HPV to help prevent HPV related OPSCC, we need to ensure increased awareness among the general population aiming to increase their adherence to the immunization protocol. This can help prevent almost 9000 cases annually.[23]

Currently there are two FDA approved HPV vaccines, Gardasil®, Cervarix®, and Gradasil®9. Gardasil is a quadrivalent HPV vaccine that is effective against HPV type 6, 11, 16, & 18, while Cervarix is a bivalent vaccine effective against HPV type 16 & 18. Gradasil®9 is the most recent vaccine and was authorized for use in Canada on February 2015 and it effective against 9 HPV types (HPV types 6, 11, 16, 18, 31, 33, 45, 52 and 58). The vaccine was developed in Australia and was approved by the food and drug administration (FDA) for preventative use in 2006. In 2007, Australia was the first country to introduce a national HPV vaccination program, but it was only directed towards young women to protect against HPV infections that can lead mainly to cervical cancers.[130] In 2009, Gardasil® was approved by the FDA to be used in males aged 9 to 26 for prevention of HPV related genital warts, anal cancer, and head and neck cancer.[131]

In Canada, Gardasil® is the mainly used vaccine and it is indicated for females between the ages of 9 & 45, but those between 9 & 21 are the primary target.[132] For males, it is indicated between the ages of 9 & 26.[133] The vaccine is administered in 3 consecutive doses of 0.5 mL suspension via an intramuscular injection at the following schedule: 0, 2 months, 6 months. The vaccine is also time sensitive, it is most effective if it is administered before the start of sexual activity, which reflects the start of

exposure to HPV virus, as it is not recommended for the adults who have already been sexually active for a number of years.[132,134] However, if an adult is already infected with HPV, the vaccine will help with protection against new infections with other strains included in the vaccine.

The prophylactic administration of the vaccine has shown to be significantly effective in deceasing the incidence of HPV related cervical cancer and preventing 90-100% of HPV infection related anogenital precancerous lesions.[135,136] However, the evidence is still unclear with regards to oral HPV infection.[27] In 2013, a multicenter study was published describing a significant decrease in the incidence of oral HPV infection 4 years after the administration of the vaccine.[129] However, based on the WHO guidelines this study does not fulfill all the required criteria in order to consider the vaccine effective against both incident and persistent cases of oral HPV infection.[27]

1.7 Treatment options & prognosis of OPSCC

The management of OPSCC depends primarily on the stage of the cancer. Based on the 2013 NCCN guidelines, early stage OPSCC can undergo a single modality treatment (surgery or definite RT), while advanced stage cases are recocommended to receive combined treatment modality.[127] In general, treatment paradigms for advanced OPSCC are either primary chemoradiotherapy (CRT) with salvage surgery if there is any residual or recurrent disease, or primary surgery with adjunctive therapy (radiation therapy (RT) alone, or CRT).[127,137] With both approaches, treatment addresses both the primary tumor site and the neck nodal disease. In the primary CRT arm, the standard RT modality used is intensity modulated radiation therapy (IMRT) and the dose to the

primary site and involved lymph nodes ranges between 66-72 gray (Gy) delivered over 6-7.2 weeks, 5 days per week, Monday-Friday.[127] The uninvolved levels of neck lymph nodes that are at risk of harboring subclinical disease or spread receive between 54-60 Gy. With regards to chemotherapy, Cetuximab and platin-based chemotherapies are the most common systemic treatments for OPSCC, which are given in total of 3-cycles during the RT.[127] However, the etiology and patient demographic of OPSCC has significantly shifted in recent decades, current treatment paradigms are controversial and protocols are continuously evolving.[27,96,103,138-140]

In the early 1980s, the main treatment of advanced stage OPSCC included primary surgery followed by adjuvant CRT in advanced stage OPSCC.[9] During that period this type of treatment provided the best cure rates compared to RT (58.2% versus 26.4%).[9] The introduction of primary CRT started in the 1990s, due to the variable and poor functional results after surgery and early reconstructive methods led many centers to experiment with organ preservation protocols using CRT in an attempt to validate the concept of functional conservation after organ preservation.[10] This concepts refers to treatment using CRT which would allow preserving the organ so it can function normally, while in surgical treatment the organ will be either partially or completely excised which leads to either partial or complete loss of function. This concept was popularization as a result of the Vetrens Affair trial, which was actually for laryngeal cancer, but this was extrapolated and assumed to be that CRT was also the best for oropharynx.[141] This type of treatment has evolved to intensifying chemotherapeutic approaches to also include neoadjuvant chemotherapy followed by CRT.[11,142-145] However, despite improved disease-related outcome this

lead to dramatic increases in the rates of treatment related early (acute) and late toxicities in terms of mucositis, xerostomia, pharyngeal strictures, osteoradionecrosis, and long-term dysphagia requiring feeding tubes.[11,36,144-152] The rates of gastrostomy tube dependence after primary CRT have ranged from 7% to as high as 31% at 1 year after treatment.[153,154]

Primary CRT became the standard of care for patients with advanced stage OPSCC despite the absence of oropharyngeal-specific trials validating this change in management.[155] On the other hand, there are several population-based,[156] retrospective,[157] and case control studies[158,159] showing equivalent survival outcomes from either primary CRT or primary surgery followed by adjuvant RT/CRT. Also, few other population based studies showing superior survival with primary surgical approaches.[160,161] The National Comprehensive Cancer Network (NCCN) guidelines suggest that primary surgery with adjunctive therapy or chemoradiotherapy (CRT) with salvage surgery are acceptable treatment regimens for advanced OPSCC.[156] The survival rates for OPSCC have significantly changed with the emergence of HPV related OPSCC. There is overwhelming evidence that HPV-OPSCC have a substantially improved prognosis, and higher overall and disease specific survival compared to HPV negative OPSCCs.[36,54,84,162] This is thought to be due to better response to therapy regardless of the treatment regimen used.[163] In addition to increased radiosensitivity and response to therapy, other factors have been proposed to play a role in the improved prognosis in HPV positive OPSCC such as absence of tobacco exposure with a reduced likelihood of field cancerization (The development of premalignant clones of the cells throughout the affected area because of repeated

exposure of the tissue to carcinogens, ex: tobacco product) and second primary tumors, and an inverse correlation with adverse tumor biomarkers such as epidermal growth factor receptor (EGFR) & p53 mutations.[72,109,164-166] Two recent papers published comparing primary surgery to primary RT in the treatment of OPSCC showed the in the HPV positive group survival is comparable with no statistical significant difference, however in the HPV negative group survival was superior in the primary surgery group.[96,167]

A landmark paper by Ang et al in 2010 demonstrated the survival differences between HPV positive and negative OPSCCs treated by non-surgical approaches.[36] They analyzed over 300 patients with OPSCC who participated in the RTOG (Radiation Therapy Oncology Group) 0219 trial. HPV-OPSCC comprised 64% of patients and was associated with improved 3-year overall survival rates (82%) compared with HPV negative OPSCC (57%); after adjusting for patient characteristics, stage, tobacco use, and treatment group, HPV-OPSCC patients had a 58% reduction in the risk of death. The risk of death increased by 1% with each additional pack-year of tobacco smoking, regardless of HPV status. This study found that HPV status was the greatest determinant of overall survival, followed by tobacco use (≤10 pack-years vs. >10 pack-years), primary tumor stage (T2/T3 vs. T4), and nodal stage (N0/N2a). In 2007, a meta-analysis comparing the overall risk of death and recurrence reduction in all patients with OPSCC based on smoking and HPV status demonstrated a 28% reduction in risk of death and a 49% reduction in risk of recurrence in HPV positive compared to HPV negative OPSCC patients.[168] This reflects that the current AJCC TNM (7^{th} edition) is unsuitable and we need to establish

a new TNM staging system for HPV positive OPSCC that is different than the HPV negative staging system.[162,169] The establishment of a new HPV specific TNM will help with future surveillance, treatment planning, counseling, and clinical trials. Two recent papers from the University of Toronto and University of Pittsburgh were published proposing a new TNM staging system for HPV positive OPSCC.[86,170] In October 2016 the 8th edition AJCC was released which included significant modification of the TNM and overall staging of OPSCC, and this to be effective from January 2017. [171,172] However, these changes provides a much more accurate assumption of survival outcome and prognosis but it still doesn't affect the available treatment guidelines which are still based on the 7th edition AJCC.

1.8 Role of primary surgical treatment in OPSCC

Similar to CRT, surgery addresses both the primary site and the neck nodal disease. The surgical treatment of the neck is in the form of neck dissection. Up until the middle of the 20th century, the main neck dissection technique was in the form of a radical neck dissection, which carries a significant postoperative morbidity and life-threatening complications.[173,174] In the early 1960s, the concept of functional neck and selective neck dissections was introduced, which helped to achieve local control rates comparable to the radical neck dissection with less postoperative morbidity and better functional results.[173,174] As for the primary site, this was in the form of open surgical approach, lip-splitting and/or mandible-splitting techniques, along with free-flap reconstruction. During the past 20 years these types of procedures carried a significant morbidity in the form of functional disabilities.[175,176] However, surgical and

reconstructive technology has dramatically advanced and improved functional outcomes. The reconstruction of oropharyngeal defect follows the standard stepwise approach depending on the size of the defect to achieve two main goals: watertight closure and functional preservation. Reconstruction can be in the form of primary closure, local flaps, and free flaps. With larger defects, free flaps has the ability to be designed in a three dimensional fashion which would help preserve the anatomical configuration and function of the different structures of the oropharynx (soft palate, tongue base, pharyngeal walls).[30] In recent publications, it has been shown patients undergoing primary surgical treatment despite presenting with advanced stage OPSCC have comparable and in some occasions better functional outcome than those undergoing primary CRT.[11,96,177,178]

Over the last decade, the advances in surgical approaches also included the introduction of transoral surgery avoiding the lip-splitting and/or mandible-splitting techniques. Transoral surgery includes two main techniques, transoral robotic surgery (TORS) and transoral laser microsurgery (TLM). It has gained popularity as a means of carrying out effective oncologic resection with much less morbidity especially in the field of OPSCC.[11,81,156,178-180] Despite using a minimally invasive approach the oncological, survival, and functional outcomes are promising as do the functional outcome.[178,180-182]

1.9 Role of postoperative adjuvant therapy in OPSCC

The principles and indications for postoperative radiotherapy and chemoradiotherapy in OPSCC are no different from other HNSCCs. Patients with OPSCC and positive nodal involvement (N+) and/or T-stage > 2 are treated with

postoperative adjuvant RT.[127,183] The RT dose to the neck and primary site in the postoperative setting can range between 60-66 Gy to the primary site and involved neck levels, and 54-60 Gy to the uninvolved at risk neck level.[127] Based on the 2013 NCCN guidelines, the main indications for the addition of chemotherapy to postoperative adjuvant RT are positive lymph node involvement with extracapsular extension and/or positive surgical resection margins.[127,184] There was a trend in favor of CRT providing a better overall survival over RT in patients with positive perineural invasion, lymphovascular invasion, and/or any stage III/IV, however it did not reach statistical significance.[184]

Recommendations by the NCCN are largely based on level one evidence showing survival benefit with the addition of RT or CRT.[183-185] However none of these studies or trials stratified patients according to their HPV and smoking status.[12] Despite the added risk and higher rates of systemic and locoregional side effects from the addition of chemotherapy in the postoperative setting, its benefit is still unclear in the literature.[12] This evidence reemphasis the importance of revising the 7th edition AJCC staging system for OPSCC according to their HPV and smoking status which will reflect on the change of their management, especially on the indications for adjuvant RT or CRT in the primary surgical cohort.[12,86,170]

1.10 De-escalation treatment strategies for HPV related OPSCC

Although arising from the same anatomic location (oropharynx), and same histological type of cancer (squamous cell carcinoma), HPV positive and HPV negative OPSCC are two different diseases with regards to their pathological characteristics, etiology,[72] clinical behavior,[186] patient's demographics,[36,41,42,81,82]

clinical presentation,[14,42,86,87] treatment response and outcome.[36,54,84,162,163] Because HPV-associated OPSCC has a much better prognosis than HPV-negative OPSCC, de-escalation of treatment has been suggested for this population to minimize acute treatment toxicity and long-term treatment-related morbidity.[14] De-escalation of treatment can be applied to both treatment modalities, primary surgery or primary RT/CRT.

In the primary RT/CRT arm, a meta-analysis of three RTOG chemoradiation trials demonstrated severe late toxicity in 43% of patients, mainly in the form of difficulty swallowing.[147] Because HPV-associated HNSCC patients are often younger, healthier, and can be expected to survive longer, they are more likely to experience significant long-term treatment-related morbidity and reduced quality of life.[187] Late and severe swallowing complications are adversely affected by increased radiation dose, the volume of pharynx radiated, and the use of concurrent chemotherapy.[147,188] O'Sullivan and colleagues reported a retrospective institutional review of 505 patients with OPSCC treated with radiation or chemoradiation based on stage.[14] Their results suggested that modifying or eliminating chemotherapy might be an appropriate de-escalation strategy in HPV-positive patients. There are ongoing clinical trials by RTOG and Eastern Cooperative Oncology Group (ECOG) with the aim to achieve similar efficacy with less toxicity and improved quality of life.[100]

In the primary surgery arm, the use of TORS and TLM coincides with the rise of HPV-OPSCC. These approaches reduce the morbidity of primary surgery and renewed the interest in primary surgery to allow de-escalation of therapy. The dose and the indications for RT and chemotherapy in the HPV positive OPSCC cohort are also

under the evaluation to undergo similar de-escalation as in the primary RT/CRT treatment arm.[86,189] The National Cancer Institute Head and Neck Cancer Steering Committee proposed two main trials to further investigate transoral surgery for OPSCC, both as a method to reduce treatment toxicity in HPV positive OPSCC and as a method of treatment intensification in the poor prognosis HPV negative OPSCC.[189] The Eastern Cooperative Oncology Group (ECOG) opened the E3311 trial, a phase II randomized trial of transoral surgical resection (TORS or TLM) followed by low-dose or standard-dose IMRT for HPV positive locally advanced OPSCC.[189] This study will investigate whether transoral surgery allows radiation dose de-escalation; in addition, risk stratification based on stage and smoking status.[189] The Radiation Therapy Oncology Group (RTOG) opened the RTOG1221 trial, a phase II randomized trial for advanced stage HPV negative OPSCC.[189] Its primary objective is to determine if surgical intensification for patients with HPV negative OPSCC will improve progression-free survival. Histologic information will be performed to direct adjuvant therapy postoperatively. These trials will provide evidence-based data for the role of surgery in treatment de-escalation or intensification based on HPV status.

1.11 Summary of the literature review

There is no clear evidence supporting the benefit of adjuvant chemotherapy in the primary surgical setting for OPSCC. The incidence and prevalence of HPV related OPSCC is significantly increasing with presumptive rates exceeding HPV related cervical cancer by 2020. The demographics, treatment response, and prognosis are significantly different the HPV negative OPSCC. The 7th edition TNM staging system and treatment protocols for OPSCC are unsuitable for HPV related OPSCC. The newly released 8th

edition AJCC staging system reflects a much more accurate prediction of prognosis and survival outcome for patient with HPV related OPSCC versus those who are not, however, it still doesn't reflect the change in the treatment protocols.[172] Current clinical trials and de-escalation protocols may help clarify the indications for adjuvant chemotherapy in the management of OPSCC.

Figure 1.1 Study Objectives

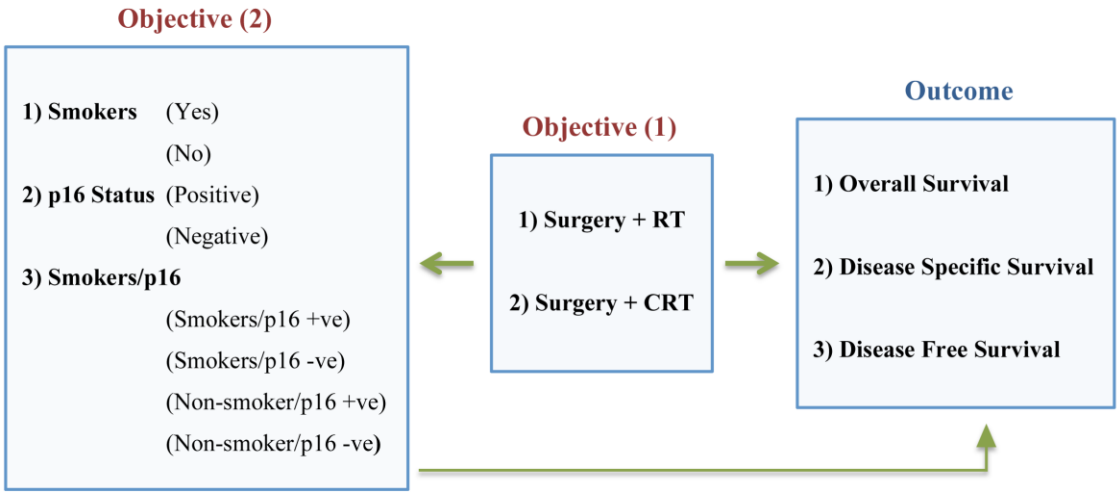


Figure 1.2 Anatomy of the pharynx

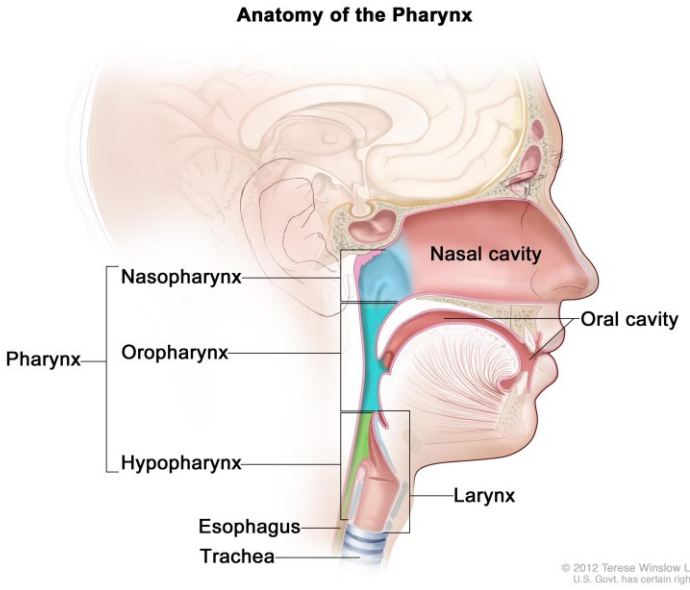


Figure 1.3 Anatomy of the oropharynx

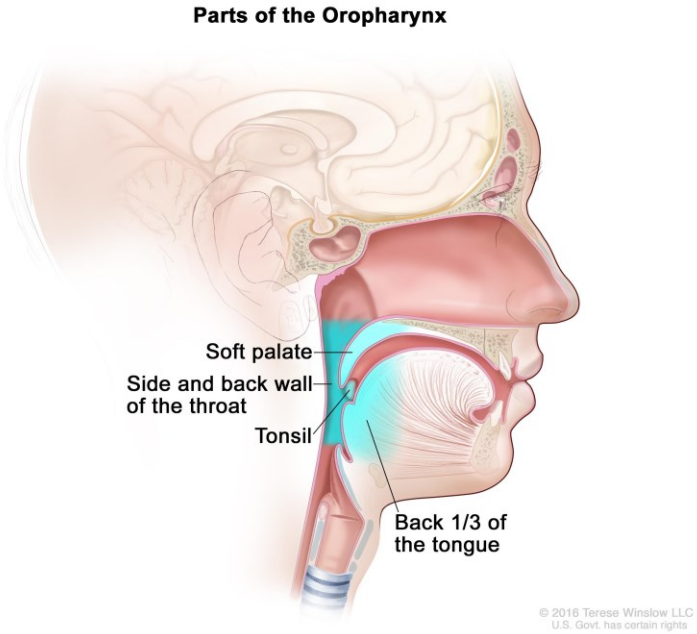
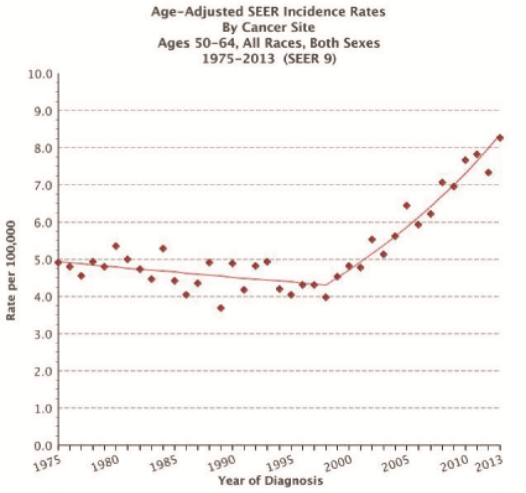
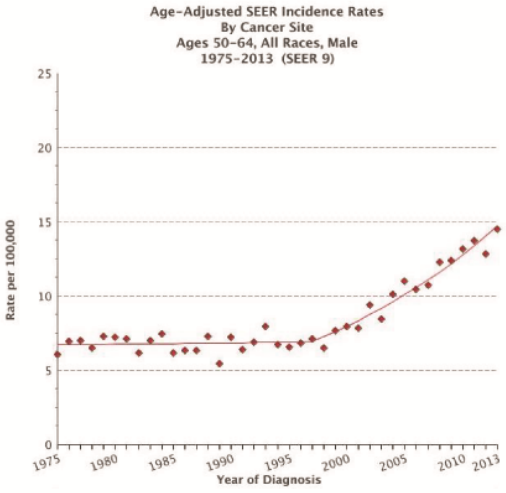
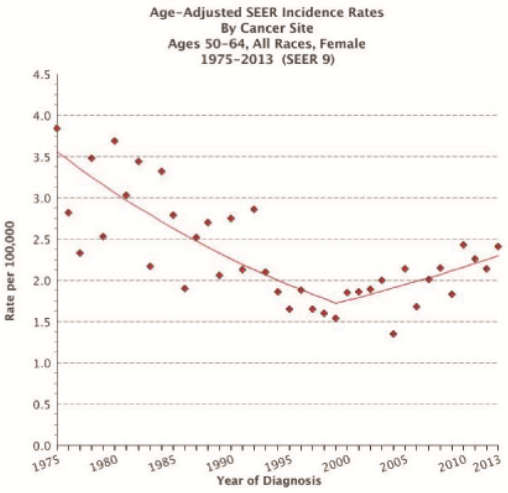
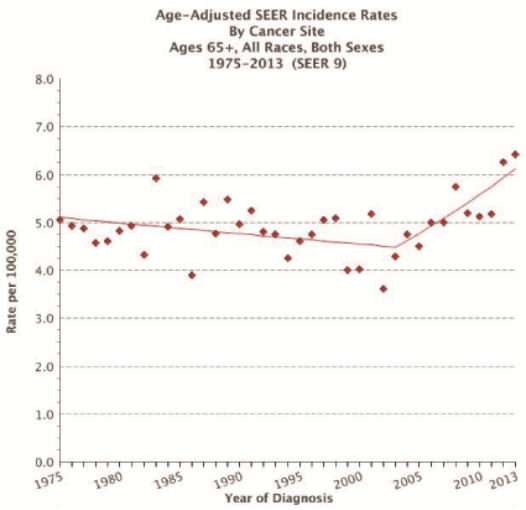


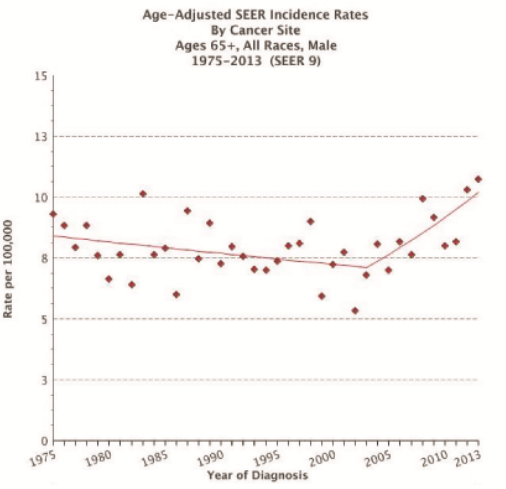
Figure 1.4 Oropharynx age-adjusted SEER incidence rates from 1975-2013

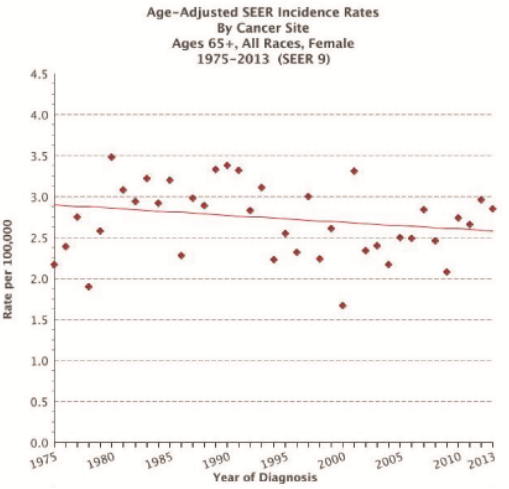


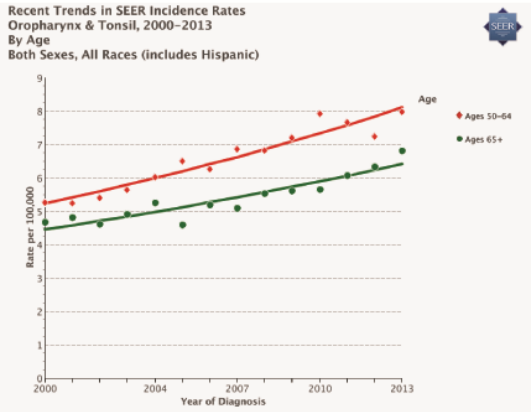


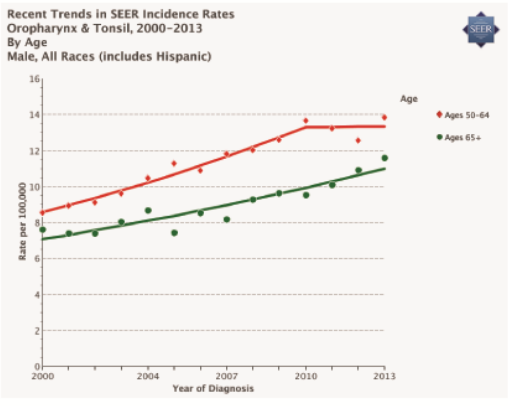


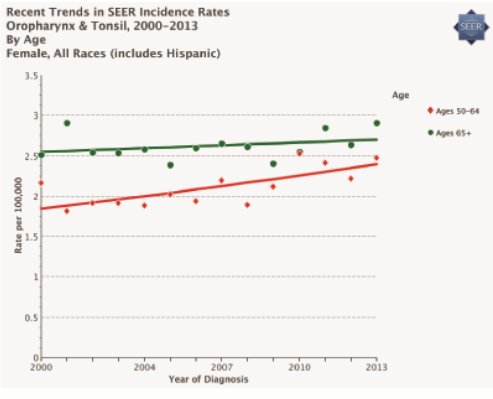












References

1. Gandini S, Botteri E, Iodice S, et al. Tobacco smoking and cancer: a meta-analysis. *Int J Cancer*. 2008;122(1):155-164. doi:10.1002/ijc.23033.

2. Hashibe M, Brennan P, Benhamou S, et al. Alcohol drinking in never users of tobacco, cigarette smoking in never drinkers, and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. *J Natl Cancer Inst*. 2007;99(10):777-789. doi:10.1093/jnci/djk179.

3. Blot WJ, McLaughlin JK, Winn DM, et al. Smoking and drinking in relation to oral and pharyngeal cancer. *Cancer Research*. 1988;48(11):3282-3287.

4. Hashibe M, Brennan P, Chuang S-C, et al. Interaction between tobacco and alcohol use and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. *Cancer Epidemiology Biomarkers & Prevention*. 2009;18(2):541-550. doi:10.1158/1055-9965.EPI-08-0347.

5. Chaturvedi AK, Engels EA, Pfeiffer RM, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *J Clin Oncol*. 2011;29(32):4294-4301. doi:10.1200/JCO.2011.36.4596.

6. Gillison ML, Broutian T, Pickard RKL, et al. Prevalence of Oral HPV Infection in the United States, 2009-2010. *JAMA*. 2012;307(7):693–11. doi:10.1001/jama.2012.101.

7. Chaturvedi AK. Epidemiology and Clinical Aspects of HPV in Head and Neck Cancers. *Head and Neck Pathol*. 2012;6(S1):16-24. doi:10.1007/s12105-012-0377-0.

8. Canadian Cancer Statistics. October 2016:1-142.

9. Carvalho AL, Magrin J, Kowalski LP. Sites of recurrence in oral and oropharyngeal cancers according to the treatment approach. *Oral Diseases*. 2003;9(3):112-118. doi:10.1034/j.1601-0825.2003.01750.x.

10. Seikaly H, Rieger J, Wolfaardt J, Moysa G, Harris J, Jha N. Functional outcomes after primary oropharyngeal cancer resection and reconstruction with the radial forearm free flap. *Laryngoscope*. 2003;113(5):897-904. doi:10.1097/00005537-200305000-00023.

11. Haughey BH, Sinha P. Prognostic factors and survival unique to surgically treated p16+ oropharyngeal cancer. *Laryngoscope*. 2012;122(S2):S13-S33. doi:10.1002/lary.23493.

12. Sinha P, Piccirillo JF, Kallogjeri D, Spitznagel EL, Haughey BH. The role of

postoperative chemoradiation for oropharynx carcinoma: A critical appraisal of the published literature and National Comprehensive Cancer Network guidelines. *Cancer*. 2015;121(11):1747-1754. doi:10.1002/cncr.29242.

13. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Human papillomaviruses. *IARC monographs on the evaluation of carcinogenic risks to humans / World Health Organization, International Agency for Research on Cancer*. 2007;90:1-636.

14. O'Sullivan B, Huang SH, Siu LL, et al. Deintensification candidate subgroups in human papillomavirus-related oropharyngeal cancer according to minimal risk of distant metastasis. *J Clin Oncol*. 2013;31(5):543-550. doi:10.1200/JCO.2012.44.0164.

15. Sinha P, Piccirillo JF, Kallogjeri D, Spitznagel EL, Haughey BH. The role of postoperative chemoradiation for oropharynx carcinoma: A critical appraisal of the published literature and National Comprehensive Cancer Network guidelines. *Cancer*. 2015;121(11):1747-1754. doi:10.1002/cncr.29242.

16. Duvvuri U, Myers JN. Cancer of the head and neck is the sixth most common cancer worldwide. *Curr Probl Surg*. 2009;46(2):114-117. doi:10.1067/j.cpsurg.2008.10.002.

17. Argiris A, Karamouzis MV, Raben D, Ferris RL. Head and neck cancer. *Lancet*. 2008;371(9625):1695-1709. doi:10.1016/S0140-6736(08)60728-X.

18. Brockstein B. Management of sarcomas of the head and neck. *Curr Oncol Rep*. 2004;6(4):321-327.

19. Speight PM, Barrett AW. Salivary gland tumours. *Oral Diseases*. 2002;8(5):229-240.

20. Shah JP, Patel SG, Singh B. *Jatin Shah's Head and Neck Surgery and Oncology*. Elsevier Health Sciences; 2012.

21. Marur S, Forastiere AA. Head and Neck Squamous Cell Carcinoma: Update on Epidemiology, Diagnosis, and Treatment. *Mayo Clinic Proceedings*. 2016;91(3):386-396. doi:10.1016/j.mayocp.2015.12.017.

22. Forte T, Niu J, Lockwood GA, Bryant HE. Incidence trends in head and neck cancers and human papillomavirus (HPV)-associated oropharyngeal cancer in Canada, 1992–2009. *Cancer Causes Control*. 2012;23(8):1343-1348. doi:10.1007/s10552-012-0013-z.

23. Luryi AL, Yarbrough WG, Niccolai LM, et al. Public Awareness of Head and Neck Cancers. *JAMA Otolaryngol Head Neck Surg*. 2014;140(7):639–8. doi:10.1001/jamaoto.2014.867.

24. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. *CA: A Cancer Journal for Clinicians*. 2013;63(1):11-30. doi:10.3322/caac.21166.

25. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA: A Cancer Journal for Clinicians*. 2015;65(1):5-29. doi:10.3322/caac.21254.

26. Blot WJ. Alcohol and cancer. *Cancer Research*. 1992;52(7 Suppl):2119s–2123s.

27. Gillison ML, Chaturvedi AK, Anderson WF, Fakhry C. Epidemiology of Human Papillomavirus-Positive Head and Neck Squamous Cell Carcinoma. *J Clin Oncol*. 2015;33(29):3235-3242. doi:10.1200/JCO.2015.61.6995.

28. Chi AC, Day TA, Neville BW. Oral cavity and oropharyngeal squamous cell carcinoma—an update. *CA: A Cancer Journal for Clinicians*. 2015;65(5):401-421. doi:10.3322/caac.21293.

29. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol*. 2010;17(6):1471-1474. doi:10.1245/s10434-010-0985-4.

30. Watkinson J, Gilbert RW. *Stell & Maran's Textbook of Head and Neck Surgery and Oncology, Fifth Edition*. CRC Press; 2011.

31. Canadian Cancer Statistics. May 2015:1-151.

32. Curado MP, Hashibe M. Recent changes in the epidemiology of head and neck cancer. *Curr Opin Oncol*. 2009;21(3):194-200. doi:10.1097/CCO.0b013e32832a68ca.

33. Propel. Tobacco Use in Canada: Patterns and Trends. April 2015:1-96.

34. Romanitan M, Näsman A, Ramqvist T, et al. Human papillomavirus frequency in oral and oropharyngeal cancer in Greece. *Anticancer Res*. 2008;28(4B):2077-2080.

35. Chaturvedi AK, Anderson WF, Lortet-Tieulent J, et al. Worldwide Trends in Incidence Rates for Oral Cavity and Oropharyngeal Cancers. *Journal of Clinical Oncology*. 2013;31(36):4550-4559. doi:10.1200/JCO.2013.50.3870.

36. Ang KK, Harris J, Wheeler R, et al. Human Papillomavirus and Survival of Patients with Oropharyngeal Cancer. *N Engl J Med*. 2010;363(1):24-35. doi:10.1056/NEJMoa0912217.

37. Mehanna H, Beech T, Nicholson T, et al. Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer--systematic review and meta-analysis of trends by time and region. Eisele DW, ed. *Head Neck*. 2013;35(5):747-755. doi:10.1002/hed.22015.

38. D'Souza G, Cullen K, Bowie J, Thorpe R, Fakhry C. Differences in oral sexual behaviors by gender, age, and race explain observed differences in prevalence of oral human papillomavirus infection. Liu X, ed. *PLoS ONE*. 2014;9(1):e86023. doi:10.1371/journal.pone.0086023.

39. Jemal A, Simard EP, Dorell C, et al. Annual Report to the Nation on the Status of Cancer, 1975-2009, featuring the burden and trends in human papillomavirus(HPV)-associated cancers and HPV vaccination coverage levels. *J Natl Cancer Inst*. 2013;105(3):175-201. doi:10.1093/jnci/djs491.

40. Heath S, Willis V, Allan K, et al. Clinically significant human papilloma virus in squamous cell carcinoma of the head and neck in UK practice. *Clin Oncol (R Coll Radiol)*. 2012;24(1):e18-e23. doi:10.1016/j.clon.2011.05.007.

41. Begum S, Cao D, Gillison M, Zahurak M, Westra WH. Tissue distribution of human papillomavirus 16 DNA integration in patients with tonsillar carcinoma. *Clin Cancer Res*. 2005;11(16):5694-5699. doi:10.1158/1078-0432.CCR-05-0587.

42. Gillison ML, D'Souza G, Westra W, et al. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. *J Natl Cancer Inst*. 2008;100(6):407-420. doi:10.1093/jnci/djn025.

43. Mackay J, Eriksen MP, World Health Organization. *The Tobacco Atlas*. World Health Organization; 2002.

44. Alavanja M, Baron J, Brownson RC, et al. Tobacco smoke and involuntary smoking. *IARC monographs on the evaluation of carcinogenic risks to humans / World Health Organization, International Agency for Research on Cancer*. 2004;83.

45. Chaturvedi AK, D'Souza G, Gillison ML, Katki HA. Burden of HPV-positive oropharynx cancers among ever and never smokers in the U.S. population. *Oral Oncology*. 2016;60:61-67. doi:10.1016/j.oraloncology.2016.06.006.

46. Smith CJ, Perfetti TA, Rumple MA, Rodgman A, Doolittle DJ. "IARC group 2A Carcinogens" reported in cigarette mainstream smoke. *Food Chem Toxicol*. 2000;38(4):371-383.

47. Smith CJ, Perfetti TA, Rumple MA, Rodgman A, Doolittle DJ. "IARC Group 2B carcinogens" reported in cigarette mainstream smoke. *Food Chem Toxicol*. 2001;39(2):183-205.

48. HA K. The Dorn study of smoking and mortality among U.S. veterans: report on eight and one-half years of observation. 1966.

49. Muñoz N, Correa P, Bock FG. Comparative carcinogenic effect of two types

of tobacco. *Cancer*. 1968;21(3):376-389. doi:10.1002/1097-0142(196803)21:3<376::AID-CNCR2820210307>3.0.CO;2-J.

50. De Stefani E, Boffetta P, Oreggia F, Mendilaharsu M, Deneo-Pellegrini H. Smoking patterns and cancer of the oral cavity and pharynx: a case–control study in Uruguay. *Oral Oncology*. 1998;34(5):340-346. doi:10.1016/S1368-8375(98)00014-1.

51. Macfarlane GJ, Zheng T, Marshall JR, et al. Alcohol, tobacco, diet and the risk of oral cancer: a pooled analysis of three case-control studies. *Eur J Cancer, B, Oral Oncol*. 1995;31B(3):181-187.

52. Duffy SA, Ronis DL, McLean S, et al. Pretreatment health behaviors predict survival among patients with head and neck squamous cell carcinoma. *J Clin Oncol*. 2009;27(12):1969-1975. doi:10.1200/JCO.2008.18.2188.

53. Chung CH, Gillison ML. Human Papillomavirus in Head and Neck Cancer: Its Role in Pathogenesis and Clinical Implications. *Clin Cancer Res*. 2009;15(22):6758-6762. doi:10.1158/1078-0432.CCR-09-0784.

54. Huang SH, Xu W, Waldron J, et al. Refining American Joint Committee on Cancer/Union for International Cancer Control TNM stage and prognostic groups for human papillomavirus-related oropharyngeal carcinomas. *J Clin Oncol*. 2015;33(8):836-845. doi:10.1200/JCO.2014.58.6412.

55. Zhang ZF, Morgenstern H, Spitz MR, et al. Marijuana use and increased risk of squamous cell carcinoma of the head and neck. *Cancer Epidemiology Biomarkers & Prevention*. 1999;8(12):1071-1078.

56. Nahas G, Latour C. The human toxicity of marijuana. *Med J Aust*. 1992;156(7):495-497.

57. Watson M, Lyu C, Unger ER, Copeland G, Peters E. *Centers for Disease Control and Prevention Human Papillomavirus Typing of Cancers Study with 7 Registries: Evaluating Representativeness*. North American Association …; 2011.

58. Snijders PJ, Scholes AG, Hart CA, et al. Prevalence of mucosotropic human papillomaviruses in squamous-cell carcinoma of the head and neck. *Int J Cancer*. 1996;66(4):464-469. doi:10.1002/(SICI)1097-0215(19960516)66:4<464::AID-IJC9>3.0.CO;2-U.

59. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiology Biomarkers & Prevention*. 2005;14(2):467-475. doi:10.1158/1055-9965.EPI-04-0551.

60. Androphy EJ. Molecular biology of human papillomavirus infection and

oncogenesis. *J Invest Dermatol*. 1994;103(2):248-256.

61. Bishop JA, Lewis JS, Rocco JW, Faquin WC. HPV-related squamous cell carcinoma of the head and neck_ An update on testing in routine pathology practice. *Seminars in Diagnostic Pathology*. 2015;32(5):344-351. doi:10.1053/j.semdp.2015.02.013.

62. Centers for Disease Control and Prevention. *How Many Cancers Are Linked with HPV Each Year*. Human papillomavirus (HPV)— …; 2014.

63. D'Souza G, Sugar E, Ruby W, Gravitt P, Gillison M. Analysis of the effect of DNA purification on detection of human papillomavirus in oral rinse samples by PCR. *J Clin Microbiol*. 2005;43(11):5526-5535. doi:10.1128/JCM.43.11.5526-5535.2005.

64. Kreimer AR, Bhatia RK, Messeguer AL, González P, Herrero R, Giuliano AR. Oral human papillomavirus in healthy individuals: a systematic review of the literature. *Sex Transm Dis*. 2010;37(6):386-391. doi:10.1097/OLQ.0b013e3181c94a3b.

65. Kreimer AR, Alberg AJ, Daniel R, et al. Oral human papillomavirus infection in adults is associated with sexual behavior and HIV serostatus. *J Infect Dis*. 2004;189(4):686-698. doi:10.1086/381504.

66. Kreimer AR, Villa A, Nyitray AG, et al. The epidemiology of oral HPV infection among a multinational sample of healthy men. *Cancer Epidemiol Biomarkers Prev*. 2011;20(1):172-182. doi:10.1158/1055-9965.EPI-10-0682.

67. Giuliano AR, Nyitray AG, Kreimer AR, et al. EUROGIN 2014 roadmap: differences in human papillomavirus infection natural history, transmission and human papillomavirus-related cancer incidence by gender and anatomic site of infection. *Int J Cancer*. 2015;136(12):2752-2760. doi:10.1002/ijc.29082.

68. Beachler DC, D'Souza G, Sugar EA, Xiao W, Gillison ML. Natural history of anal vs oral HPV infection in HIV-infected men and women. *J Infect Dis*. 2013;208(2):330-339. doi:10.1093/infdis/jit170.

69. Kreimer AR, Pierce Campbell CM, Lin H-Y, et al. Incidence and clearance of oral human papillomavirus infection in men: the HIM cohort study. *Lancet*. 2013;382(9895):877-887. doi:10.1016/S0140-6736(13)60809-0.

70. D'Souza G, Fakhry C, Sugar EA, et al. Six-month natural history of oral versus cervical human papillomavirus infection. *Int J Cancer*. 2007;121(1):143-150. doi:10.1002/ijc.22667.

71. Syrjänen K, Syrjänen S, Lamberg M, Pyrhönen S, Nuutinen J. Morphological and immunohistochemical evidence suggesting human papillomavirus (HPV)

involvement in oral squamous cell carcinogenesis. *International Journal of Oral Surgery*. 1983;12(6):418-424. doi:10.1016/S0300-9785(83)80033-7.

72. Gillison ML, Koch WM, Capone RB, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst*. 2000;92(9):709-720. doi:10.1093/jnci/92.9.709.

73. Smith EM, Hoffman HT, Summersgill KS, Kirchner HL, Turek LP, Haugen TH. Human papillomavirus and risk of oral cancer. *Laryngoscope*. 1998;108(7):1098-1103.

74. Gillison ML. Human papillomavirus-associated head and neck cancer is a distinct epidemiologic, clinical, and molecular entity. *Semin Oncol*. 2004;31(6):744-754.

75. D'Souza G, Kreimer AR, Viscidi R, et al. Case-control study of human papillomavirus and oropharyngeal cancer. *N Engl J Med*. 2007;356(19):1944-1956. doi:10.1056/NEJMoa065497.

76. Lingen MW, Xiao W, Schmitt A, Jiang B, Pickard R. Low etiologic fraction for high-risk human papillomavirus in oral cavity squamous cell carcinomas. *Oral Oncology*. 2013.

77. Isayeva T, Li Y, Maswahu D, Brandwein-Gensler M. Human Papillomavirus in Non-Oropharyngeal Head and Neck Cancers: A Systematic Literature Review. *Head and Neck Pathol*. 2012;6(1):104-120. doi:10.1007/s12105-012-0368-1.

78. Castellsague X, Alemany L, Quer M, et al. HPV Involvement in Head and Neck Cancers: Comprehensive Assessment of Biomarkers in 3680 Patients. *J Natl Cancer Inst*. 2016;108(6):djv403. doi:10.1093/jnci/djv403.

79. Stein AP, Saha S, Yu M, Kimple RJ, Lambert PF. Prevalence of Human Papillomavirus in Oropharyngeal Squamous Cell Carcinoma in the United States Across Time. *Chem Res Toxicol*. 2014;27(4):462-469. doi:10.1021/tx500034c.

80. Chaturvedi AK, Engels EA, Anderson WF, Gillison ML. Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. *J Clin Oncol*. 2008;26(4):612-619. doi:10.1200/JCO.2007.14.1713.

81. Deschler DG, Richmon JD, Khariwala SS, Ferris RL, Wang MB. The "New" Head and Neck Cancer Patient--Young, Nonsmoker, Nondrinker, and HPV Positive: Evaluation. *Otolaryngology -- Head and Neck Surgery*. 2014;151(3):375-380. doi:10.1177/0194599814538605.

82. Schwartz SR, Yueh B, McDougall JK, Daling JR, Schwartz SM. Human

Papillomavirus Infection and Survival in Oral Squamous Cell Cancer: A Population-Based Study. *Otolaryngology -- Head and Neck Surgery*. 2001;125(1):1-9. doi:10.1067/mhn.2001.116979.

83. Gillison ML. *HPV and Prognosis for Patients with Oropharynx Cancer*. European Journal of Cancer; 2009.

84. Fakhry C, Westra WH, Li S, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst*. 2008;100(4):261-269. doi:10.1093/jnci/djn011.

85. Settle K, Posner MR, Schumaker LM, et al. Racial survival disparity in head and neck cancer results from low prevalence of human papillomavirus infection in black oropharyngeal cancer patients. *Cancer Prev Res (Phila)*. 2009;2(9):776-781. doi:10.1158/1940-6207.CAPR-09-0149.

86. MD PBO, MD SHH, MSc JS, et al. Development and validation of a staging system for HPV-related oropharyngeal cancer by the International Collaboration on Oropharyngeal cancer Network for Staging (ICON-S): a multicentre cohort study. *Lancet Oncology*. 2016;17(4):440-451. doi:10.1016/S1470-2045(15)00560-4.

87. Vent J, Haidle B, Wedemeyer I, et al. p16 Expression in carcinoma of unknown primary: Diagnostic indicator and prognostic marker. *Head Neck*. 2013;35(11):1521-1526. doi:10.1002/hed.23190.

88. Jereczek-Fossa BA, Jassem J, Orecchia R. Cervical lymph node metastases of squamous cell carcinoma from an unknown primary. *Cancer Treatment Reviews*. 2004;30(2):153-164. doi:10.1016/j.ctrv.2003.10.001.

89. Mendenhall WM, Mancuso AA, Parsons JT. Diagnostic evaluation of squamous cell carcinoma metastatic to cervical lymph nodes from an unknown head and neck primary site. *Head Neck.* 1998.

90. Cianchetti M, Mancuso AA, Amdur RJ, et al. Diagnostic evaluation of squamous cell carcinoma metastatic to cervical lymph nodes from an unknown head and neck primary site. *Laryngoscope*. 2009;119(12):2348-2354. doi:10.1002/lary.20638.

91. Iganej S, Kagan R, Anderson P, et al. Metastatic squamous cell carcinoma of the neck from an unknown primary: Management options and patterns of relapse. *Head Neck*. 2002;24(3):236-246. doi:10.1002/hed.10017.

92. Kingma DW, Allen RA, Moore W, et al. HPV genotype distribution in oral and oropharyngeal squamous cell carcinoma using seven in vitro amplification assays. *Anticancer Res*. 2010;30(12):5099-5104.

93. Goodman MT, Saraiya M, Thompson TD, et al. Human papillomavirus genotype and oropharynx cancer survival in the United States of America. *European Journal of Cancer*. 2015;51(18):2759-2767. doi:10.1016/j.ejca.2015.09.005.

94. Lewis A, Kang R, Levine A, Maghami E. The New Face of Head and Neck Cancer: The HPV Epidemic. *Oncology (Williston Park, NY)*. 2015;29(9):616-626.

95. Biron VL, Mohamed A, Hendzel MJ, Alan Underhill D, Seikaly H. Epigenetic differences between human papillomavirus-positive and -negative oropharyngeal squamous cell carcinomas. 2012;41 Suppl 1:S65-S70.

96. Seikaly H, Biron VL, Zhang H, et al. Role of primary surgery in the treatment of advanced oropharyngeal cancer. *Head Neck*. July 2015:n/a–n/a. doi:10.1002/hed.24042.

97. Xu CC, Biron VL, Puttagunta L, Seikaly H. HPV Status and second primary tumours in Oropharyngeal Squamous Cell Carcinoma. 2013;42(1):1. doi:10.1186/1916-0216-42-36.

98. Begum S, Gillison ML, Ansari-Lari MA, Shah K, Westra WH. Detection of Human Papillomavirus in Cervical Lymph Nodes. *Clin Cancer Res*. 2003;9(17):6469-6475. doi:10.3322/canjclin.51.1.15.

99. Bishop JA, Ogawa T, Chang X, et al. HPV analysis in distinguishing second primary tumors from lung metastases in patients with head and neck squamous cell carcinoma. *The American Journal of Surgical Pathology*. 2012;36(1):142-148. doi:10.1097/PAS.0b013e3182395c7b.

100. Mirghani H, Amen F, Blanchard P, et al. Treatment de-escalation in HPV-positive oropharyngeal carcinoma: ongoing trials, critical issues and perspectives. *Int J Cancer*. 2015;136(7):1494-1503. doi:10.1002/ijc.28847.

101. Bonilla-Velez J, Mroz EA, Hammon RJ, Rocco JW. Impact of human papillomavirus on oropharyngeal cancer biology and response to therapy: implications for treatment. *Otolaryngol Clin North Am*. 2013;46(4):521-543. doi:10.1016/j.otc.2013.04.009.

102. Moody CA, Laimins LA. Human papillomavirus oncoproteins: pathways to transformation. *Nature Reviews Cancer*. 2010;10(8):550-560. doi:10.1038/nrc2886.

103. Hayes DN, Van Waes C, Seiwert TY. Genetic landscape of human papillomavirus–associated head and neck cancer and comparison to tobacco-related tumors. *Journal of Clinical Oncology*. 2015.

104. Nishat R, Behura SS, Ramachandra S, Kumar H, Bandyopadhyay A. Human

Papilloma Virus (HPV) Induced Head & Neck Squamous Cell Carcinoma: A Comprehensive Retrospect. *Journal of Clinical and Diagnostic Research : JCDR*. 2015;9(6):ZE01-ZE04. doi:10.7860/JCDR/2015/13948.6056.

105. Walline HM, Komarck C, McHugh JB, et al. High-Risk Human Papillomavirus Detection in Oropharyngeal, Nasopharyngeal, and Oral Cavity Cancers: Comparison of Multiple Methods. *JAMA Otolaryngol Head Neck Surg*. 2013;139(12):1320-1327. doi:10.1001/jamaoto.2013.5460.

106. Kreimer AR, Johansson M, Waterboer T, et al. Evaluation of human papillomavirus antibodies and risk of subsequent head and neck cancer. *J Clin Oncol*. 2013;31(21):2708-2715. doi:10.1200/JCO.2012.47.2738.

107. Flint PW, Haughey BH, Robbins KT, et al. *Cummings Otolaryngology - Head and Neck Surgery*. Elsevier Health Sciences; 2014.

108. Smeets SJ, Hesselink AT, Speel E-JM, et al. A novel algorithm for reliable detection of human papillomavirus in paraffin embedded head and neck cancer specimen. *Int J Cancer*. 2007;121(11):2465-2472. doi:10.1002/ijc.22980.

109. Weinberger PM, Yu Z, Haffty BG, et al. Molecular classification identifies a subset of human papillomavirus--associated oropharyngeal cancers with favorable prognosis. *J Clin Oncol*. 2006;24(5):736-747. doi:10.1200/JCO.2004.00.3335.

110. Leemans CR, Braakhuis BJM, Brakenhoff RH. The molecular biology of head and neck cancer. *Nature Reviews Cancer*. 2011;11(1):9-22. doi:10.1038/nrc2982.

111. van Houten VM, Snijders PJ, van den Brekel MW, et al. Biological evidence that human papillomaviruses are etiologically involved in a subgroup of head and neck squamous cell carcinomas. *Int J Cancer*. 2001;93(2):232-235. doi:10.1002/ijc.1313.

112. Bishop JA, Ma X-J, Wang H, et al. Detection of transcriptionally active high-risk HPV in patients with head and neck squamous cell carcinoma as visualized by a novel E6/E7 mRNA in situ hybridization method. *The American Journal of Surgical Pathology*. 2012;36(12):1874-1882. doi:10.1097/PAS.0b013e318265fb2b.

113. Ukpo OC, Flanagan JJ, Ma X-J, Luo Y, Thorstad WL, Lewis JS. High-risk human papillomavirus E6/E7 mRNA detection by a novel in situ hybridization assay strongly correlates with p16 expression and patient outcomes in oropharyngeal squamous cell carcinoma. *The American Journal of Surgical Pathology*. 2011;35(9):1343-1350. doi:10.1097/PAS.0b013e318220e59d.

114. Schache AG, Liloglou T, Risk JM, et al. Validation of a novel diagnostic standard in HPV-positive oropharyngeal squamous cell carcinoma. *British Journal of Cancer*. 2013;108(6):1332-1339. doi:10.1038/bjc.2013.63.

115. Lewis JS Jr. p16 Immunohistochemistry As a Standalone Test for Risk Stratification in Oropharyngeal Squamous Cell Carcinoma. *Head and Neck Pathol*. 2012;6(1):75-82. doi:10.1007/s12105-012-0369-0.

116. Lewis JS, Jr, Thorstad WL, et al. p16 Positive Oropharyngeal Squamous Cell Carcinoma: An Entity With a Favorable Prognosis Regardless of Tumor HPV Status. *The American Journal of Surgical Pathology*. 2010;34(8):1088-1096. doi:10.1097/PAS.0b013e3181e84652.

117. Naggar El AK, Westra WH. p16 expression as a surrogate marker for HPV-related oropharyngeal carcinoma: A guide for interpretative relevance and consistency. *Head Neck*. 2012;34(4):459-461. doi:10.1002/hed.21974.

118. Thomas J, Primeaux T. Is p16 immunohistochemistry a more cost-effective method for identification of human papilloma virus–associated head and neck squamous cell carcinoma? *Annals of Diagnostic Pathology*. 2012;16(2):91-99. doi:10.1016/j.anndiagpath.2011.09.002.

119. Oguejiofor KK, Hall JS, Mani N, et al. The Prognostic Significance of the Biomarker p16 in Oropharyngeal Squamous Cell Carcinoma. *Clinical Oncology*. 2013;25(11):630-638. doi:10.1016/j.clon.2013.07.003.

120. Jordan RC, Lingen MW, Perez-Ordonez B, et al. Validation of Methods for Oropharyngeal Cancer HPV Status Determination in US Cooperative Group Trials. *The American Journal of Surgical Pathology*. 2012;36(7):945-954. doi:10.1097/PAS.0b013e318253a2d1.

121. Mroz EA, Baird AH, Michaud WA, Rocco JW. COOH-terminal binding protein regulates expression of the p16INK4A tumor suppressor and senescence in primary human cells. *Cancer Research*. 2008;68(15):6049-6053. doi:10.1158/0008-5472.CAN-08-1279.

122. Münger K, Baldwin A, Edwards KM, et al. Mechanisms of human papillomavirus-induced oncogenesis. *J Virol*. 2004;78(21):11451-11460. doi:10.1128/JVI.78.21.11451-11460.2004.

123. Reed AL, Califano J, Cairns P, et al. High frequency of p16 (CDKN2/MTS-1/INK4A) inactivation in head and neck squamous cell carcinoma. *Cancer Research*. 1996;56(16):3630-3633.

124. Schache AG, Liloglou T, Risk JM, et al. Evaluation of human papilloma virus diagnostic testing in oropharyngeal squamous cell carcinoma: sensitivity, specificity, and prognostic discrimination. *Clin Cancer Res*.

2011;17(19):6262-6271. doi:10.1158/1078-0432.CCR-11-0388.

125. Cao D, Begum S, Ali SZ, Westra WH. Expression of p16 in benign and malignant cystic squamous lesions of the neck. *Hum Pathol*. 2010;41(4):535-539. doi:10.1016/j.humpath.2009.09.006.

126. Singhi AD, Westra WH. Comparison of human papillomavirus in situ hybridization and p16 immunohistochemistry in the detection of human papillomavirus-associated head and neck cancer based on a prospective clinical experience. *Cancer*. 2010;116(9):2166-2173. doi:10.1002/cncr.25033.

127. National Comprehensive Cancer Network. *NCCN Head and Neck - Google Search*. Fort Washington; 2013. doi:10.1111/j.1464-410X.2011.10693.x/full.

128. Muñoz N, Kjaer SK, Sigurdsson K, et al. Impact of Human Papillomavirus (HPV)-6/11/16/18 Vaccine on All HPV-Associated Genital Diseases in Young Women. *J Natl Cancer Inst*. 2010;102(5):325-339. doi:10.1093/jnci/djp534.

129. Herrero R, Quint W, Hildesheim A, et al. Reduced Prevalence of Oral Human Papillomavirus (HPV) 4 Years after Bivalent HPV Vaccination in a Randomized Clinical Trial in Costa Rica. Ramqvist T, ed. *PLoS ONE*. 2013;8(7):e68329. doi:10.1371/journal.pone.0068329.

130. Tabrizi SN, Brotherton JML, Kaldor JM, et al. Fall in human papillomavirus prevalence following a national vaccination program. *J Infect Dis*. 2012;206(11):1645-1651. doi:10.1093/infdis/jis590.

131. Centers for Disease Control and Prevention (CDC). FDA licensure of quadrivalent human papillomavirus vaccine (HPV4, Gardasil) for use in males and guidance from the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep*. 2010;59(20):630-632.

132. Seifert B, Quach-Thanh C, Skowronski D, Tan B. Update on Human Papillomavirus (HPV) Vaccines. *phac-aspcgcca*

133. Seifert B, Quach-Thanh C, Skowronski D, Tan B. Update on Human Papillomavirus (HPV) Vaccines. *phac-aspcgcca*

134. Approved Products > Gardasil.

135. Lehtinen M, Dillner J. Clinical trials of human papillomavirus vaccines and beyond. *Nat Rev Clin Oncol*. 2013;10(7):400-410. doi:10.1038/nrclinonc.2013.84.

136. Jit M, Brisson M, Portnoy A, Hutubessy R. Cost-effectiveness of female human papillomavirus vaccination in 179 countries: a PRIME modelling study. *Lancet Glob Health*. 2014;2(7):e406-e414. doi:10.1016/S2214-

109X(14)70237-2.

137. Denis F, Garaud P, Bardet E, et al. Final results of the 94-01 French Head and Neck Oncology and Radiotherapy Group randomized trial comparing radiotherapy alone with concomitant radiochemotherapy in advanced-stage oropharynx carcinoma. *Journal of Clinical Oncology*. 2004;22(1):69-76. doi:10.1200/JCO.2004.08.021.

138. Gillison ML, Zhang Q, Jordan R, et al. Tobacco Smoking and Increased Risk of Death and Progression for Patients With p16-Positive and p16-Negative Oropharyngeal Cancer. *Journal of Clinical Oncology*. 2012;30(17):JCO.2011.38.4099–2111. doi:10.1200/JCO.2011.38.4099.

139. Braakhuis BJM, Snijders PJF, Keune W-JH, et al. Genetic patterns in head and neck cancers that contain or lack transcriptionally active human papillomavirus. *J Natl Cancer Inst*. 2004;96(13):998-1006. doi:10.1093/jnci/djh183.

140. Chai RC, Lambie D, Verma M, Punyadeera C. Current trends in the etiology and diagnosis of HPV- related head and neck cancers. *Cancer Medicine*. 2015;4(4):596-607. doi:10.1002/cam4.424.

141. Department of Veterans Affairs Laryngeal Cancer Study Group, Wolf GT, Fisher SG, et al. Induction chemotherapy plus radiation compared with surgery plus radiation in patients with advanced laryngeal cancer. *N Engl J Med*. 1991;324(24):1685-1690. doi:10.1056/NEJM199106133242402.

142. Pignon JP, Syz N, Posner M, Olivares R. … selection suggests the addition of docetaxel to 5-fluorouracil–cisplatin induction therapy may offer survival benefit in squamous cell cancer of the head and neck. ... *-cancer drugs*. 2004.

143. Posner MR, Hershock DM, Blajman CR, et al. Cisplatin and fluorouracil alone or with docetaxel in head and neck cancer. *N Engl J Med*. 2007;357(17):1705-1715. doi:10.1056/NEJMoa070956.

144. Garden AS, Harris J, Trotti A, et al. Long-Term Results of Concomitant Boost Radiation Plus Concurrent Cisplatin for Advanced Head and Neck Carcinomas: A Phase II Trial of the Radiation Therapy Oncology Group (RTOG 99-14). *International Journal of Radiation Oncology*Biology*Physics*. 2008;71(5):1351-1355.

145. Prestwich RJD, Kancherla K, Oksuz DC, et al. A single centre experience with sequential and concomitant chemoradiotherapy in locally advanced stage IV tonsillar cancer. *Radiation Oncology 2010 5:1*. 2010;5(1):121. doi:10.1186/1748-717X-5-121.

146. Denis F, Garaud P, Bardet E. ... *And Radiotherapy Group Randomized Trial*

Comparing Radiotherapy Alone with Concomitant Radiochemotherapy in Advanced-Stage Oropharynx Carcinoma. … Oncology; 2004.

147. Machtay M, Moughan J, Trotti A, et al. Factors associated with severe late toxicity after concurrent chemoradiation for locally advanced head and neck cancer: an RTOG analysis. *J Clin Oncol*. 2008;26(21):3582-3589. doi:10.1200/JCO.2007.14.8841.

148. Greven KM, White DR, Browne JD, Williams DWI, McGuirt WFS, D'Agostino RBJ. Swallowing Dysfunction is a Common Sequelae After Chemoradiation for Oropharynx Carcinoma. *American Journal of Clinical Oncology*. 2008;31(3):209-212. doi:10.1097/COC.0b013e3181595b10.

149. Wilson JA, Carding PN, Patterson JM. Dysphagia after Nonsurgical Head and Neck Cancer Treatment Patients' Perspectives. *Otolaryngology -- Head and Neck Surgery*. 2011;145(5):767-771. doi:10.1177/0194599811414506.

150. Best SR, Ha PK, Blanco RG, et al. Factors associated with pharyngoesophageal stricture in patients treated with concurrent chemotherapy and radiation therapy for oropharyngeal squamous cell carcinoma. *Head & Neck*. 2011;33(12):1727-1734. doi:10.1002/hed.21657.

151. Nien HH, Sturgis EM, Kies MS, et al. Comparison of systemic therapies used concurrently with radiation for the treatment of human papillomavirus–associated oropharyngeal cancer. *Head & Neck*. 2016;38(S1):E1554-E1561. doi:10.1002/hed.24278.

152. Vainshtein JM, Samuels S, Tao Y, et al. Impact of xerostomia on dysphagia after chemotherapy–intensity- modulated radiotherapy for oropharyngeal cancer: Prospective longitudinal study. *Head & Neck*. 2016;38(S1):E1605-E1612. doi:10.1002/hed.24286.

153. Shiley SG, Hargunani CA, Skoner JM, Holland JM, Wax MK. Swallowing Function after Chemoradiation for Advanced Stage Oropharyngeal Cancer. *Otolaryngology -- Head and Neck Surgery*. 2006;134(3):455-459. doi:10.1016/j.otohns.2005.10.054.

154. Setton J, Lee NY, Riaz N, et al. A multi- institution pooled analysis of gastrostomy tube dependence in patients with oropharyngeal cancer treated with definitive intensity- modulated radiotherapy. *Cancer*. 2015;121(2):294-301. doi:10.1002/cncr.29022.

155. Chen AY, Schrag N, Hao Y, Stewart A, Ward E. Changes in treatment of advanced oropharyngeal cancer, 1985-2001. *Laryngoscope*. 2007;117(1):16-21. doi:10.1097/01.mlg.0000240182.61922.31.

156. Chen AY, Zhu J, Fedewa S. Temporal trends in oropharyngeal cancer treatment and survival: 1998-2009. *Laryngoscope*. 2014;124(1):131-138. doi:10.1002/lary.24296.

157. Park G, Lee S-W, Kim SY, et al. Can concurrent chemoradiotherapy replace surgery and postoperative radiation for locally advanced stage III/IV tonsillar squamous cell carcinoma? *Anticancer Res*. 2013;33(3):1237-1243.

158. Kano S, Homma A, Hayashi R, et al. Matched-pair analysis in patients with advanced oropharyngeal cancer: surgery versus concurrent chemoradiotherapy. *Oncology*. 2013;84(5):290-298. doi:10.1159/000346908.

159. D PB-RM, D AGM, D VBM, et al. Matched Survival Analysis in Patients with Locoregionally Advanced Resectable Oropharyngeal Carcinoma: Platinum-Based Induction and Concurrent Chemoradiotherapy Versus Primary Surgical Resection. *Radiation Oncology Biology*. 2011;80(1):154-160. doi:10.1016/j.ijrobp.2010.01.032.

160. Zhen W, Karnell LH, Hoffman HT, Funk GF, Buatti JM, Menck HR. The National Cancer Data Base report on squamous cell carcinoma of the base of tongue. *Head Neck*. 2004;26(8):660-674. doi:10.1002/hed.20064.

161. O'Connell D, Seikaly H, Murphy R, et al. Primary surgery versus chemoradiotherapy for advanced oropharyngeal cancers: a longitudinal population study. 2013;42(1):31. doi:10.1186/1916-0216-42-31.

162. Dahlstrom KR, Calzada G, Hanby JD, et al. An evolution in demographics, treatment, and outcomes of oropharyngeal cancer at a major cancer center: a staging system in need of repair. *Cancer*. 2013;119(1):81-89. doi:10.1002/cncr.27727.

163. Dayyani F, Etzel CJ, Liu M, Ho C-H, Lippman SM, Tsao AS. Meta-analysis of the impact of human papillomavirus (HPV) on cancer risk and overall survival in head and neck squamous cell carcinomas (HNSCC). *Head & Neck Oncology 2010 2:1*. 2010;2(1):1. doi:10.1186/1758-3284-2-15.

164. Adelstein DJ, Ridge JA, Gillison ML, et al. Head and neck squamous cell cancer and the human papillomavirus: Summary of a National Cancer Institute State of the Science Meeting, November 9–10, 2008, Washington, D.C. *Head & Neck*. 2009;31(11):1393-1422. doi:10.1002/hed.21269.

165. Chandarana SP, Lee JS, Chanowski EJP, et al. Prevalence and predictive role of p16 and epidermal growth factor receptor in surgically treated oropharyngeal and oral cavity cancer. *Head & Neck*. 2013;35(8):1083-1090. doi:10.1002/hed.23087.

166. Bristow RG, Benchimol S, Hill RP. The p53 gene as a modifier of intrinsic radiosensitivity: implications for radiotherapy. *Radiotherapy and Oncology*.

1996;40(3):197-223. doi:10.1016/0167-8140(96)01806-3.

167. Wang MB, Liu IY, Gornbein JA, Nguyen CT. HPV-Positive Oropharyngeal Carcinoma: A Systematic Review of Treatment and Prognosis. *Otolaryngology -- Head and Neck Surgery*. 2015;153(5):758-769. doi:10.1177/0194599815592157.

168. Ragin CCR, Taioli E. Survival of squamous cell carcinoma of the head and neck in relation to human papillomavirus infection: Review and meta-analysis. *Int J Cancer*. 2007;121(8):1813-1820. doi:10.1002/ijc.22851.

169. Brizel DM. Different Strokes for Different Folks: New Paradigms for Staging Oropharynx Cancer. *Journal of Clinical Oncology*. 2015;33(8):JCO.2014.60.1757-JCO.2014.60.1818. doi:10.1200/JCO.2014.60.1757.

170. Horne ZD, Glaser SM, Vargo JA, et al. Confirmation of proposed human papillomavirus risk-adapted staging according to AJCC/UICC TNM criteria for positive oropharyngeal carcinomas. *Cancer*. April 2016:1-10. doi:10.1002/cncr.30021.

171. Amin MB, Edge S, Greene FL, et al. *AJCC Cancer Staging Manual*. Springer; 2016.

172. Lydiatt WM, Patel SG, O'Sullivan B, et al. Head and Neck cancers-major changes in the American Joint Committee on cancer eighth edition cancer staging manual. *CA: A Cancer Journal for Clinicians*. 2017;67(2):122-137. doi:10.3322/caac.21389.

173. Teymoortash A, Werner JA. Current advances in diagnosis and surgical treatment of lymph node metastasis in head and neck cancer. *GMS Curr Top Otorhinolaryngol Head Neck Surg*. 2012;11:Doc04. doi:10.3205/cto000086.

174. Subramanian S, Chiesa F, Lyubaev V, Aidarbekova A. *The Evolution of Surgery in the Management of Neck Metastases.* Vol 26. Pacini Editore; 2006:309-316.

175. Parsons JT, Mendenhall WM, Stringer SP, et al. Squamous cell carcinoma of the oropharynx: surgery, radiation therapy, or both. *Cancer*. 2002;94(11):2967-2980. doi:10.1002/cncr.10567.

176. Parsons J, Mendenhall W, Million R, Stringer S, Cassisi N. The Management of Primary Cancers of the Oropharynx: Combined Treatment or Irradiation Alone? *Semin Radiat Oncol*. 1992;2(3):142-148. doi:10.1053/SRAO00200142.

177. Kumar B, Cipolla MJ, Old MO, et al. Surgical management of oropharyngeal

squamous cell carcinoma: Survival and functional outcomes. *Head Neck*. 2015;38(S1):E1794-E1802. doi:10.1002/hed.24319.

178. Stucken CL, de Almeida JR, Sikora AG, Tong CCL, Genden EM. Impact of human papillomavirus and smoking on survival outcomes after transoral robotic surgery. *Head Neck*. 2015;38(3):380-386. doi:10.1002/hed.23915.

179. Kass JI, Giraldez L, Gooding W, et al. Oncologic outcomes of surgically treated early-stage oropharyngeal squamous cell carcinoma. *Head Neck*. April 2016:1-5. doi:10.1002/hed.24456.

180. Camp AA, Fundakowski C, Petruzzelli GJ, Emami B. Functional and oncologic results following transoral laser microsurgical excision of base of tongue carcinoma. *Otolaryngology -- Head and Neck Surgery*. 2009;141(1):66-69. doi:10.1016/j.otohns.2009.02.028.

181. Smith RV, Schiff BA, Garg M, Haigentz M. The impact of transoral robotic surgery on the overall treatment of oropharyngeal cancer patients. *Laryngoscope*. 2015;125:S1-S15. doi:10.1002/lary.25534.

182. Monnier Y, Simon C. Surgery Versus Radiotherapy for Early Oropharyngeal Tumors: a Never-Ending Debate. *Curr Treat Options in Oncol*. 2015;16(9):42–13. doi:10.1007/s11864-015-0362-4.

183. Bernier J, Domenge C, Ozsahin M, et al. Postoperative irradiation with or without concomitant chemotherapy for locally advanced head and neck cancer. *N Engl J Med*. 2004;350(19):1945-1952. doi:10.1056/NEJMoa032641.

184. Bernier J, Cooper JS, Pajak TF, et al. Defining risk levels in locally advanced head and neck cancers: a comparative analysis of concurrent postoperative radiation plus chemotherapy trials of the EORTC (#22931) and RTOG (# 9501). *Head Neck*. 2005;27(10):843-850. doi:10.1002/hed.20279.

185. Cooper JS, Pajak TF, Forastiere AA, et al. Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck. *N Engl J Med*. 2004;350(19):1937-1944. doi:10.1056/NEJMoa032646.

186. Huang SH, Perez-Ordonez B, Liu F-F, et al. Atypical clinical behavior of p16-confirmed HPV-related oropharyngeal squamous cell carcinoma treated with radical radiotherapy. *Int J Radiat Oncol Biol Phys*. 2012;82(1):276-283. doi:10.1016/j.ijrobp.2010.08.031.

187. Langendijk JA, Doornaert P, Verdonck-de Leeuw IM, Leemans CR, Aaronson NK, Slotman BJ. Impact of late treatment-related toxicity on quality of life among patients with head and neck cancer treated with radiotherapy. *J Clin Oncol*. 2008;26(22):3770-3776.

doi:10.1200/JCO.2007.14.6647.

188. Sanguineti G, Sormani MP, Marur S, et al. Effect of radiotherapy and chemotherapy on the risk of mucositis during intensity-modulated radiation therapy for oropharyngeal cancer. *Int J Radiat Oncol Biol Phys*. 2012;83(1):235-242. doi:10.1016/j.ijrobp.2011.06.2000.

189. Adelstein DJ, Ridge JA, Brizel DM, et al. Transoral resection of pharyngeal cancer: Summary of a National Cancer Institute Head and Neck Cancer Steering Committee Clinical Trials Planning Meeting, November 6–7, 2011, Arlington, Virginia. *Head & Neck*. 2012;34(12):1681-1703. doi:10.1002/hed.23136.

Chapter 2: Methods

This is a retrospective population based cohort study. Ethics approval was obtained from the University of Alberta Health Research Ethics Board (No: HREB / Pro00016426, 2015-11-30).

2.1 Patient cohort:

Patient date were obtained from 1) Alberta Cancer Registry Edmonton site, and 2) Medical records (electronic & paper) at two institutions (University of Alberta hospital and Cross Cancer Institute) where all of the treatment and follow-up occurred. All the required data were collected as part of prior research work on patients with OPSCC. In this current study, data were further stratified and analyzed based on our objectives: 1) Compare patients with advanced stage OPSCC treated with primary surgery followed by RT or CRT to determine whether the addition of chemotherapy has a significant survival advantage, 2) Evaluate the benefit of chemotherapy on survival dependent on p16 status and tobacco smoking history.

A retrospective chart review of patients identified from the Alberta Cancer Registry was performed. The Alberta Cancer Registry is a population based disease registry with gold certification from the North American Association for Central Cancer Registries during the period of consideration. It is responsible for recording and tracking all new diagnoses of cancer, their initial treatments, and vital status, through mandatory reporting enforced by legislation in the Province of Alberta. Patients diagnosed with OPSCC at the University of Alberta Hospital and/or Cross Cancer Institute between January 1st 1998, and December 31st 2009 were identified

and their medical records reviewed. The condition of all patients included in the study were assessed for treatment in a consistent manner by the Northern Alberta Multidisciplinary Head and Neck Oncology Tumor Board at the University of Alberta Hospital, which is the standard of care for treatment planning for any patient with head and neck cancer. Eligibility criteria included all patients with advanced stage OPSCC (stage III or IV according to American Joint Committee on Cancer seventh edition)[1] who received primary surgery followed by either radiation (S+RT) or chemoradiation therapy (S+CRT) for curative intent. As chemotherapy is not indicated in early stage OPSCC that group of patients, they were excluded.

2.2 Data collection:

Medical records were reviewed and data were extracted (electronic and paper charts) at the University of Alberta Hospital and/or the Cross Cancer Institute. Database accuracy was reviewed and audited by three head and neck oncologic surgeons directly involved in the study and any inconsistencies found were resolved by consensus. The data obtained from the charts included patient demographics (eg. age, sex), Eastern Cooperative Oncology Group (ECOG) quality of life score, smoking status, pack-years, tumor characteristics (ex: subsite, side, size, TNM staging), treatment details (eg. S+RT, S+CRT), procedure details (extent of the primary resection, type of neck dissection, type of reconstruction performed), radiation and chemotherapy details (chemotherapy agents, number of cycles, RT dose, RT field) pathological description (eg. p16 status, perineural invasion, lymphovascular invasion, margin status, and neck node status, number of positive neck nodes, extracapsular

extension (ECE)), and outcome data (5-year survival, recurrence, time to recurrence, death, time to death, and cause of death).

2.3 Data definitions & treatment groups classifications:

Cause of death was classified as either disease specific or from other causes as determined by the Alberta Cancer Registry and chart review for calculation of overall survival (OS; death from any cause), disease specific survival (DSS; accounting only for death from OPSCC), and disease free survival (DFS; accounting only for death from OPSCC or alive with local recurrence or distant metastasis).

Patients were categorized into two intent-to-treat subgroups: (1) Surgery + Post-operative Radiation (S+RT), (2) Surgery + Post-operative chemoradiation (S+CRT). Patients were further subdivided based on their smoking status, p16, extracapsular extension (ECE), and surgical margin status. Smoking status was based on data collected from the patient on their first visit to the cancer clinic at both institutes (University of Alberta Hospital and Cross Cancer institute). The questions addressed if they smoked tobacco at any time of their life and for how long they smoked to be able to calculate the pack-year smoking history. The cutoff-point used to define patients as smokers was > 10 pack-years,[2] and p16 status was determined by immunohistochemistry of formalin-fixed paraffin-embedded (FFPE) tumor tissue blocks.

2.4 Classification/definition of p16 groups:

Tissue microarrays (TMAs) were constructed with FFPE tumor tissue blocks from either pretreatment biopsies or primary surgical specimen, as previously reported and

described[3-5] A head and neck pathologist (L.P.) reviewed the blocks and excluded cases with inadequate tissue. Six negative controls were incorporated on the arrays using FFPE tissue blocks from placenta, kidney, pancreas, liver, and HPV-negative tonsils. HPV-positive control tissue was included for reference intensity staining determined by HPV16/18 by *in situ* hybridization and p16 positivity by 2 pathologists. Five TMAs were constructed with duplicate or triplicate cores of FFPE blocks as per the TMA protocol previously described.[6,7] Immunohistochemistry (IHC) for p16 (clone JC8; Santa Cruz Biotechnology, Dallas, TX) was performed using the diaminobenzidine staining method, as previously reported and scored for p16 status using previously established standards.[7-9]

2.5 Outcome variables & covariates:

Our outcome variables were divided into three different survival categories: 1) Overall Survival (OS; death from any cause), 2) Disease Specific Survival (DSS; accounting only for death from OPSCC), and Disease Free Survival (DFS; accounting only for death from OPSCC or alive with local recurrence or distant metastasis). Our covariates were demographic (eg. age, sex), Eastern Cooperative Oncology Group (ECOG) quality of life score, smoking status, pack-years, tumor characteristics (ex: subsite, side, size, TNM staging), treatment details (eg. S+RT, S+CRT), procedure details (extent of the primary resection, type of neck dissection, type of reconstruction performed), radiation and chemotherapy details (chemotherapy agents, number of cycles, RT dose, RT field) pathological description (eg. p16 status, perineural

invasion, lymphovascular invasion, margin status, and neck node status, number of positive neck nodes, extracapsular extension (ECE)).

2.6 Statistical analysis:

Demographic, pathologic, treatment and staging information for all patients included in the study were tabulated. Differences between the two treatment groups were tested for any statistical significance using Student's t-test, Pearson's chi-square, and Fisher's exact test. Statistical significance was defined as $p < 0.05$.

Follow-up using electronic medical records and paper charts included up to five years from patient diagnosis for each patient. Survival time was calculated in years from the date of a biopsy proven OPSCC to the date last known alive by follow-up or electronic medical records, or date of death. If the patient was alive at the end of the of follow-up, the survival time considered as censored.[10] A Cox proportional hazards model was used to perform multivariate analyses for overall survival, disease specific survival and disease free survival with respect prognostic factors and variables.[11]

Variables for the proportional hazards model were chosen using purposeful selection method. The variables for the survival analysis included age, sex, ECOG score, smoking status, treatment, p16 positivity, ECE status, margins status, and TNM overall stage. We started by fitting a univariate Cox's regression model for each of the variables then we fit a multivariate Cox's regression model for the same group of variables (as they were all clinically important to keep in the model even if they were statistically non-significant). Confounding was not tested as none of the variables were removed from the model after multivariate analysis. Proportional hazard

assumption was tested for all the variables retained in the final model and none violated the assumption of constant risk across survival time period. All clinically plausible interactions were tested, none of which were statistically significant, which included: age*sex, p16*smoking, margins*ECE, treatment*age, treatment*sex, treatment*ECOG, treatment*smoking, treatment*p16, treatment*ECE, treatment*margins, treatment*overall stage.

Kaplan–Meier curves and log-rank tests were used to estimate and compare 5-year OS, DSS, and DFS times between patients who received S+RT and S+CRT, stratified by p16 status and smoking history. Post-hoc power analysis was carried out to estimate the sample size required detecting a difference of ≥ 5%, as well as the estimated sample size for 80% power for those with observed power less than 80%.

Statistical analysis was performed using SPSS version 22.0 (SPSS, Chicago, IL), and STATA 13 software (StataCorp. 2013. *Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP).

References

1. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol. 2010;17(6):1471-1474. doi:10.1245/s10434-010-0985-4.

2. Ang KK, Harris J, Wheeler R, et al. Human Papillomavirus and Survival of Patients with Oropharyngeal Cancer. N Engl J Med. 2010;363(1):24-35. doi:10.1056/NEJMoa0912217.

3. Cooper T, Biron V, Adam B, Klimowicz AC, Puttagunta L, Seikaly H. Prognostic utility of basaloid differentiation in oropharyngeal cancer. 2013;42(1):57. doi:10.1186/1916-0216-42-57.

4. Barber BR, Biron VL, Klimowicz AC, Puttagunta L, Côté DWJ, Seikaly H. Molecular predictors of locoregional and distant metastases in oropharyngeal squamous cell carcinoma. 2013;42(1):53. doi:10.1186/1916-0216-42-53.

5. Seikaly H, Biron VL, Zhang H, et al. Role of primary surgery in the treatment of advanced oropharyngeal cancer. Head Neck. July 2015:n/a–n/a. doi:10.1002/hed.24042.

6. Forastiere AA, Goepfert H, Maor M, et al. Concurrent chemotherapy and radiotherapy for organ preservation in advanced laryngeal cancer. N Engl J Med. 2003;349(22):2091-2098. doi:10.1056/NEJMoa031317.

7. Lau HY, Brar S, Klimowicz AC, et al. Prognostic significance of p16 in locally advanced squamous cell carcinoma of the head and neck treated with concurrent cisplatin and radiotherapy. Head Neck. 2011;33(2):251-256. doi:10.1002/hed.21439.

8. Begum S, Gillison ML, Ansari-Lari MA, Shah K, Westra WH. Detection of human papillomavirus in cervical lymph nodes: a highly effective strategy for localizing site of tumor origin. Clin Cancer Res. 2003;9(17):6469-6475.

9. Liang C, Marsit CJ, McClean MD, et al. Biomarkers of HPV in Head and Neck Squamous Cell Carcinoma. Cancer Research. 2012;72(19):5004-5013. doi:10.1158/0008-5472.CAN-11-3277.

10. Clark TG, Bradburn MJ, Love SB, Altman DG. Survival analysis part I: basic concepts and first analyses. British Journal of Cancer. 2003;89(2):232-238. doi:10.1038/sj.bjc.6601118.

11. Bradburn MJ, Clark TG, Love SB, Altman DG. Survival analysis part II: multivariate data analysis--an introduction to concepts and methods. British Journal of Cancer. 2003;89(3):431-436. doi:10.1038/sj.bjc.6601119.

Chapter 3: Results

3.1 Patient cohort:

Three hundred and forty consecutive patients with OPSCC were curatively treated at the University of Alberta Hospital and the Cross Cancer Institute from January 1, 1998, to December 31, 2009. Two hundred seventy-nine patients had stage 3 or 4 disease and were included in the study. Patients with single modality treatment and those undergoing primary CRT were excluded from the analysis files. Thirty-three patients were missing P16 data. The patient categories for inclusion and exclusion in the analysis files are summarized in Figure 3.1.

The total number of patients with data available for inclusion in our analysis was 138, with full 5-year survival history available for 126 (91.3%) patients and 80 (58%) patients alive for at 5 years post diagnosis (Figure 3.2). Demographics, clinical characteristics, and risk factors and characteristics are summarized in Table 3.1. The majority of patients were male (81%), stage 4 (86%), p16 positive (59%), smoker of ≥ 10 pack-years (72%) with a mean age of 54.5 years. Tonsils were the most common tumor subsite (59%) followed by base of tongue (28%). Based on the treatment modality patients were categorized as patients who received S+CRT (N=71, 51%) or S+RT (N=67, 49%). Patients were further subdivided based on p16 positivity and smoking status into four groups with largest group being those who were p16 positive smokers (42%) followed p16 negative and smokers (30%) (Table 3.2). A total of 31 patients (22%) had either a recurrence and/or distant metastasis within 5-years after their primary treatment (Table 3.3). Extracapsular extension and margin status data was obtained from final pathology reports, however 19 patients were missing ECE status. Out of the 119 patients

with ECE and margin status data there were 54 patients (45%) who had either ECE or positive margins or both (Table 3.4).

When comparing demographics of the two treatment groups there was a statistically significant difference with regards to their p16 status (32% p16 positive in the S+CRT vs. 49% in the S+RT), N-stage (90% ≥ N2a in the S+CRT vs. 60% in the S+RT) and overall stage (93% stage 4 in the S+CRT vs. 78% in the S+RT) indicating the need of chemotherapy in that group. There was also a statistically significant difference in their p16 status and subsite involved. The details of both radiation therapy and chemotherapy are summarized in Table 3.5. Patients who received S+RT more commonly received conventional RT (43%) compared to S+CRT (7.0%) treatment group. Platinum-based chemotherapy was used in all patients treated with S+CRT with the dose being either 70 or 100 mg/m^2. Surgical resections were performed through transoral or open approaches for the primary disease and level I to IV neck dissections were performed for any neck disease (N1–3) with selective neck dissections reserved for N0 neck disease.

A total of 45 patients were excluded from the study as they had received a single modality treatment (RT or surgery) with different characteristics than the study population (Table 3.6). This group of patients tended to be older (mean age of 64.2 years) with higher rates of comorbidities. They also had higher percentages of smokers (73%) and p16 negative (69%) as compared to the final group included in the study that received a multimodality treatment.

Thirty-three patients with advanced-stage oropharyngeal SCC did not have p16 data because of the lack of adequate specimen for TMA construction or an inconclusive

result after IHC (Table 3.7). These 33 subjects were compared to the remaining cohort of 138 patients with complete p16 data, as seen in Table 7. There was no statistically significant difference in the demographics or survival outcomes between the 2 groups, suggesting the cohort of 138 patients with complete data were representative of the entire advanced-stage oropharyngeal SCC cohort.

3.2 Predictors of survival (Role of chemotherapy and p16 status)

(Multivariate Cox's regression & proportional hazard analysis):

A univariate followed by a multivariate Cox's regression analysis was performed for the same group of variables (as they were all clinically important to keep in the model even if some were statistically non-significant) for all three-outcome variables (Table 3.8 & 3.9). Initially we tested the whole cohort regardless to all the other covariates. On univariate Cox's regression analysis patients receiving S+CRT were 0.48 times less likely to die from any cause in the analysis of overall survival (OS) [hazard ratio (HR): 0.48, range: 0.26-0.87] and this was statistically significant for OS with a p-value of < 0.05. However, the analysis was non-significant for disease specific survival (DSS) and disease free survival (DFS). On multivariate Cox's regression analysis S+CRT was borderline significant with p –value of 0.06 for OS, and not a significant predictor for DSS and DFS.

Univariate and multivariate Cox's regression analysis were performed on the remaining variables including p16 and smoking status. Multivariate Cox regression analysis showed that both positive smoking history of $\geq$ 10 pack-years and positive ECE were significant determinants of survival for all three survival outcomes (Table 3.9). Patients with a positive smoking history of $\geq$ 10 pack-year were 2.7 times more likely to

die from any cause in the analysis of OS time [HR: 2.7, range: 1.2-6.3], 3.3 time more likely to die from their disease in the analysis of disease specific survival times (DSS) [HR: 3.3, range: 0.95-11.5], and 3.5 times more likely to either die from their disease or alive with recurrence and/or distant metastasis in the analysis of disease free survival time (DFS) [HR: 3.5, range: 1.2-10.4] compared to non-smokers. This was statistically significant for OS and DFS with a p-value of < 0.05, and borderline significance for DSS with a p-value of 0.06. Patients with positive ECE had a significantly higher risk of death with an OS HR of 2.2 (95% confidence interval: 1.1-4.4), DSS HR of 5.0 (95% confidence interval: 1.7-15), and DFS HR of 3.9 (95% confidence interval: 1.6-10.0). This was all statistically significant with a p-value < 0.05.

3.3 Kaplan–Meier survival analysis

(Based on treatment received, p16 status, and smoking status):

Starting with the whole cohort divided by treatment status (S+CRT vs. S+RT) (Figure 3.3), the addition of chemotherapy was associated with a statistically significant better 5-year OS (77% vs. 58.1%), however it was not statistically significant for DSS (85.4% vs. 74.9%) and DFS (84.2% vs. 69.1%) with a *post hoc* power calculation of 84% and 91% respectively (Table 3.10).

When stratifying by smoking status (Figure 3.4), the non-smokers group did not show any statistically significant differences in all three different 5-years survival analyses, with a *post hoc* power calculation for OS being only 3.8%, and could not be calculated for DSS and DFS (Table 3.10), as no events (deaths) occurred for these analyses. With regards to the smoking group there was a significantly higher 5-year OS (73.8% vs. 48.1%) and DFS (82.1% vs. 59.5%), but not DSS (84.5% vs. 66.8%), with a

post hoc power calculation of 95% (Table 3.10). When stratifying by p16 status (Figure 3.5), neither of the two groups showed any statistical significant 5-year survival difference (OS, DSS, & DFS) between S+CRT vs. S+RT, with *post hoc* power calculation ranging between 16-83% (Table 3.10). We then combined both ECE and margins status stratifying them into two groups: (1) both negative and (2) either or both positive (Table 3.4). Both groups did not show any statistically significant 5-year survival differences (OS, DSS, & DFS) between S+CRT vs. S+RT (Figure 3.6), with power calculation ranging between 6-93% (Table 3.10). Lastly, we combined both p16 and smoking status stratifying them into four groups (Table 3.2). The only group that showed statistically significant 5-year better OS (83.9% vs. 57.7%) & DFS (93.4% vs. 67.4%) with the addition of chemotherapy, and borderline significance for DSS (93.4% vs. 75.0%) was the p16 positive & smokers (group 1) (Figure 3.7). For the other 3 groups that showed no statistically significant differences, power calculations were either not possible to calculate due to the absence of events or ranged between 6-11% (Table 3.10).

Table 3.1 Demographics of 138 patients with advanced stage oropharyngeal squamous cell carcinoma with multimodality treatment. (Using two sample t-test, Chi square, Fisher exact)

	Variable	S+CRT* (n=71)	S+RT* (n=67)	Total (n=138)	*p*-value
Demographic	**Age**				
	Mean	54.3	54.7	54.5	0.74
	SD	7.3	9.16	8.2	
	Gender				
	Male	57 (80%)	55 (82%)	112 (81%)	0.79
	Female	14 (20%)	12 (18%)	26 (19%)	
Clinical	**T-Stage**				
	1	14 (20%)	16 (24%)	30 (22%)	
	2	24 (34%)	17 (25%)	41 (30%)	0.33
	3	22 (31%)	28 (42%)	50 (36%)	
	4a	11 (15%)	6 (9%)	17 (12%)	
	N-Stage				
	0	2 (3%)	13 (20%)	15 (11%)	
	1	5 (7%)	15 (22%)	20 (14%)	
	2a	12 (17%)	6 (9%)	18 (13%)	***0.002***
	2b	30 (42%)	19 (28%)	49 (36%)	
	2c	16 (23%)	10 (15%)	26 (19%)	
	3	6 (8%)	4 (6%)	10 (7%)	
	M-Stage				
	0	64 (90%)	62 (93%)	126 (91%)	0.62
	1	7 (10%)	5 (7%)	12 (9%)	
	Overall Stage				
	3	5 (7%)	15 (22%)	20 (14%)	***0.01***
	4	66 (93%)	52 (78%)	118 (86%)	
	Subsite				
	Tonsil	44 (62%)	38 (57%)	82 (59%)	
	Base of Tongue	22 (31%)	17 (25%)	39 (28%)	***0.04***
	Soft Palate	3 (4%)	1 (2%)	4 (3%)	
	Posterior Wall	2 (3%)	11 (16%)	13 (10%)	
Risk Factors	**P16**				
	Positive	48 (68%)	34 (51%)	82 (59%)	***0.04***
	Negative	23 (32%)	33 (49%)	56 (41%)	
	Smoking				
	Yes	47 (66%)	52 (78%)	99 (72%)	0.14
	No	24 (34%)	15 (22%)	39 (28%)	
	ECOG*				
	0 & 1	64 (90%)	61 (91%)	125 (91%)	0.86
	2 & 3	7 (10%)	6 (9%)	13 (9%)	

**S+CRT*: Surgery and chemo-radiotherapy, **S+RT*: Surgery and radiotherapy, **ECOG*: Eastern Cooperative Oncology Group.

Table 3.2 Number of patients in each treatment group based on p16 & smoking status

P16 / Smoking Status	Treatment		Total
	S + CRT*	S + RT*	
P16 +ve & Smokers	32 (45%)	26 (39%)	58 (42%)
P16 +ve & Non-Smokers	16 (23%)	8 (12%)	24 (17%)
P16 -ve & Smokers	15 (21%)	26 (39%)	41 (30%)
P16 -ve & Non-Smokers	8 (11%)	7 (10%)	15 (11%)
Total	**71**	**67**	**138**

****S+CRT***: Surgery and chemo-radiotherapy, ****S+RT***: Surgery and radiotherapy,
****ECOG***: Eastern Cooperative Oncology Group,

Table 3.3 Number of patients in each treatment group based on recurrence & distant metastasis status

Recurrence / Distant Metastasis Status	Treatment		Total
	S + CRT	S + RT	
Both Negative	59 (83%)	48 (72%)	107 (78%)
Either or Both Positive	12 (17%)	19 (28%)	31 (22%)
Total	**71**	**67**	**138**

****S+CRT***: Surgery and chemo-radiotherapy, ****S+RT***: Surgery and radiotherapy,

Table 3.4 Number of patients in each treatment group based on ECE* & margin status

ECE* / Margin Status	Treatment		Total
	S + CRT	S + RT	
Both Negative	34 (55%)	31 (54%)	65 (55%)
Either or Both Positive	28 (45%)	26 (46%)	54 (45%)
Total	**62**	**57**	**119**

****ECE:*** Extracapsular extension
****S+CRT***: Surgery and chemo-radiotherapy, ****S+RT***: Surgery and radiotherapy,

Table 3.5 Radiation and chemotherapy type and dosage for patients with advanced-stage oropharyngeal squamous cell carcinoma treated with multimodality.

Variable	S+CRT* (n=71)	S+RT* (n=67)	Total (n=138)
Radiation Type *(%)*			
Conventional	5 (7%)	29 (43%)	34 (25%)
*IMRT**	66 (93%)	38 (57%)	104 (75%)
Radiation Dose *(mean Gy)*			
Primary	63.3	60.4	63.2
Neck	57	49.8	53.4
Chemotherapy Type *(%)*			
Cisplatin	51 (72%)	NA	NA
Carboplatin	20 (28%)		
Chemotherapy Dose *(mg/m^2)*			
Cisplatin 70 mg/m^2	2 (3%)	NA	NA
Cisplatin 100 mg/m^2	49 (69%)		
Carboplatin 70 mg/m^2	17 (24%)		
Carboplatin 100 mg/m^2	3 (4%)		

*__S+CRT__: Surgery and chemo-radiotherapy, *__S+RT__: Surgery and radiotherapy,
*__IMRT:__ Intensity modulated radiation therapy

Table 3.6 Demographics of patients with advanced-stage oropharyngeal squamous cell carcinoma with single-modality treatment.

Variable	Single-Modality (n=45)	Multimodality (n=138)	*p*-value
Age			
Mean	64.2	54.5	< 0.001
SD	12.2	8.2	
Gender *(%)*			
Male	36 (80 %)	112 (81%)	0.8
Female	9 (20 %)	26 (19%)	
Overall Stage *(%)*			
3	19 (42 %)	20 (14%)	< 0.001
4	26 (58 %)	118 (86%)	
Subsite *(%)*			
Tonsil	22 (49 %)	82 (59%)	
Base of Tongue	15 (33 %)	39 (28%)	0.5
Soft Palate	1 (2 %)	4 (3%)	
Posterior Wall	7 (16 %)	13 (10%)	
P16 *(%)*			
Positive	14 (31 %)	56 (41%)	0.2
Negative	31 (69 %)	82 (59%)	
Smoking *(%)*			
Yes	33 (73 %)	99 (72%)	1.0
No	12 (27 %)	39 (28%)	
ECOG* *(%)*			
0 & 1	24 (53 %)	125 (91%)	< 0.001
2 & 3	21 (47 %)	13 (9%)	

**ECOG*: Eastern Cooperative Oncology Group.

Table 3.7 Demographics of 33 patients with advanced-stage oropharyngeal squamous cell carcinoma and missing p16 data.

Variable	Excluded (n=33)	Included (n=138)	*p*-value
Age			
Mean	54.9	54.5	0.8
SD	8.7	8.2	
Gender			
Male	27 (82%)	112 (81%)	1.0
Female	6 (18)	26 (19%)	
Overall Stage			
3	8 (24 %)	20 (14%)	0.2
4	25 (76 %)	118 (86%)	
Subsite			
Tonsil	11 (68%)	82 (59%)	
Base of Tongue	9 (28%)	39 (28%)	0.6
Soft Palate	0 (0 %)	4 (3%)	
Posterior Wall	2 (5.0%)	13 (10%)	
P16			
Positive	-	56 (41%)	NA
Negative	-	82 (59%)	
Smoking			
Yes	25 (76%)	99 (72%)	0.8
No	8 (24%)	39 (28%)	
ECOG*			
0 & 1	2 (87%)	125 (91%)	< 0.001
2 & 3	6 (18%)	13 (9%)	

**ECOG*: Eastern Cooperative Oncology Group.

Table 3.8 Univariate Cox's Proportional Hazard Model of survival in 138 patients with advanced oropharyngeal squamous cell carcinoma

Covariate	Overall Survival		Disease Specific Survival		Disease Free Survival	
	Hazard Ratio (95 % CI)	*p*-value	Hazard Ratio (95 % CI)	*p*-value	Hazard Ratio (95 % CI)	*p*-value
Age	0 .98 (0.86-3.2)	0.14	1.1 (1.0-1.1)	***0.02***	1.1 (1.0-1.1)	***0.004***
Female Gender (vs. Male)	1.7 (0.86-3.2)	0.14	1.8 (0.74-4.3)	0.2	1.6 (0.72-3.6)	0.25
ECOG* 2 & 3 (vs. 0 & 1)	3.0 (1.5-6.3)	***0.003***	3.6 (1.4-9.1)	***0.006***	2.9 (1.2-7.1)	***0.02***
Smoking (vs. Non-smoker)	2.6 (1.2-5.8)	***0.02***	3.3 (1.0-11.2)	***0.05***	3.1 (1.1-8.9)	***0.034***
S + CRT* Treatment (vs. S+RT*)	0.48 (0.26-0.87)	***0.016***	0.6 (0.27-1.3)	0.2	0.57 (0.27-1.2)	0.12
P16 Negative (vs. Positive)	2.16 (1.21-3.88)	***0.009***	2.07 (0.94-4.57)	0.07	2.0 (0.99-4.06)	0.06
ECE* Positive (vs. Negative)	2.5 (1.4-4.5)	***0.003***	4.9 (1.9-12.3)	**0.001**	4.5 (2.0-10.1)	***<0.001***
Margins Positive (vs. Negative)	1.6 (0.5-5.2)	0.42	0.99 (0.13-7.3)	0.99	2.5 (0.76-8.3)	0.1
TNM Stage IV (vs. Stage III)	2.2 (0.78-6.1)	0.13	4.9 (0.7-36.1)	0.12	6.2 (0.84-45.2)	0.07

* ***ECOG***: Eastern Cooperative Oncology Group, * ***S+CRT:*** Surgery + Chemoradiation, * ***S+RT:*** Surgery + Radiation, * ***ECE***: Extracapsular extension

Table 3.9 Multivariate Cox's Proportional Hazard Model of survival in 138 patients with advanced oropharyngeal squamous cell carcinoma

Covariate	Overall Survival		Disease Specific Survival		Disease Free Survival	
	Hazard Ratio (95 % CI)	*p*-value	Hazard Ratio (95 % CI)	*p*-value	Hazard Ratio (95 % CI)	*p*-value
Age	1.0 (0.97 -1.1)	0.72	0.99 (0.93-1.0)	0.72	1.0 (0.96-1.1)	0.84
Female Gender (vs. Male)	1.4 (0.69-2.9)	0.34	1.3 (0.46-3.7)	0.63	1.4 (0.56-3.7)	0.45
ECOG* 2 & 3 (vs. 0 & 1)	1.5 (0.68-3.5)	0.30	1.7 (0.59-5.1)	0.32	1.3 (0.48-3.6)	0.59
Smoking (vs. Non-smoker)	2.7 (1.2-6.3)	***0.02***	3.3 (0.95-11.5)	0.06	3.5 (1.2-10.4)	***0.03***
S + CRT* Treatment (vs. S+RT*)	0.53 (0.28-1.0)	0.06	0.69 (0.29-1.6)	0.4	0.66 (0.29-1.4)	0.29
P16 Negative (vs. Positive)	1.74 (0.92-3.3)	0.09	1.58 (0.64-3.9)	0.31	1.7 (0.78-3.8)	0.18
ECE* Positive (vs. Negative)	2.2 (1.1-4.4)	***0.02***	5.0 (1.7-15.0)	**0.004**	3.9 (1.6-10.0)	***0.003***
Margins Positive (vs. Negative)	1.9 (0.54-6.4)	0.33	1.1 (0.13-8.6)	0.95	2.8 (0.76-10.2)	0.12
TNM Stage IV (vs. Stage III)	1.5 (0.48-4.8)	0.47	1.6 (0.18-15.2)	0.66	2.6 (0.29-21.9)	0.39

**ECOG*: Eastern Cooperative Oncology Group, **S+CRT:* Surgery + Chemoradiation, **S+RT:* Surgery + Radiation, **ECE*: Extracapsular extension

Table 3.10 Post-hoc power calculation using Log-rank test.

Log-rank Test Comparing Two Survival Rates Based on Treatment	Hazard Ratio (95 % CI)	*p*-value	Sample Size	Power	Estimated Sample Size for 80% power
OS*: All Cohort	0 .48 (0.26-.87)	***0.014***	138	***99 %***	---
DSS*: All Cohort	0.6 (0.27-1.3)	0.21	138	84 %	---
DFS*: All Cohort	0.57 (0.27-1.2)	0.12	138	91 %	---
OS: Non-Smoker	0.94 (0.2-4.2)	0.94	39	3.8 %	8702
OS: Smokers	0.45 (0.23-0.88)	***0.017***	99	***97 %***	---
DSS: Non-Smoker	--------	0.16	39	--- %	---
DSS: Smoker	0.48 (0.2-1.2)	0.097	99	95 %	---
DFS: Non-Smoker	--------	0.10	39	---- %	---
DFS: Smoker	0.42 (0.19-0.97)	***0.036***	99	***99 %***	---
OS: P16 -ve	0 .5 (0.2-1.2)	0.10	56	72 %	68
OS: P16 +ve	0.56 (0.23-1.4)	0.19	82	73 %	97
DSS: P16 -ve	0.77 (0.26-2.3)	0.63	56	16 %	475
DSS: P16 +ve	0.58 (0.18-1.9)	0.36	82	68 %	110
DFS: P16 -ve	0.74 (0.27-1.9)	0.55	56	18 %	358
DFS: P16 +ve	0.52 (0.18-1.5)	0.22	82	83 %	---
OS: ECE* -ve & Margins -ve	1.2 (0.38-4.0)	0.08	65	11 %	946
OS: ECE +ve &/or Margins +ve	0.39 (0.13-1.2)	0.13	54	93 %	---
DSS: ECE -ve & Margins -ve	1.1 (0.2-6.0)	0.25	65	6 %	3462
DSS: ECE +ve &/or Margins +ve	0 .52 (0.14-1.9)	0.63	54	67 %	74
DFS: ECE -ve & Margins -ve	1.7 (0.33-8.2)	0.07	65	57 %	112
DFS: ECE +ve &/or Margins +ve	0.52 (0.14-1.9)	0.58	54	67 %	74
OS: P16 +ve & Smoker	0.33 (0.1-0.9)	***0.029***	58	***98 %***	---
OS: P16 +ve & Non-Smoker	---	0.136	24	--- %	---
OS: P16 -ve & Smoker	0.79 (0.32-1.9)	0.598	41	11 %	606
OS: P16 -ve & Non-Smoker	---	***0.049***	15	--- %	---
DSS: P16 +ve & Smoker	0.25 (0.05-1.2)	***0.066***	58	***99 %***	---
DSS: P16 +ve & Non-Smoker	---	0.194	24	--- %	---
DSS: P16 -ve & Smoker	0 .99 (0.3-2.9)	0.997	41	3 %	333353
DFS: P16 +ve & Smoker	0.19 (0.04-0.89)	***0.018***	58	***100 %***	30
DFS: P16 +ve & Non-Smoker	---	0.14	24	--- %	---
DFS: P16 -ve & Smoker	0.97 (0.37-2.6)	0.95	41	3 %	36294

*__OS:__ Overall Survival, *__DSS:__ Disease Specific Survival,
*__DFS:__ Disease Free Survival, *__ECE__: Extracapsular extension

Figure 3.1 Summary of all patients with OPSCC.

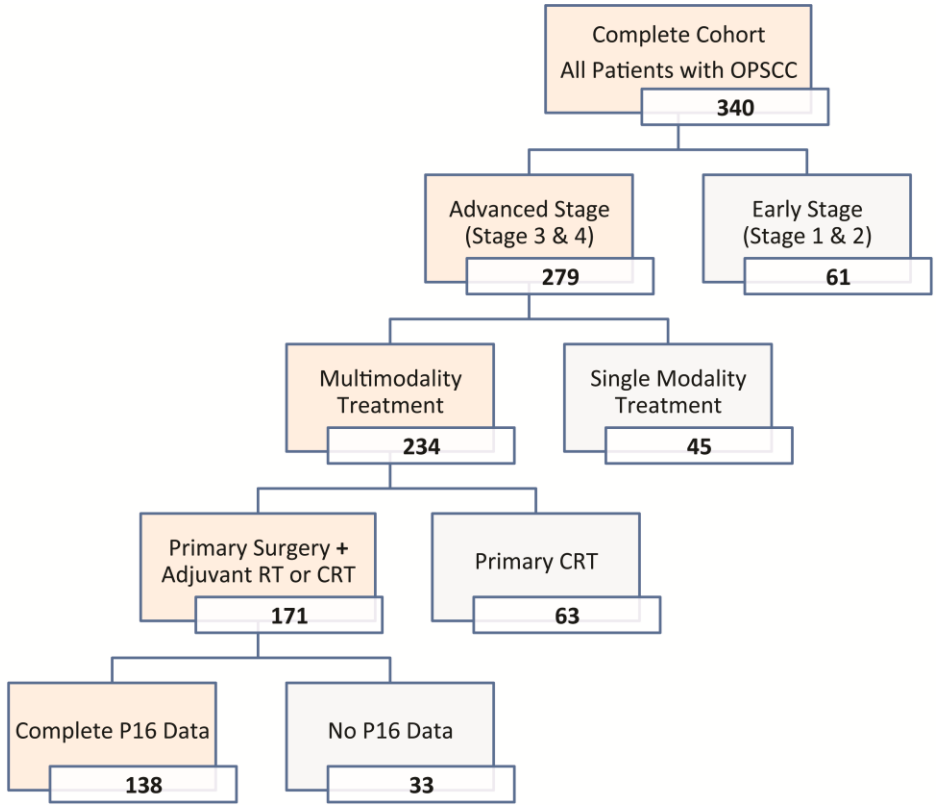


Figure 3.2 Summary of patients included in the study.

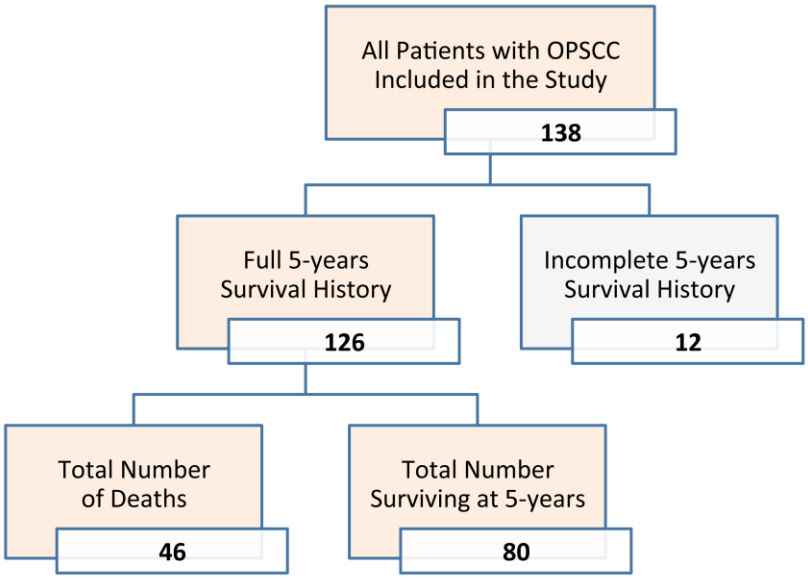
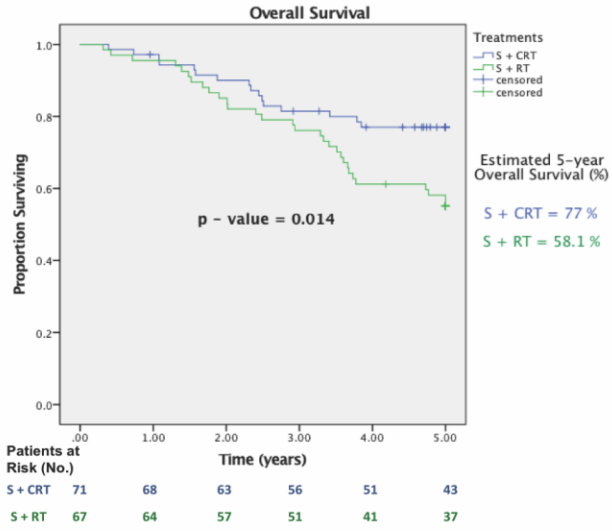
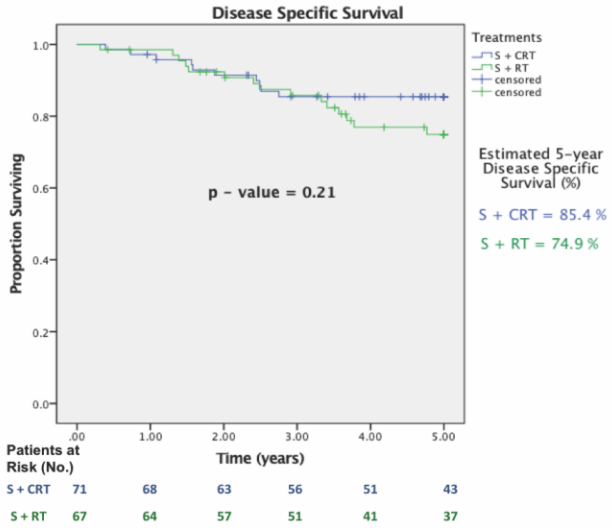


Figure 3.3 Survival analysis of all patient with advanced OPSCC





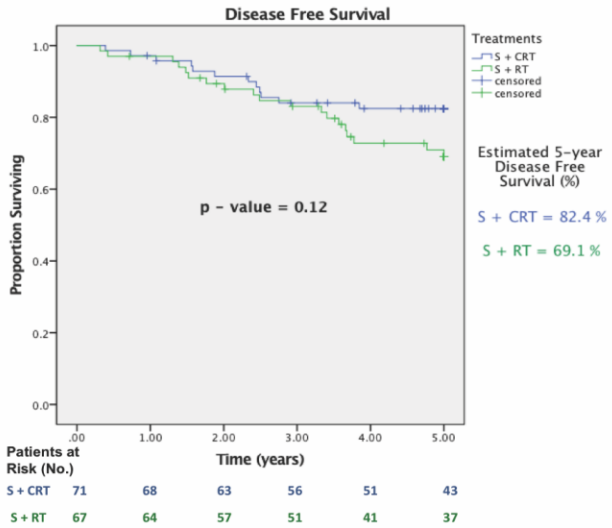


Figure 3.4 Survival of advanced OPSCC patients according to smoking status.

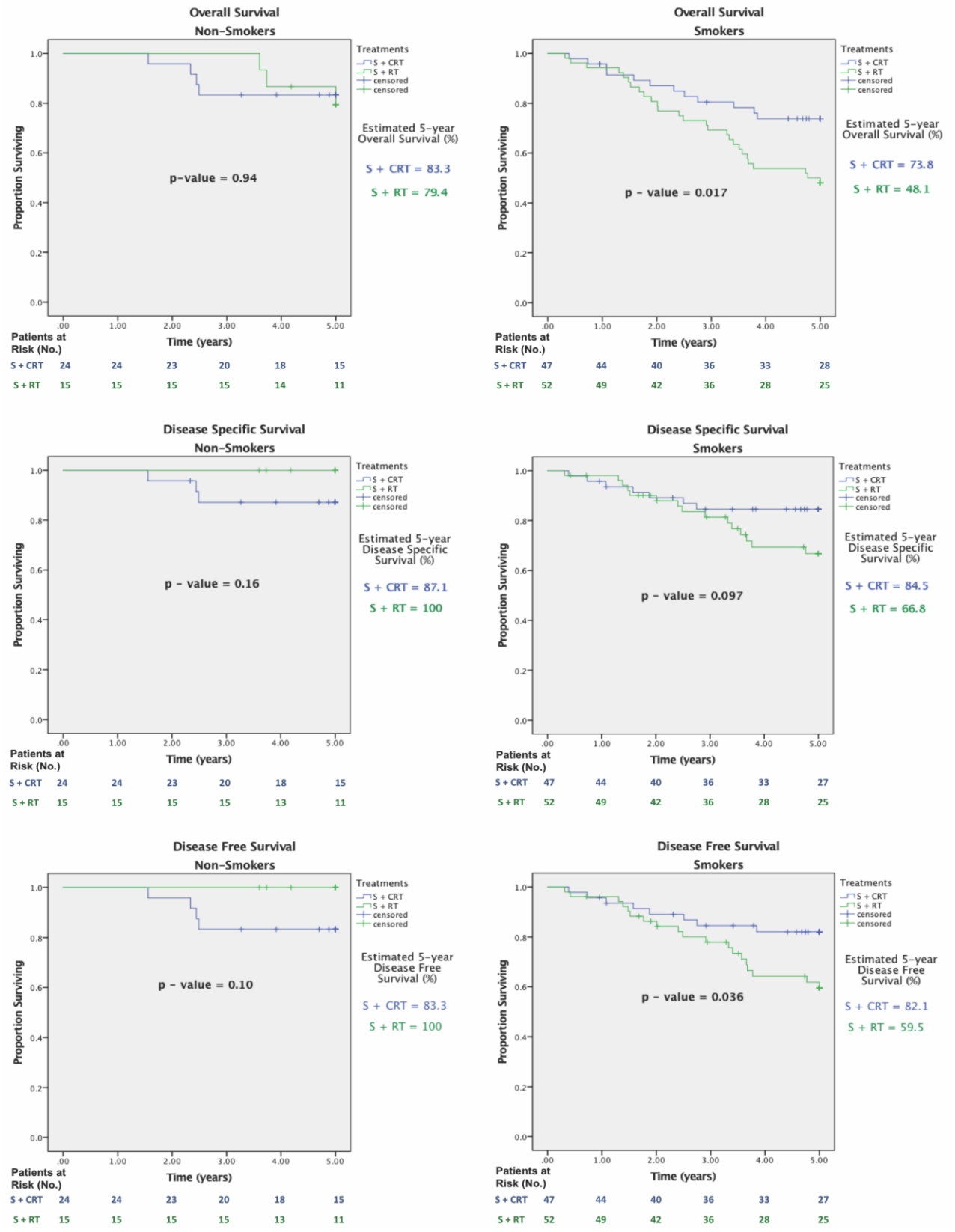


Figure 3.5 Survival of advanced OPSCC patients according to P16 status.

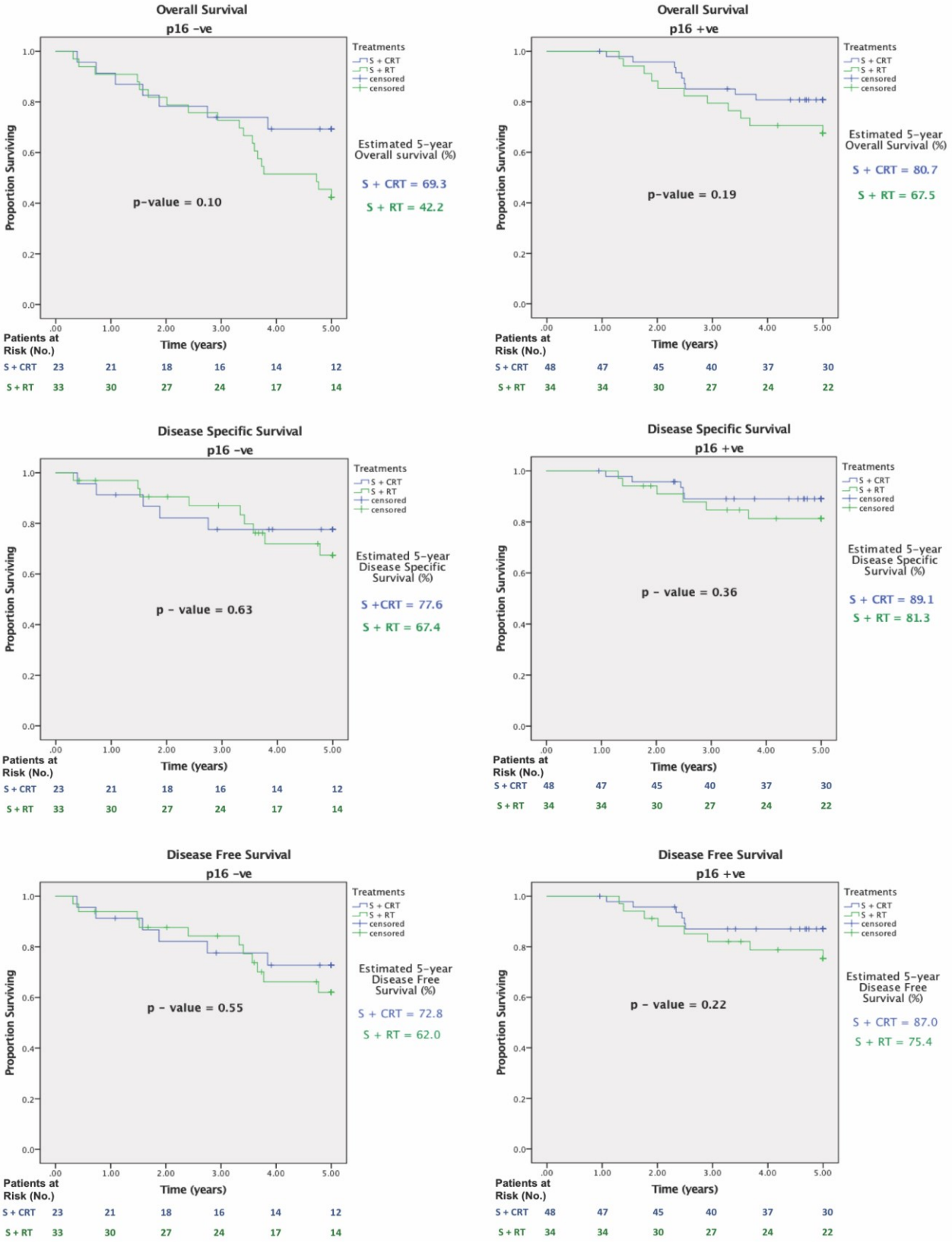


Figure 3.6 Survival of advanced OPSCC patients according to ECE & margins status

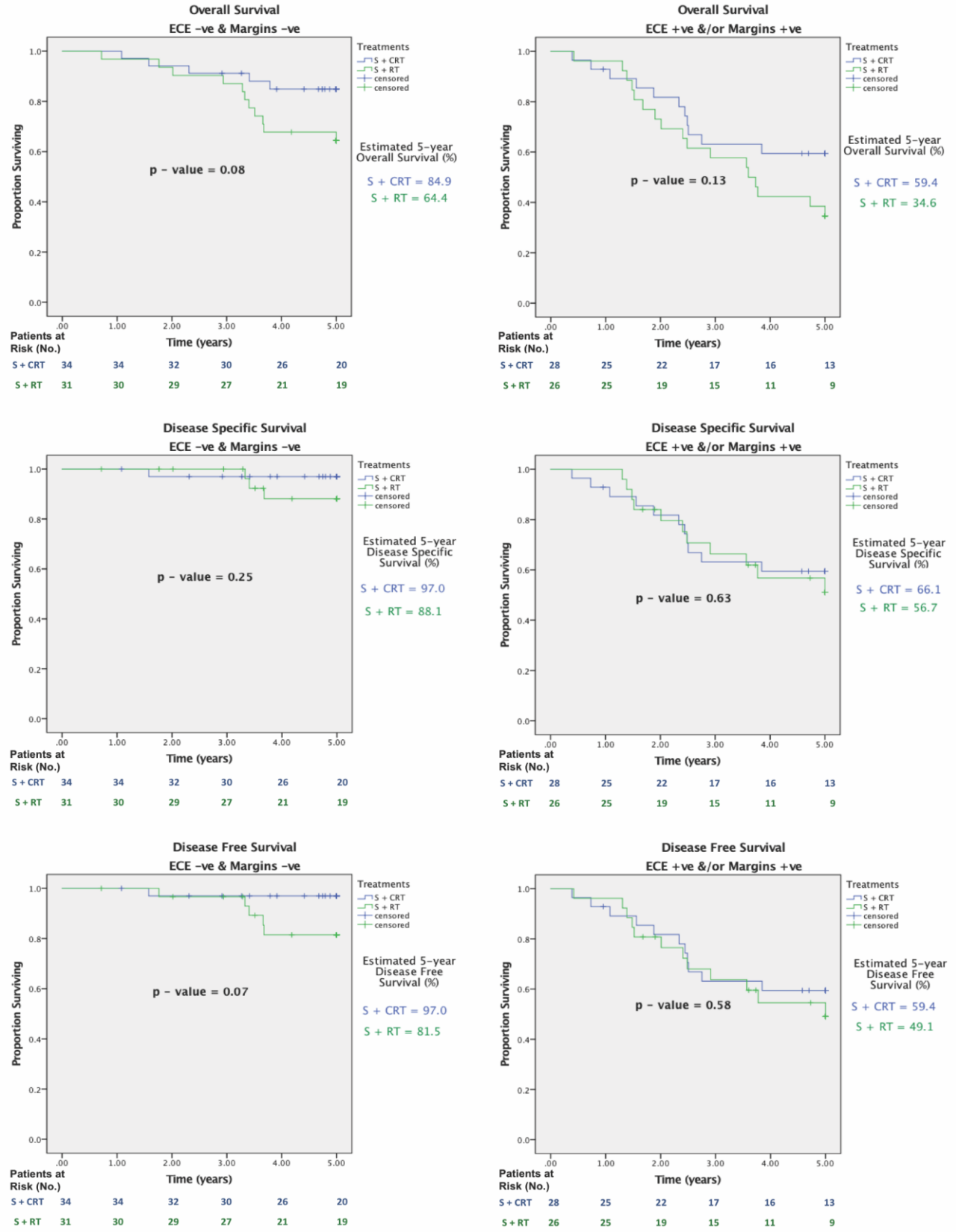
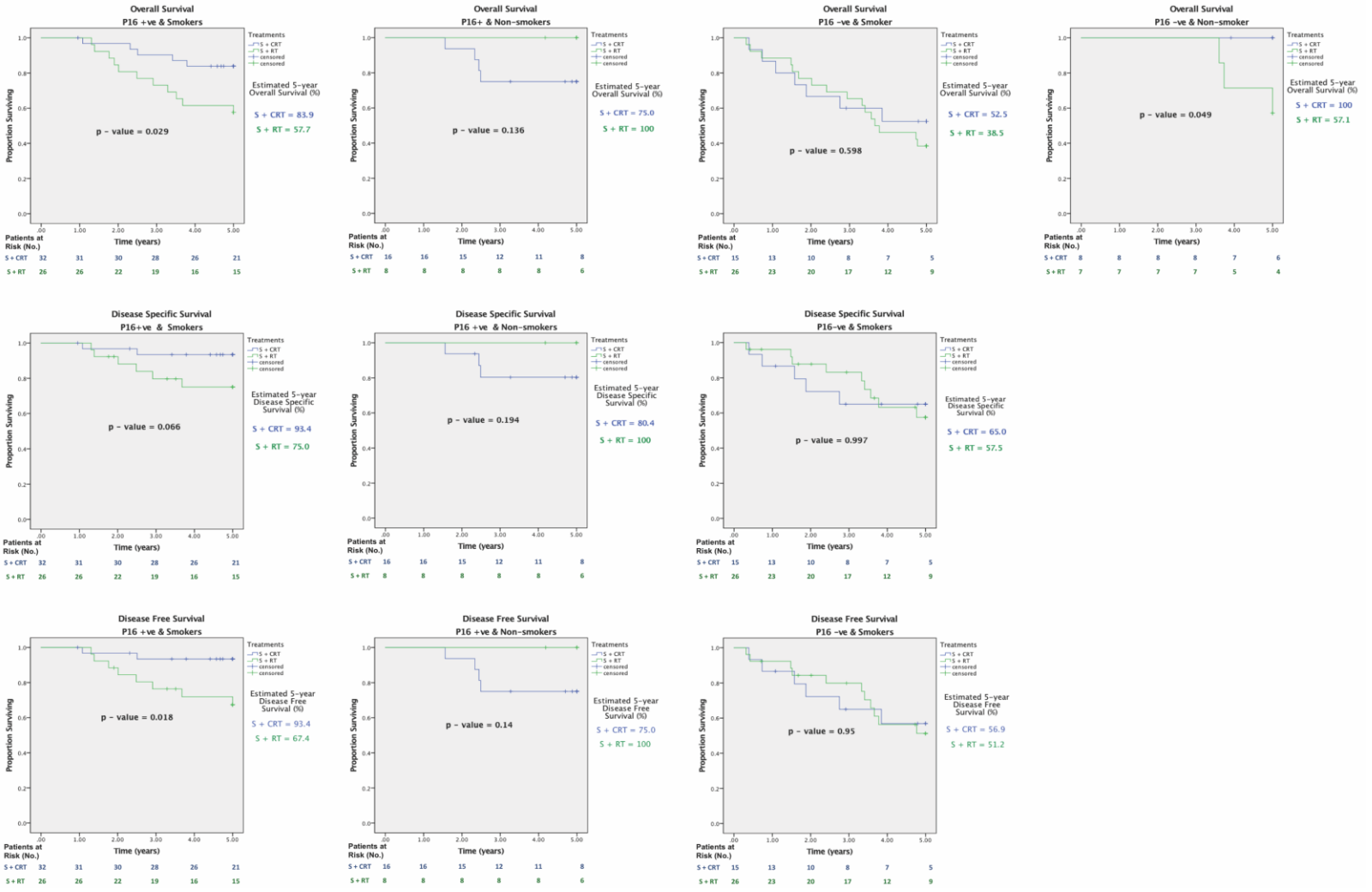


Figure 3.7 Survival of advanced OPSCC patients according to combined p16 & smoking status. No Kaplan Meier curves generated for p16-ve & nonsmokers because there were no events under DSS (disease specific deaths), or DFS (disease specific deaths, recurrences, or distant metastasis).



Chapter 4: Discussion & Conclusions

4.1 Discussion & summary of findings

HNSCC is the is the fifth most common human cancer worldwide.[1] Based on the United States 2016 cancer statistics it accounts for approximately 62,000 cases and 13,000 deaths annually.[2] Despite the decrease in the incidence of HNSCC over the past 30 years in Canada and most of the world,[3-5] OPSCC incidence has increased by 70% in the United States and 60% in Canada between 1983 and 2002.[6-9] The increasing incidence of OPSCC is thought to be driven largely by HPV-16 infection.[7,10,11] It is expected that by 2020 the incidence of HPV related OPSCC will exceed the incidence HPV related cervical cancer.[7] This trend is expected to continue despite national HPV vaccination campaigns, with epidemiological estimates suggesting the impacts of vaccination on HPV-OPSCC incidence may not result in a decline until 2060.[12]

Patients with HPV-OPSCCs have favorable survival outcomes compared to non-HPV-OPSCC.[8,13-15] The available staging system and treatment guidelines of OPSCC are based on a historical cohort of patients mostly comprised of smokers with non-HPV-OPSCC.[16,17] These results therefore poses an important and controversial question to be addressed regarding the staging and treatment of OPSCC with the current majority of patients harboring HPV positive disease, many of which are non-smokers. With the knowledge that HPV positive vs. negative OPSCCs are distinct from molecular, pathologic and clinical perspectives, should these cancers be diagnosed and treated differently? In recent years, there has been a substantial amount of clinical research focused on addressing this question. In patients undergoing primary surgery for HPV-

OPSCC, de-escalation of post-operative treatment can be considered by minimizing the use of chemotherapy and reducing radiation doses. There are three ongoing clinical trials investigating the use of treatment de-escalating strategies in the management of HPV-OPSCC in those undergoing primary surgery,[18] namely the ECOG E3311, (NCT01898494), ADEPT (NCT01687413), and the U.K.-based PATHOS (NCT02215265). The E3311 and PATHOS trials are investigating the effects reducing the total dose of post-operative RT for those with advanced stage HPV-OPSCC, with negative margins and ECE, from 60 Gy to 50 Gy. With regards to chemotherapy, the current NCCN guidelines state the main indications for post-operative adjuvant chemotherapy are either positive margins and/or positive ECE. The ADEPT & PATHOS trials are investigating the survival benefit of chemotherapy in patients with advanced stage HPV-OPSCC with ECE (ADEPT & PATHOS) and positive (< 1 mm) (PATHOS).

This thesis investigated the survival benefit of adding chemotherapy in the primary surgical setting followed by adjuvant RT in the management of all patients with advanced stage OPSCC. In a recent publication summarizing the NCCN guidelines and the current evidence supporting the use of adjuvant chemotherapy in addition to S+RT, the only two variables were associated with survival benefit included positive surgical margins and positive ECE.[19]

This thesis provides additional information by stratifying patients based on p16 and smoking status separately or combined. Starting with our multivariable analysis (n=138), after adjustment for all covariates, smoking status and ECE were both independent predictors of OS and DFS, while ECE was also a predictor of DSS (Table 3.9). The p16 in our cohort was not a significant independent predictor for survival at p-

value < 0.05 but was significant at p-value < 0.1 in our multivariate analysis after adjusting for all covariates included in the analysis (age, gender, ECOG, smoking, treatment, ECE, margins, and overall TNM stage) (Table 3.9), which contradicts much of the current literature, whereas in the univariate analysis it was a significant predictor for OS and borderline significant at 0.07 & 0.06 for DSS & DFS respectively (Table 3.8). The p16 status in OPSCC is an important predictor of survival, however due to two main limitations in our cohort it did not reach significance. First, the small number of our cohort (138) decreases significance in a multivariate analysis especially in the setting of other covariates such as smoking or ECE, which are nearly synonymous with p16 negativity. Secondly, the analysis took place from 1998 to 2009, in a cohort where the majority of p16 positive OPSCC were also smokers (≥10 pack/year smokers, 71%) (p16 + Smokers/p16+ Non-smokers = 58/24). In such a cohort, the pre-test probability of p16 positivity predicting oncogenic HPV positivity should be lower than in a cohort of non-smokers, as has been demonstrated in p16 positive oral cavity cancers.[20,21] Despite survival among HPV positive OPSCC are significantly better than HPV negative, the positive history of smoking exposure decreases survival among HPV positive OPSCC by approximately 1% per pack-year of smoking exposure.[22,23] In support of p16 positivity still being a significant predictor of survival, a prior publication on the same cohort including patients treated with RT/CRT with a total of 200 patients, p16 was a significant predictor of survival on multivariate analysis.[24] Another study published by a group at Ohio State University comparing patients undergoing primary surgery versus primary RT/CRT in OPSCC demonstrated p16 positivity to be a significant predictor of overall survival on univariate analysis, however on multivariate analysis p16 status was not a

significant independent predictor of survival.[25] In their multivariate analysis they included HPV status based on in situ hybridization (ISH) testing not p16 testing, as fifty-three patients were p16- positive/HPV-negative by ISH and three were p16-negative/HPV-positive. For better illustration, we have summarized both our results and the Ohio State university results (Table 4.1, 4.2, and 4.3). On the other hand, another study by a group from Mount Sinai Hospital showed almost similar results to ours in their multivariate analysis where p16 was not a significant predictor for OS and DSS and their total cohort was only 55 patients with 24 patients with p16 positive OPSCC compared to 30 patients with OPSCC who are smokers.[26]

In our survival analysis we compared three different survival outcomes: OS, DSS, & DFS for the whole cohort regardless to the other covariates, the addition of chemotherapy was associated with a statistically significant better 5-year OS (77% vs. 58.1%); however, it was not statistically significant for DSS (85.4% vs. 74.9%) and DFS (84.2% vs. 69.1%) with a post hoc power calculation of 84% and 91% respectively (Table 3.10).

We stratified patients based on their p16 and smoking status separate and combined. Based on smoking status, the survival benefit from the addition of chemotherapy in post-operative setting was significant in smokers OS & DFS and borderline for DSS. In P16 positive patients, the addition of chemotherapy was not associated with improved survival outcomes (Table 3.10). When stratifying patients based on both P16 and smoking the power of our analysis significantly decreases due to the small groups being compared. Despite these small subgroups, p16 positive smokers who received chemotherapy had a significantly higher OS & DFS at < 0.05 and DSS at $<$

0.1 (Table 3.10). A group from Ohio State University recently reported similar results supporting our findings; demonstrating no survival benefit (OS) when comparing S+RT versus S+CRT in HPV-OPSCC patients.[25] However, they did not stratify patients based on smoking and/or p16 positivity and only reported OS as a survival outcome. Taken together, our study is the first to provide evidence with regards to the survival benefit when comparing S+CRT versus S+RT stratifying by p16 and smoking status separate and combined. Our survival results coincide with the published literature showing no survival benefit in the HPV-OPSCC, however we were also able to show that a certain subgroup with advanced stage OPSCC might have a survival benefit from the addition of chemotherapy (smokers and p16+ve/smokers).

Based on the NCCN guidelines the only two indications for adjuvant postoperative chemotherapy in patients with OPSCC, regardless of HPV and smoking status, are positive margins and/or ECE. In our dataset, when investigating the survival benefit of post-operative chemotherapy no significant survival benefit is seen when patients are not stratified according to p16 or smoking status. A recent critical appraisal of the current NCCN clinical practice guidelines with regards to the indications of post-operative adjuvant chemotherapy in the management of OPSCC.[27] The NCCN reports their consensus on the indication of post-operative adjuvant chemotherapy for margin and/or ECE positivity as a category one, which is based upon high-level evidence and the uniform consensus that the intervention is appropriate. Their consensus was based on 2 main clinical trails [Radiation Therapy Oncology Group (RTOG 9501) trial, and the European Organization for Research and Treatment of Cancer (EORTC 22931) trial], and a collaborative, comparative analysis of both trials. A review of these trials noted

significant limitations in their methodology, most importantly a lack of reporting HPV/p16 status. These studies lack adequate validity to be extrapolated to OPSCC patients, who must be categorized based on HPV/p16 positivity. This highlights the importance of further studies investigating the role of postoperative chemotherapy in the management of OPSCC, in the HPV positive cohort in particular. Our study is therefore an important first step in this regard.

Regarding chemotherapy toxicity, the National Cancer Institute has published standardized definitions for adverse events to describe the severity of organ toxicity for patients receiving cancer therapy. They graded toxicity as mild (Grade 1), moderate (Grade 2), severe (Grade 3), or life-threatening (Grade 4), with specific parameters according to the organ system involved (ex: lung, kidney, heart, gastrointestinal tract, and neurological). Death (Grade 5) is used for some of the criteria to denote a fatality. In 2004, a study was published comparing RT versus CRT in the management of advanced stage OPSCC and in their final analysis found significantly higher rates of teeth-related problems and numerically higher rates of several other side effects in the CRT group.[28] The percentage of patients with no evidence of disease but grade 3/4 late effects was 30% with RT alone and 56% with CRT (although this was not statistically significant; P = .12).[28] Other studies have indicated that as more chemotherapy is added, the relative risk of toxicity increases.[8,29-33] With our work and more ongoing research we can establish new guidelines with more clear indication for the addition of chemotherapy for the right group of patients were the benefit overcomes the toxicity.

4.2 Strengths & limitations

Our main strengths in this study are that this is the 2nd largest cohort of OPSCC undergoing primary surgical modality treatment analyzed in the English literature, and the largest in Canada. Despite this represent the 2nd largest cohort after the paper from the Ohio State University,[25] we were the only to further stratify our cohort based smoking status and analyze their survival (OS, DSS, & DFS) and still maintain a post-hoc power of > 95% for smokers across all three survival periods. This was reflected in a new conclusion of showing a significant survival benefit of the addition of chemotherapy in the smokers' cohort. We were also able to stratify patients based on p16 and smoking status combined and assess for survival accordingly, however we did not have adequate power to test a true association. The survival analysis used in their paper was only from an OS perspective only, whereas our analysis investigated DSS & DFS in addition to OS. This provided insight into the benefit of chemotherapy with regards to locoregional recurrence, distant metastasis, and death from OPSCC.

Our main limitations included the retrospective design of the study and the small numbers of patients in subgroups especially when stratifying by p16 and smoking status combined. Survival history was complete for 126 out of the 138 patients. Covariate data was complete for all 138 patients except for ECE/Margin status, which was missing for 19 patients out of the 138. The survival analysis with regards to the effect of the ECE/Margin status was performed for only 119 patients. Also, our cohort of patients were from 1998 to 2009 with more than 70% smokers, which has an impact on those who are P16 positive and the accuracy of this test as a surrogate marker for HPV-OPSCC.

4.3 Conclusions & future directions

In advanced stage OPSCC treated with primary surgery and RT, the addition of chemotherapy may improve disease specific survival in select patients, mainly smokers and p16+ve smokers. The indications for adjuvant chemotherapy need to be revised for the whole cohort of OPSCC in general and for the HPV related OPSCC in particular, due to its distinct tumor behavior, clinical aspects, patient demographics, and prognosis. This also reflects the need to a new staging and treatment guidelines that consider HPV positive and negative OPSCC distinct malignant diseases. Further prospective trials that include p16 status would be recommended to address this hypothesis.

Table 4.1 Demographics comparison between University of Alberta and Ohio State University data

	Variable	UoA* Total (n=138)	OSU* Total (n=296)
Demographic	**Age**		
	Mean	54.5	57.8
	SD	8.2	9.2
	Gender		
	Male	112 (81%)	235 (79.4%)
	Female	26 (19%)	61 (20.6%)
Clinical	**T-Stage**		
	1	30 (22%)	77 (26%)
	2	41 (30%)	126 (42.6%)
	3	50 (36%)	48 (16.2%)
	4a	17 (12%)	45 (15.2%)
	N-Stage		
	0	15 (11%)	38 (11%)
	1	20 (14%)	57 (14%)
	2	93 (68%)	186 (63.3%)
	3	10 (7%)	13 (7%)
	Overall Stage		
	1	-	11 (3.7%)
	2	-	12 (4.1%)
	3	20 (14%)	62 (21.1%)
	4	118 (86%)	209 (71.1%)
	Subsite		
	Tonsil	82 (59%)	198 (67.1%)
	Base of Tongue	39 (28%)	74 (25.1%)
	Soft Palate	4 (3%)	-
	Posterior Wall	13 (10%)	-
	Other		23 (7.8%)
Risk Factors	**P16**		
	Positive	82 (59%)	222 (77.1%)
	Negative	56 (41%)	66 (22.9%)
	HPV		
	Yes	-	172 (40.5%)
	No	-	117 (59.5%)
	Smoking		
	Yes	99 (72%)	201 (71%)
	No	39 (28%)	82 (29%)
	P16 & Smoking		
	P16 +ve & Smokers	58 (42%)	-
	P16 +ve & Non-Smokers	24 (17%)	-
	P16 -ve & Smokers	41 (30%)	-
	P16 -ve & Non-Smokers	15 (11%)	-

*__UOA:__ University of Alberta, *__OSU:__ Ohio State University

Table 4.2 Comparison of Univariate Cox's Proportional Hazard Model of survival between University of Alberta and Ohio State University data

Covariate	UoA* - Overall Survival		OSU* - Overall Survival	
	Hazard Ratio (95 % CI)	*p*-value	Hazard Ratio (95 % CI)	*p*-value
Smoking (vs. Non-smoker)	2.6 (1.2-5.8)	***0.02***	2.0 (1.2-3.3)	***0.006***
P16 Negative (vs. Positive)	2.16 (1.21-3.88)	***0.009***	2.98 (1.99-4.45)	***< 0.001***
HPV Negative (vs. Positive)	-	-	3.39 (2.26-5.1)	***< 0.001***
ECE* Positive (vs. Negative)	2.5 (1.4-4.5)	***0.003***	2.36 (1.58-3.53)	***< 0.001***
Margins Positive (vs. Negative)	1.6 (0.5-5.2)	0.42	1.59 (1.0-2.5)	0.047

****UOA:*** University of Alberta, ****OSU:*** Ohio State University

Table 4.3 Comparison of Multivariate Cox's Proportional Hazard Model of survival between University of Alberta and Ohio State University data

Covariate	UoA* - Overall Survival		OSU* - Overall Survival	
	Hazard Ratio (95 % CI)	*p*-value	Hazard Ratio (95 % CI)	*p*-value
Smoking (vs. Non-smoker)	2.7 (1.2-6.3)	***0.02***	1.3 (0.76-2.27)	0.32
P16 Negative (vs. Positive)	1.74 (0.92-3.3)	0.09	-	-
HPV Negative (vs. Positive)	-	-	2.36 (1.49-3.73)	***0.0002***
ECE* Positive (vs. Negative)	2.2 (1.1-4.4)	***0.02***	1.9 (1.26-2.98)	**0.0025**
Margins Positive (vs. Negative)	1.9 (0.54-6.4)	0.33	1.6 (0.97-2.7)	0.066

****UOA:*** University of Alberta, ****OSU:*** Ohio State University

References

1. McDonald JT, Johnson-Obaseki S, Hwang E, Connell C, Corsten M. The relationship between survival and socio-economic status for head and neck cancer in Canada. *Journal of Otolaryngology - Head & Neck Surgery 2014 43:1*. 2014;43(1):2. doi:10.1186/1916-0216-43-2.

2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA: A Cancer Journal for Clinicians*. 2016;66(1):7-30. doi:10.3322/caac.21332.

3. Canadian Cancer Statistics. May 2015:1-151.

4. Curado MP, Hashibe M. Recent changes in the epidemiology of head and neck cancer. *Curr Opin Oncol*. 2009;21(3):194-200. doi:10.1097/CCO.0b013e32832a68ca.

5. Propel. Tobacco Use in Canada: Patterns and Trends. April 2015:1-96.

6. Chaturvedi AK, Anderson WF, Lortet-Tieulent J, et al. Worldwide Trends in Incidence Rates for Oral Cavity and Oropharyngeal Cancers. *Journal of Clinical Oncology*. 2013;31(36):4550-4559. doi:10.1200/JCO.2013.50.3870.

7. Chaturvedi AK, Engels EA, Pfeiffer RM, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *J Clin Oncol*. 2011;29(32):4294-4301. doi:10.1200/JCO.2011.36.4596.

8. Ang KK, Harris J, Wheeler R, et al. Human Papillomavirus and Survival of Patients with Oropharyngeal Cancer. *N Engl J Med*. 2010;363(1):24-35. doi:10.1056/NEJMoa0912217.

9. Mehanna H, Beech T, Nicholson T, et al. Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer--systematic review and meta-analysis of trends by time and region. Eisele DW, ed. *Head Neck*. 2013;35(5):747-755. doi:10.1002/hed.22015.

10. Chaturvedi AK. Epidemiology and Clinical Aspects of HPV in Head and Neck Cancers. *Head and Neck Pathol*. 2012;6(S1):16-24. doi:10.1007/s12105-012-0377-0.

11. Romanitan M, Näsman A, Ramqvist T, et al. Human papillomavirus frequency in oral and oropharyngeal cancer in Greece. *Anticancer Res*. 2008;28(4B):2077-2080.

12. Gillison ML, Chaturvedi AK, Anderson WF, Fakhry C. Epidemiology of Human Papillomavirus-Positive Head and Neck Squamous Cell Carcinoma. *J Clin Oncol*. 2015;33(29):3235-3242. doi:10.1200/JCO.2015.61.6995.

13. Fakhry C, Westra WH, Li S, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective

clinical trial. *J Natl Cancer Inst*. 2008;100(4):261-269. doi:10.1093/jnci/djn011.

14. Dahlstrom KR, Calzada G, Hanby JD, et al. An evolution in demographics, treatment, and outcomes of oropharyngeal cancer at a major cancer center: a staging system in need of repair. *Cancer*. 2013;119(1):81-89. doi:10.1002/cncr.27727.

15. Huang SH, Xu W, Waldron J, et al. Refining American Joint Committee on Cancer/Union for International Cancer Control TNM stage and prognostic groups for human papillomavirus-related oropharyngeal carcinomas. *J Clin Oncol*. 2015;33(8):836-845. doi:10.1200/JCO.2014.58.6412.

16. MD PBO, MD SHH, MSc JS, et al. Development and validation of a staging system for HPV-related oropharyngeal cancer by the International Collaboration on Oropharyngeal cancer Network for Staging (ICON-S): a multicentre cohort study. March 2016:1-12. doi:10.1016/S1470-2045(15)00560-4.

17. Horne ZD, Glaser SM, Vargo JA, et al. Confirmation of proposed human papillomavirus risk-adapted staging according to AJCC/UICC TNM criteria for positive oropharyngeal carcinomas. *Cancer*. April 2016:1-10. doi:10.1002/cncr.30021.

18. Wilkie MD, Upile NS, Lau AS, et al. Transoral laser microsurgery for oropharyngeal squamous cell carcinoma: A paradigm shift in therapeutic approach. *Head & Neck*. 2016;38(8):1263-1270. doi:10.1002/hed.24432.

19. Sinha P, Piccirillo JF, Kallogjeri D, Spitznagel EL, Haughey BH. The role of postoperative chemoradiation for oropharynx carcinoma: A critical appraisal of the published literature and National Comprehensive Cancer Network guidelines. *Cancer*. 2015;121(11):1747-1754. doi:10.1002/cncr.29242.

20. Castellsague X, Alemany L, Quer M, et al. HPV Involvement in Head and Neck Cancers: Comprehensive Assessment of Biomarkers in 3680 Patients. *J Natl Cancer Inst*. 2016;108(6):djv403. doi:10.1093/jnci/djv403.

21. Chaturvedi AK, Anderson WF, Lortet-Tieulent J, et al. Worldwide Trends in Incidence Rates for Oral Cavity and Oropharyngeal Cancers. *Journal of Clinical Oncology*. 2013;31(36):4550-4559. doi:10.1200/JCO.2013.50.3870.

22. Gillison ML, Zhang Q, Jordan R, et al. Tobacco Smoking and Increased Risk of Death and Progression for Patients With p16-Positive and p16-Negative Oropharyngeal Cancer. *Journal of Clinical Oncology*. 2012;30(17):JCO.2011.38.4099–2111. doi:10.1200/JCO.2011.38.4099.

23. Chaturvedi AK, D'Souza G, Gillison ML, Katki HA. Burden of HPV-positive oropharynx cancers among ever and never smokers in the U.S. population. *Oral Oncology*. 2016;60:61-67. doi:10.1016/j.oraloncology.2016.06.006.

24. Seikaly H, Biron VL, Zhang H, et al. Role of primary surgery in the treatment of advanced oropharyngeal cancer. *Head Neck*. 2015;38(S1):E571-E579. doi:10.1002/hed.24042.

25. Kumar B, Cipolla MJ, Old MO, et al. Surgical management of oropharyngeal squamous cell carcinoma: Survival and functional outcomes. *Head Neck*. 2015;38(S1):E1794-E1802. doi:10.1002/hed.24319.

26. Stucken CL, de Almeida JR, Sikora AG, Tong CCL, Genden EM. Impact of human papillomavirus and smoking on survival outcomes after transoral robotic surgery. *Head Neck*. 2015;38(3):380-386. doi:10.1002/hed.23915.

27. Sinha P, Piccirillo JF, Kallogjeri D, Spitznagel EL, Haughey BH. The role of postoperative chemoradiation for oropharynx carcinoma: A critical appraisal of the published literature and National Comprehensive Cancer Network guidelines. *Cancer*. 2015;121(11):1747-1754. doi:10.1002/cncr.29242.

28. Denis F, Garaud P, Bardet E, et al. Final results of the 94-01 French Head and Neck Oncology and Radiotherapy Group randomized trial comparing radiotherapy alone with concomitant radiochemotherapy in advanced-stage oropharynx carcinoma. *Journal of Clinical Oncology*. 2004;22(1):69-76. doi:10.1200/JCO.2004.08.021.

29. Haughey BH, Sinha P. Prognostic factors and survival unique to surgically treated p16+ oropharyngeal cancer. *Laryngoscope*. 2012;122(S2):S13-S33. doi:10.1002/lary.23493.

30. Machtay M, Moughan J, Trotti A, et al. Factors associated with severe late toxicity after concurrent chemoradiation for locally advanced head and neck cancer: an RTOG analysis. *J Clin Oncol*. 2008;26(21):3582-3589. doi:10.1200/JCO.2007.14.8841.

31. Greven KM, White DR, Browne JD, Williams DWI, McGuirt WFS, D'Agostino RBJ. Swallowing Dysfunction is a Common Sequelae After Chemoradiation for Oropharynx Carcinoma. *American Journal of Clinical Oncology*. 2008;31(3):209-212. doi:10.1097/COC.0b013e3181595b10.

32. Nien HH, Sturgis EM, Kies MS, et al. Comparison of systemic therapies used concurrently with radiation for the treatment of human papillomavirus–associated oropharyngeal cancer. *Head & Neck*. 2016;38(S1):E1554-E1561. doi:10.1002/hed.24278.

33. Vainshtein JM, Samuels S, Tao Y, et al. Impact of xerostomia on dysphagia after chemotherapy–intensity- modulated radiotherapy for oropharyngeal cancer: Prospective longitudinal study. *Head & Neck*. 2016;38(S1):E1605-E1612. doi:10.1002/hed.24286.

Bibliography

1. Gandini S, Botteri E, Iodice S, et al. Tobacco smoking and cancer: a meta-analysis. Int J Cancer. 2008;122(1):155-164. doi:10.1002/ijc.23033.

2. Hashibe M, Brennan P, Benhamou S, et al. Alcohol drinking in never users of tobacco, cigarette smoking in never drinkers, and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. J Natl Cancer Inst. 2007;99(10):777-789. doi:10.1093/jnci/djk179.

3. Blot WJ, McLaughlin JK, Winn DM, et al. Smoking and drinking in relation to oral and pharyngeal cancer. Cancer Research. 1988;48(11):3282-3287.

4. Hashibe M, Brennan P, Chuang S-C, et al. Interaction between tobacco and alcohol use and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. Cancer Epidemiology Biomarkers & Prevention. 2009;18(2):541-550. doi:10.1158/1055-9965.EPI-08-0347.

5. Chaturvedi AK, Engels EA, Pfeiffer RM, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol. 2011;29(32):4294-4301. doi:10.1200/JCO.2011.36.4596.

6. Gillison ML, Broutian T, Pickard RKL, et al. Prevalence of Oral HPV Infection in the United States, 2009-2010. JAMA. 2012;307(7):693–11. doi:10.1001/jama.2012.101.

7. Chaturvedi AK. Epidemiology and Clinical Aspects of HPV in Head and Neck Cancers. Head and Neck Pathol. 2012;6(S1):16-24. doi:10.1007/s12105-012-0377-0.

8. Canadian Cancer Statistics. October 2016:1-142.

9. Carvalho AL, Magrin J, Kowalski LP. Sites of recurrence in oral and oropharyngeal cancers according to the treatment approach. Oral Diseases. 2003;9(3):112-118. doi:10.1034/j.1601-0825.2003.01750.x.

10. Seikaly H, Rieger J, Wolfaardt J, Moysa G, Harris J, Jha N. Functional outcomes after primary oropharyngeal cancer resection and reconstruction with the radial forearm free flap. Laryngoscope. 2003;113(5):897-904. doi:10.1097/00005537-200305000-00023.

11. Haughey BH, Sinha P. Prognostic factors and survival unique to surgically treated p16+ oropharyngeal cancer. Laryngoscope. 2012;122(S2):S13-S33. doi:10.1002/lary.23493.

12. Sinha P, Piccirillo JF, Kallogjeri D, Spitznagel EL, Haughey BH. The role of postoperative chemoradiation for oropharynx carcinoma: A critical appraisal of the published literature and National Comprehensive Cancer Network guidelines. Cancer. 2015;121(11):1747-1754. doi:10.1002/cncr.29242.

13. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Human papillomaviruses. IARC monographs on the evaluation of carcinogenic risks to humans / World Health Organization, International Agency for Research on Cancer. 2007;90:1-636.

14. O'Sullivan B, Huang SH, Siu LL, et al. Deintensification candidate subgroups in human papillomavirus-related oropharyngeal cancer according to minimal risk of distant metastasis. J Clin Oncol. 2013;31(5):543-550. doi:10.1200/JCO.2012.44.0164.

15. Sinha P, Piccirillo JF, Kallogjeri D, Spitznagel EL, Haughey BH. The role of postoperative chemoradiation for oropharynx carcinoma: A critical appraisal of the published literature and National Comprehensive Cancer Network guidelines. Cancer. 2015;121(11):1747-1754. doi:10.1002/cncr.29242.

16. Duvvuri U, Myers JN. Cancer of the head and neck is the sixth most common cancer worldwide. Curr Probl Surg. 2009;46(2):114-117. doi:10.1067/j.cpsurg.2008.10.002.

17. Argiris A, Karamouzis MV, Raben D, Ferris RL. Head and neck cancer. Lancet. 2008;371(9625):1695-1709. doi:10.1016/S0140-6736(08)60728-X.

18. Brockstein B. Management of sarcomas of the head and neck. Curr Oncol Rep. 2004;6(4):321-327.

19. Speight PM, Barrett AW. Salivary gland tumours. Oral Diseases. 2002;8(5):229-240.

20. Shah JP, Patel SG, Singh B. Jatin Shah's Head and Neck Surgery and Oncology. Elsevier Health Sciences; 2012.

21. Marur S, Forastiere AA. Head and Neck Squamous Cell Carcinoma: Update on Epidemiology, Diagnosis, and Treatment. Mayo Clinic Proceedings. 2016;91(3):386-396. doi:10.1016/j.mayocp.2015.12.017.

22. Forte T, Niu J, Lockwood GA, Bryant HE. Incidence trends in head and neck cancers and human papillomavirus (HPV)-associated oropharyngeal cancer in Canada, 1992–2009. Cancer Causes Control. 2012;23(8):1343-1348. doi:10.1007/s10552-012-0013-z.

23. Luryi AL, Yarbrough WG, Niccolai LM, et al. Public Awareness of Head and Neck Cancers. JAMA Otolaryngol Head Neck Surg. 2014;140(7):639–8. doi:10.1001/jamaoto.2014.867.

24. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA: A Cancer Journal for Clinicians. 2013;63(1):11-30. doi:10.3322/caac.21166.

25. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA: A Cancer Journal for Clinicians. 2015;65(1):5-29. doi:10.3322/caac.21254.

26. Blot WJ. Alcohol and cancer. Cancer Research. 1992;52(7 Suppl):2119s–2123s.

27. Gillison ML, Chaturvedi AK, Anderson WF, Fakhry C. Epidemiology of Human Papillomavirus-Positive Head and Neck Squamous Cell Carcinoma. J Clin Oncol. 2015;33(29):3235-3242. doi:10.1200/JCO.2015.61.6995.

28. Chi AC, Day TA, Neville BW. Oral cavity and oropharyngeal squamous cell carcinoma—an update. CA: A Cancer Journal for Clinicians. 2015;65(5):401-421. doi:10.3322/caac.21293.

29. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol. 2010;17(6):1471-1474. doi:10.1245/s10434-010-0985-4.

30. Watkinson J, Gilbert RW. Stell & Maran's Textbook of Head and Neck Surgery and Oncology, Fifth Edition. CRC Press; 2011.

31. Canadian Cancer Statistics. May 2015:1-151.

32. Curado MP, Hashibe M. Recent changes in the epidemiology of head and neck cancer. Curr Opin Oncol. 2009;21(3):194-200. doi:10.1097/CCO.0b013e32832a68ca.

33. Propel. Tobacco Use in Canada: Patterns and Trends. April 2015:1-96.

34. Romanitan M, Näsman A, Ramqvist T, et al. Human papillomavirus frequency in oral and oropharyngeal cancer in Greece. Anticancer Res. 2008;28(4B):2077-2080.

35. Chaturvedi AK, Anderson WF, Lortet-Tieulent J, et al. Worldwide Trends in Incidence Rates for Oral Cavity and Oropharyngeal Cancers. Journal of Clinical Oncology. 2013;31(36):4550-4559. doi:10.1200/JCO.2013.50.3870.

36. Ang KK, Harris J, Wheeler R, et al. Human Papillomavirus and Survival of Patients with Oropharyngeal Cancer. N Engl J Med. 2010;363(1):24-35. doi:10.1056/NEJMoa0912217.

37. Mehanna H, Beech T, Nicholson T, et al. Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer--systematic review and meta-analysis of trends by time and region. Eisele DW, ed. Head Neck. 2013;35(5):747-755. doi:10.1002/hed.22015.

38. D'Souza G, Cullen K, Bowie J, Thorpe R, Fakhry C. Differences in oral sexual behaviors by gender, age, and race explain observed differences in prevalence of oral human papillomavirus infection. Liu X, ed. PLoS ONE. 2014;9(1):e86023. doi:10.1371/journal.pone.0086023.

39. Jemal A, Simard EP, Dorell C, et al. Annual Report to the Nation on the Status of Cancer, 1975-2009, featuring the burden and trends in human papillomavirus(HPV)-associated cancers and HPV vaccination coverage levels. J Natl Cancer Inst. 2013;105(3):175-201. doi:10.1093/jnci/djs491.

40. Heath S, Willis V, Allan K, et al. Clinically significant human papilloma virus in squamous cell carcinoma of the head and neck in UK practice. Clin Oncol (R Coll Radiol). 2012;24(1):e18-e23. doi:10.1016/j.clon.2011.05.007.

41. Begum S, Cao D, Gillison M, Zahurak M, Westra WH. Tissue distribution of human papillomavirus 16 DNA integration in patients with tonsillar carcinoma. Clin Cancer Res. 2005;11(16):5694-5699. doi:10.1158/1078-0432.CCR-05-0587.

42. Gillison ML, D'Souza G, Westra W, et al. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. J Natl Cancer Inst. 2008;100(6):407-420. doi:10.1093/jnci/djn025.

43. Mackay J, Eriksen MP, World Health Organization. The Tobacco Atlas. World Health Organization; 2002.

44. Alavanja M, Baron J, Brownson RC, et al. Tobacco smoke and involuntary smoking. IARC monographs on the evaluation of carcinogenic risks to humans / World Health Organization, International Agency for Research on Cancer. 2004;83.

45. Chaturvedi AK, D'Souza G, Gillison ML, Katki HA. Burden of HPV-positive oropharynx cancers among ever and never smokers in the U.S. population. Oral Oncology. 2016;60:61-67. doi:10.1016/j.oraloncology.2016.06.006.

46. Smith CJ, Perfetti TA, Rumple MA, Rodgman A, Doolittle DJ. "IARC group 2A Carcinogens" reported in cigarette mainstream smoke. Food Chem Toxicol. 2000;38(4):371-383.

47. Smith CJ, Perfetti TA, Rumple MA, Rodgman A, Doolittle DJ. "IARC Group 2B carcinogens" reported in cigarette mainstream smoke. Food Chem Toxicol. 2001;39(2):183-205.

48. HA K. The Dorn study of smoking and mortality among U.S. veterans: report on eight and one-half years of observation. 1966.

49. Muñoz N, Correa P, Bock FG. Comparative carcinogenic effect of two types

of tobacco. Cancer. 1968;21(3):376-389. doi:10.1002/1097-0142(196803)21:3<376::AID-CNCR2820210307>3.0.CO;2-J.

50. De Stefani E, Boffetta P, Oreggia F, Mendilaharsu M, Deneo-Pellegrini H. Smoking patterns and cancer of the oral cavity and pharynx: a case–control study in Uruguay. Oral Oncology. 1998;34(5):340-346. doi:10.1016/S1368-8375(98)00014-1.

51. Macfarlane GJ, Zheng T, Marshall JR, et al. Alcohol, tobacco, diet and the risk of oral cancer: a pooled analysis of three case-control studies. Eur J Cancer, B, Oral Oncol. 1995;31B(3):181-187.

52. Duffy SA, Ronis DL, McLean S, et al. Pretreatment health behaviors predict survival among patients with head and neck squamous cell carcinoma. J Clin Oncol. 2009;27(12):1969-1975. doi:10.1200/JCO.2008.18.2188.

53. Chung CH, Gillison ML. Human Papillomavirus in Head and Neck Cancer: Its Role in Pathogenesis and Clinical Implications. Clin Cancer Res. 2009;15(22):6758-6762. doi:10.1158/1078-0432.CCR-09-0784.

54. Huang SH, Xu W, Waldron J, et al. Refining American Joint Committee on Cancer/Union for International Cancer Control TNM stage and prognostic groups for human papillomavirus-related oropharyngeal carcinomas. J Clin Oncol. 2015;33(8):836-845. doi:10.1200/JCO.2014.58.6412.

55. Zhang ZF, Morgenstern H, Spitz MR, et al. Marijuana use and increased risk of squamous cell carcinoma of the head and neck. Cancer Epidemiology Biomarkers & Prevention. 1999;8(12):1071-1078.

56. Nahas G, Latour C. The human toxicity of marijuana. Med J Aust. 1992;156(7):495-497.

57. Watson M, Lyu C, Unger ER, Copeland G, Peters E. Centers for Disease Control and Prevention Human Papillomavirus Typing of Cancers Study with 7 Registries: Evaluating Representativeness. North American Association …; 2011.

58. Snijders PJ, Scholes AG, Hart CA, et al. Prevalence of mucosotropic human papillomaviruses in squamous-cell carcinoma of the head and neck. Int J Cancer. 1996;66(4):464-469. doi:10.1002/(SICI)1097-0215(19960516)66:4<464::AID-IJC9>3.0.CO;2-U.

59. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiology Biomarkers & Prevention. 2005;14(2):467-475. doi:10.1158/1055-9965.EPI-04-0551.

60. Androphy EJ. Molecular biology of human papillomavirus infection and

oncogenesis. J Invest Dermatol. 1994;103(2):248-256.

61. Bishop JA, Lewis JS, Rocco JW, Faquin WC. HPV-related squamous cell carcinoma of the head and neck_ An update on testing in routine pathology practice. Seminars in Diagnostic Pathology. 2015;32(5):344-351. doi:10.1053/j.semdp.2015.02.013.

62. Centers for Disease Control and Prevention. How Many Cancers Are Linked with HPV Each Year. Human papillomavirus (HPV)— …; 2014.

63. D'Souza G, Sugar E, Ruby W, Gravitt P, Gillison M. Analysis of the effect of DNA purification on detection of human papillomavirus in oral rinse samples by PCR. J Clin Microbiol. 2005;43(11):5526-5535. doi:10.1128/JCM.43.11.5526-5535.2005.

64. Kreimer AR, Bhatia RK, Messeguer AL, González P, Herrero R, Giuliano AR. Oral human papillomavirus in healthy individuals: a systematic review of the literature. Sex Transm Dis. 2010;37(6):386-391. doi:10.1097/OLQ.0b013e3181c94a3b.

65. Kreimer AR, Alberg AJ, Daniel R, et al. Oral human papillomavirus infection in adults is associated with sexual behavior and HIV serostatus. J Infect Dis. 2004;189(4):686-698. doi:10.1086/381504.

66. Kreimer AR, Villa A, Nyitray AG, et al. The epidemiology of oral HPV infection among a multinational sample of healthy men. Cancer Epidemiol Biomarkers Prev. 2011;20(1):172-182. doi:10.1158/1055-9965.EPI-10-0682.

67. Giuliano AR, Nyitray AG, Kreimer AR, et al. EUROGIN 2014 roadmap: differences in human papillomavirus infection natural history, transmission and human papillomavirus-related cancer incidence by gender and anatomic site of infection. Int J Cancer. 2015;136(12):2752-2760. doi:10.1002/ijc.29082.

68. Beachler DC, D'Souza G, Sugar EA, Xiao W, Gillison ML. Natural history of anal vs oral HPV infection in HIV-infected men and women. J Infect Dis. 2013;208(2):330-339. doi:10.1093/infdis/jit170.

69. Kreimer AR, Pierce Campbell CM, Lin H-Y, et al. Incidence and clearance of oral human papillomavirus infection in men: the HIM cohort study. Lancet. 2013;382(9895):877-887. doi:10.1016/S0140-6736(13)60809-0.

70. D'Souza G, Fakhry C, Sugar EA, et al. Six-month natural history of oral versus cervical human papillomavirus infection. Int J Cancer. 2007;121(1):143-150. doi:10.1002/ijc.22667.

71. Syrjänen K, Syrjänen S, Lamberg M, Pyrhönen S, Nuutinen J. Morphological and immunohistochemical evidence suggesting human papillomavirus (HPV)

involvement in oral squamous cell carcinogenesis. International Journal of Oral Surgery. 1983;12(6):418-424. doi:10.1016/S0300-9785(83)80033-7.

72. Gillison ML, Koch WM, Capone RB, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst. 2000;92(9):709-720. doi:10.1093/jnci/92.9.709.

73. Smith EM, Hoffman HT, Summersgill KS, Kirchner HL, Turek LP, Haugen TH. Human papillomavirus and risk of oral cancer. Laryngoscope. 1998;108(7):1098-1103.

74. Gillison ML. Human papillomavirus-associated head and neck cancer is a distinct epidemiologic, clinical, and molecular entity. Semin Oncol. 2004;31(6):744-754.

75. D'Souza G, Kreimer AR, Viscidi R, et al. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med. 2007;356(19):1944-1956. doi:10.1056/NEJMoa065497.

76. Lingen MW, Xiao W, Schmitt A, Jiang B, Pickard R. Low etiologic fraction for high-risk human papillomavirus in oral cavity squamous cell carcinomas. Oral Oncology. 2013.

77. Isayeva T, Li Y, Maswahu D, Brandwein-Gensler M. Human Papillomavirus in Non-Oropharyngeal Head and Neck Cancers: A Systematic Literature Review. Head and Neck Pathol. 2012;6(1):104-120. doi:10.1007/s12105-012-0368-1.

78. Castellsague X, Alemany L, Quer M, et al. HPV Involvement in Head and Neck Cancers: Comprehensive Assessment of Biomarkers in 3680 Patients. J Natl Cancer Inst. 2016;108(6):djv403. doi:10.1093/jnci/djv403.

79. Stein AP, Saha S, Yu M, Kimple RJ, Lambert PF. Prevalence of Human Papillomavirus in Oropharyngeal Squamous Cell Carcinoma in the United States Across Time. Chem Res Toxicol. 2014;27(4):462-469. doi:10.1021/tx500034c.

80. Chaturvedi AK, Engels EA, Anderson WF, Gillison ML. Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. J Clin Oncol. 2008;26(4):612-619. doi:10.1200/JCO.2007.14.1713.

81. Deschler DG, Richmon JD, Khariwala SS, Ferris RL, Wang MB. The “New” Head and Neck Cancer Patient--Young, Nonsmoker, Nondrinker, and HPV Positive: Evaluation. Otolaryngology -- Head and Neck Surgery. 2014;151(3):375-380. doi:10.1177/0194599814538605.

82. Schwartz SR, Yueh B, McDougall JK, Daling JR, Schwartz SM. Human

Papillomavirus Infection and Survival in Oral Squamous Cell Cancer: A Population-Based Study. Otolaryngology -- Head and Neck Surgery. 2001;125(1):1-9. doi:10.1067/mhn.2001.116979.

83. Gillison ML. HPV and Prognosis for Patients with Oropharynx Cancer. European Journal of Cancer; 2009.

84. Fakhry C, Westra WH, Li S, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. J Natl Cancer Inst. 2008;100(4):261-269. doi:10.1093/jnci/djn011.

85. Settle K, Posner MR, Schumaker LM, et al. Racial survival disparity in head and neck cancer results from low prevalence of human papillomavirus infection in black oropharyngeal cancer patients. Cancer Prev Res (Phila). 2009;2(9):776-781. doi:10.1158/1940-6207.CAPR-09-0149.

86. MD PBO, MD SHH, MSc JS, et al. Development and validation of a staging system for HPV-related oropharyngeal cancer by the International Collaboration on Oropharyngeal cancer Network for Staging (ICON-S): a multicentre cohort study. Lancet Oncology. 2016;17(4):440-451. doi:10.1016/S1470-2045(15)00560-4.

87. Vent J, Haidle B, Wedemeyer I, et al. p16 Expression in carcinoma of unknown primary: Diagnostic indicator and prognostic marker. Head Neck. 2013;35(11):1521-1526. doi:10.1002/hed.23190.

88. Jereczek-Fossa BA, Jassem J, Orecchia R. Cervical lymph node metastases of squamous cell carcinoma from an unknown primary. Cancer Treatment Reviews. 2004;30(2):153-164. doi:10.1016/j.ctrv.2003.10.001.

89. Mendenhall WM, Mancuso AA, Parsons JT. Diagnostic evaluation of squamous cell carcinoma metastatic to cervical lymph nodes from an unknown head and neck primary site. Head Neck. 1998.

90. Cianchetti M, Mancuso AA, Amdur RJ, et al. Diagnostic evaluation of squamous cell carcinoma metastatic to cervical lymph nodes from an unknown head and neck primary site. Laryngoscope. 2009;119(12):2348-2354. doi:10.1002/lary.20638.

91. Iganej S, Kagan R, Anderson P, et al. Metastatic squamous cell carcinoma of the neck from an unknown primary: Management options and patterns of relapse. Head Neck. 2002;24(3):236-246. doi:10.1002/hed.10017.

92. Kingma DW, Allen RA, Moore W, et al. HPV genotype distribution in oral and oropharyngeal squamous cell carcinoma using seven in vitro amplification assays. Anticancer Res. 2010;30(12):5099-5104.

93. Goodman MT, Saraiya M, Thompson TD, et al. Human papillomavirus genotype and oropharynx cancer survival in the United States of America. European Journal of Cancer. 2015;51(18):2759-2767. doi:10.1016/j.ejca.2015.09.005.

94. Lewis A, Kang R, Levine A, Maghami E. The New Face of Head and Neck Cancer: The HPV Epidemic. Oncology (Williston Park, NY). 2015;29(9):616-626.

95. Biron VL, Mohamed A, Hendzel MJ, Alan Underhill D, Seikaly H. Epigenetic differences between human papillomavirus-positive and -negative oropharyngeal squamous cell carcinomas. 2012;41 Suppl 1:S65-S70.

96. Seikaly H, Biron VL, Zhang H, et al. Role of primary surgery in the treatment of advanced oropharyngeal cancer. Head Neck. July 2015:n/a–n/a. doi:10.1002/hed.24042.

97. Xu CC, Biron VL, Puttagunta L, Seikaly H. HPV Status and second primary tumours in Oropharyngeal Squamous Cell Carcinoma. 2013;42(1):1. doi:10.1186/1916-0216-42-36.

98. Begum S, Gillison ML, Ansari-Lari MA, Shah K, Westra WH. Detection of Human Papillomavirus in Cervical Lymph Nodes. Clin Cancer Res. 2003;9(17):6469-6475. doi:10.3322/canjclin.51.1.15.

99. Bishop JA, Ogawa T, Chang X, et al. HPV analysis in distinguishing second primary tumors from lung metastases in patients with head and neck squamous cell carcinoma. The American Journal of Surgical Pathology. 2012;36(1):142-148. doi:10.1097/PAS.0b013e3182395c7b.

100. Mirghani H, Amen F, Blanchard P, et al. Treatment de-escalation in HPV-positive oropharyngeal carcinoma: ongoing trials, critical issues and perspectives. Int J Cancer. 2015;136(7):1494-1503. doi:10.1002/ijc.28847.

101. Bonilla-Velez J, Mroz EA, Hammon RJ, Rocco JW. Impact of human papillomavirus on oropharyngeal cancer biology and response to therapy: implications for treatment. Otolaryngol Clin North Am. 2013;46(4):521-543. doi:10.1016/j.otc.2013.04.009.

102. Moody CA, Laimins LA. Human papillomavirus oncoproteins: pathways to transformation. Nature Reviews Cancer. 2010;10(8):550-560. doi:10.1038/nrc2886.

103. Hayes DN, Van Waes C, Seiwert TY. Genetic landscape of human papillomavirus–associated head and neck cancer and comparison to tobacco-related tumors. Journal of Clinical Oncology. 2015.

104. Nishat R, Behura SS, Ramachandra S, Kumar H, Bandyopadhyay A. Human

Papilloma Virus (HPV) Induced Head & Neck Squamous Cell Carcinoma: A Comprehensive Retrospect. Journal of Clinical and Diagnostic Research : JCDR. 2015;9(6):ZE01-ZE04. doi:10.7860/JCDR/2015/13948.6056.

105. Walline HM, Komarck C, McHugh JB, et al. High-Risk Human Papillomavirus Detection in Oropharyngeal, Nasopharyngeal, and Oral Cavity Cancers: Comparison of Multiple Methods. JAMA Otolaryngol Head Neck Surg. 2013;139(12):1320-1327. doi:10.1001/jamaoto.2013.5460.

106. Kreimer AR, Johansson M, Waterboer T, et al. Evaluation of human papillomavirus antibodies and risk of subsequent head and neck cancer. J Clin Oncol. 2013;31(21):2708-2715. doi:10.1200/JCO.2012.47.2738.

107. Flint PW, Haughey BH, Robbins KT, et al. Cummings Otolaryngology - Head and Neck Surgery. Elsevier Health Sciences; 2014.

108. Smeets SJ, Hesselink AT, Speel E-JM, et al. A novel algorithm for reliable detection of human papillomavirus in paraffin embedded head and neck cancer specimen. Int J Cancer. 2007;121(11):2465-2472. doi:10.1002/ijc.22980.

109. Weinberger PM, Yu Z, Haffty BG, et al. Molecular classification identifies a subset of human papillomavirus--associated oropharyngeal cancers with favorable prognosis. J Clin Oncol. 2006;24(5):736-747. doi:10.1200/JCO.2004.00.3335.

110. Leemans CR, Braakhuis BJM, Brakenhoff RH. The molecular biology of head and neck cancer. Nature Reviews Cancer. 2011;11(1):9-22. doi:10.1038/nrc2982.

111. van Houten VM, Snijders PJ, van den Brekel MW, et al. Biological evidence that human papillomaviruses are etiologically involved in a subgroup of head and neck squamous cell carcinomas. Int J Cancer. 2001;93(2):232-235. doi:10.1002/ijc.1313.

112. Bishop JA, Ma X-J, Wang H, et al. Detection of transcriptionally active high-risk HPV in patients with head and neck squamous cell carcinoma as visualized by a novel E6/E7 mRNA in situ hybridization method. The American Journal of Surgical Pathology. 2012;36(12):1874-1882. doi:10.1097/PAS.0b013e318265fb2b.

113. Ukpo OC, Flanagan JJ, Ma X-J, Luo Y, Thorstad WL, Lewis JS. High-risk human papillomavirus E6/E7 mRNA detection by a novel in situ hybridization assay strongly correlates with p16 expression and patient outcomes in oropharyngeal squamous cell carcinoma. The American Journal of Surgical Pathology. 2011;35(9):1343-1350. doi:10.1097/PAS.0b013e318220e59d.

114. Schache AG, Liloglou T, Risk JM, et al. Validation of a novel diagnostic standard in HPV-positive oropharyngeal squamous cell carcinoma. British Journal of Cancer. 2013;108(6):1332-1339. doi:10.1038/bjc.2013.63.

115. Lewis JS Jr. p16 Immunohistochemistry As a Standalone Test for Risk Stratification in Oropharyngeal Squamous Cell Carcinoma. Head and Neck Pathol. 2012;6(1):75-82. doi:10.1007/s12105-012-0369-0.

116. Lewis JS, Jr, Thorstad WL, et al. p16 Positive Oropharyngeal Squamous Cell Carcinoma: An Entity With a Favorable Prognosis Regardless of Tumor HPV Status. The American Journal of Surgical Pathology. 2010;34(8):1088-1096. doi:10.1097/PAS.0b013e3181e84652.

117. Naggar El AK, Westra WH. p16 expression as a surrogate marker for HPV-related oropharyngeal carcinoma: A guide for interpretative relevance and consistency. Head Neck. 2012;34(4):459-461. doi:10.1002/hed.21974.

118. Thomas J, Primeaux T. Is p16 immunohistochemistry a more cost-effective method for identification of human papilloma virus–associated head and neck squamous cell carcinoma? Annals of Diagnostic Pathology. 2012;16(2):91-99. doi:10.1016/j.anndiagpath.2011.09.002.

119. Oguejiofor KK, Hall JS, Mani N, et al. The Prognostic Significance of the Biomarker p16 in Oropharyngeal Squamous Cell Carcinoma. Clinical Oncology. 2013;25(11):630-638. doi:10.1016/j.clon.2013.07.003.

120. Jordan RC, Lingen MW, Perez-Ordonez B, et al. Validation of Methods for Oropharyngeal Cancer HPV Status Determination in US Cooperative Group Trials. The American Journal of Surgical Pathology. 2012;36(7):945-954. doi:10.1097/PAS.0b013e318253a2d1.

121. Mroz EA, Baird AH, Michaud WA, Rocco JW. COOH-terminal binding protein regulates expression of the p16INK4A tumor suppressor and senescence in primary human cells. Cancer Research. 2008;68(15):6049-6053. doi:10.1158/0008-5472.CAN-08-1279.

122. Münger K, Baldwin A, Edwards KM, et al. Mechanisms of human papillomavirus-induced oncogenesis. J Virol. 2004;78(21):11451-11460. doi:10.1128/JVI.78.21.11451-11460.2004.

123. Reed AL, Califano J, Cairns P, et al. High frequency of p16 (CDKN2/MTS-1/INK4A) inactivation in head and neck squamous cell carcinoma. Cancer Research. 1996;56(16):3630-3633.

124. Schache AG, Liloglou T, Risk JM, et al. Evaluation of human papilloma virus diagnostic testing in oropharyngeal squamous cell carcinoma: sensitivity, specificity, and prognostic discrimination. Clin Cancer Res.

2011;17(19):6262-6271. doi:10.1158/1078-0432.CCR-11-0388.

125. Cao D, Begum S, Ali SZ, Westra WH. Expression of p16 in benign and malignant cystic squamous lesions of the neck. Hum Pathol. 2010;41(4):535-539. doi:10.1016/j.humpath.2009.09.006.

126. Singhi AD, Westra WH. Comparison of human papillomavirus in situ hybridization and p16 immunohistochemistry in the detection of human papillomavirus-associated head and neck cancer based on a prospective clinical experience. Cancer. 2010;116(9):2166-2173. doi:10.1002/cncr.25033.

127. National Comprehensive Cancer Network. NCCN Head and Neck - Google Search. Fort Washington; 2013. doi:10.1111/j.1464-410X.2011.10693.x/full.

128. Muñoz N, Kjaer SK, Sigurdsson K, et al. Impact of Human Papillomavirus (HPV)-6/11/16/18 Vaccine on All HPV-Associated Genital Diseases in Young Women. J Natl Cancer Inst. 2010;102(5):325-339. doi:10.1093/jnci/djp534.

129. Herrero R, Quint W, Hildesheim A, et al. Reduced Prevalence of Oral Human Papillomavirus (HPV) 4 Years after Bivalent HPV Vaccination in a Randomized Clinical Trial in Costa Rica. Ramqvist T, ed. PLoS ONE. 2013;8(7):e68329. doi:10.1371/journal.pone.0068329.

130. Tabrizi SN, Brotherton JML, Kaldor JM, et al. Fall in human papillomavirus prevalence following a national vaccination program. J Infect Dis. 2012;206(11):1645-1651. doi:10.1093/infdis/jis590.

131. Centers for Disease Control and Prevention (CDC). FDA licensure of quadrivalent human papillomavirus vaccine (HPV4, Gardasil) for use in males and guidance from the Advisory Committee on Immunization Practices (ACIP). MMWR Morb Mortal Wkly Rep. 2010;59(20):630-632.

132. Seifert B, Quach-Thanh C, Skowronski D, Tan B. Update on Human Papillomavirus (HPV) Vaccines. phac-aspcgcca

133. Seifert B, Quach-Thanh C, Skowronski D, Tan B. Update on Human Papillomavirus (HPV) Vaccines. phac-aspcgcca

134. Approved Products > Gardasil.

135. Lehtinen M, Dillner J. Clinical trials of human papillomavirus vaccines and beyond. Nat Rev Clin Oncol. 2013;10(7):400-410. doi:10.1038/nrclinonc.2013.84.

136. Jit M, Brisson M, Portnoy A, Hutubessy R. Cost-effectiveness of female human papillomavirus vaccination in 179 countries: a PRIME modelling study. Lancet Glob Health. 2014;2(7):e406-e414. doi:10.1016/S2214-

109X(14)70237-2.

137. Denis F, Garaud P, Bardet E, et al. Final results of the 94-01 French Head and Neck Oncology and Radiotherapy Group randomized trial comparing radiotherapy alone with concomitant radiochemotherapy in advanced-stage oropharynx carcinoma. Journal of Clinical Oncology. 2004;22(1):69-76. doi:10.1200/JCO.2004.08.021.

138. Gillison ML, Zhang Q, Jordan R, et al. Tobacco Smoking and Increased Risk of Death and Progression for Patients With p16-Positive and p16-Negative Oropharyngeal Cancer. Journal of Clinical Oncology. 2012;30(17):JCO.2011.38.4099–2111. doi:10.1200/JCO.2011.38.4099.

139. Braakhuis BJM, Snijders PJF, Keune W-JH, et al. Genetic patterns in head and neck cancers that contain or lack transcriptionally active human papillomavirus. J Natl Cancer Inst. 2004;96(13):998-1006. doi:10.1093/jnci/djh183.

140. Chai RC, Lambie D, Verma M, Punyadeera C. Current trends in the etiology and diagnosis of HPV- related head and neck cancers. Cancer Medicine. 2015;4(4):596-607. doi:10.1002/cam4.424.

141. Department of Veterans Affairs Laryngeal Cancer Study Group, Wolf GT, Fisher SG, et al. Induction chemotherapy plus radiation compared with surgery plus radiation in patients with advanced laryngeal cancer. N Engl J Med. 1991;324(24):1685-1690. doi:10.1056/NEJM199106133242402.

142. Pignon JP, Syz N, Posner M, Olivares R. … selection suggests the addition of docetaxel to 5-fluorouracil–cisplatin induction therapy may offer survival benefit in squamous cell cancer of the head and neck. … -cancer drugs. 2004.

143. Posner MR, Hershock DM, Blajman CR, et al. Cisplatin and fluorouracil alone or with docetaxel in head and neck cancer. N Engl J Med. 2007;357(17):1705-1715. doi:10.1056/NEJMoa070956.

144. Garden AS, Harris J, Trotti A, et al. Long-Term Results of Concomitant Boost Radiation Plus Concurrent Cisplatin for Advanced Head and Neck Carcinomas: A Phase II Trial of the Radiation Therapy Oncology Group (RTOG 99-14). International Journal of Radiation Oncology*Biology*Physics. 2008;71(5):1351-1355.

145. Prestwich RJD, Kancherla K, Oksuz DC, et al. A single centre experience with sequential and concomitant chemoradiotherapy in locally advanced stage IV tonsillar cancer. Radiation Oncology 2010 5:1. 2010;5(1):121. doi:10.1186/1748-717X-5-121.

146. Denis F, Garaud P, Bardet E. … And Radiotherapy Group Randomized Trial

Comparing Radiotherapy Alone with Concomitant Radiochemotherapy in Advanced-Stage Oropharynx Carcinoma. … Oncology; 2004.

147. Machtay M, Moughan J, Trotti A, et al. Factors associated with severe late toxicity after concurrent chemoradiation for locally advanced head and neck cancer: an RTOG analysis. J Clin Oncol. 2008;26(21):3582-3589. doi:10.1200/JCO.2007.14.8841.

148. Greven KM, White DR, Browne JD, Williams DWI, McGuirt WFS, D'Agostino RBJ. Swallowing Dysfunction is a Common Sequelae After Chemoradiation for Oropharynx Carcinoma. American Journal of Clinical Oncology. 2008;31(3):209-212. doi:10.1097/COC.0b013e3181595b10.

149. Wilson JA, Carding PN, Patterson JM. Dysphagia after Nonsurgical Head and Neck Cancer Treatment Patients' Perspectives. Otolaryngology -- Head and Neck Surgery. 2011;145(5):767-771. doi:10.1177/0194599811414506.

150. Best SR, Ha PK, Blanco RG, et al. Factors associated with pharyngoesophageal stricture in patients treated with concurrent chemotherapy and radiation therapy for oropharyngeal squamous cell carcinoma. Head & Neck. 2011;33(12):1727-1734. doi:10.1002/hed.21657.

151. Nien HH, Sturgis EM, Kies MS, et al. Comparison of systemic therapies used concurrently with radiation for the treatment of human papillomavirus–associated oropharyngeal cancer. Head & Neck. 2016;38(S1):E1554-E1561. doi:10.1002/hed.24278.

152. Vainshtein JM, Samuels S, Tao Y, et al. Impact of xerostomia on dysphagia after chemotherapy–intensity- modulated radiotherapy for oropharyngeal cancer: Prospective longitudinal study. Head & Neck. 2016;38(S1):E1605-E1612. doi:10.1002/hed.24286.

153. Shiley SG, Hargunani CA, Skoner JM, Holland JM, Wax MK. Swallowing Function after Chemoradiation for Advanced Stage Oropharyngeal Cancer. Otolaryngology -- Head and Neck Surgery. 2006;134(3):455-459. doi:10.1016/j.otohns.2005.10.054.

154. Setton J, Lee NY, Riaz N, et al. A multi- institution pooled analysis of gastrostomy tube dependence in patients with oropharyngeal cancer treated with definitive intensity- modulated radiotherapy. Cancer. 2015;121(2):294-301. doi:10.1002/cncr.29022.

155. Chen AY, Schrag N, Hao Y, Stewart A, Ward E. Changes in treatment of advanced oropharyngeal cancer, 1985-2001. Laryngoscope. 2007;117(1):16-21. doi:10.1097/01.mlg.0000240182.61922.31.

156. Chen AY, Zhu J, Fedewa S. Temporal trends in oropharyngeal cancer treatment and survival: 1998-2009. Laryngoscope. 2014;124(1):131-138. doi:10.1002/lary.24296.

157. Park G, Lee S-W, Kim SY, et al. Can concurrent chemoradiotherapy replace surgery and postoperative radiation for locally advanced stage III/IV tonsillar squamous cell carcinoma? Anticancer Res. 2013;33(3):1237-1243.

158. Kano S, Homma A, Hayashi R, et al. Matched-pair analysis in patients with advanced oropharyngeal cancer: surgery versus concurrent chemoradiotherapy. Oncology. 2013;84(5):290-298. doi:10.1159/000346908.

159. D PB-RM, D AGM, D VBM, et al. Matched Survival Analysis in Patients with Locoregionally Advanced Resectable Oropharyngeal Carcinoma: Platinum-Based Induction and Concurrent Chemoradiotherapy Versus Primary Surgical Resection. Radiation Oncology Biology. 2011;80(1):154-160. doi:10.1016/j.ijrobp.2010.01.032.

160. Zhen W, Karnell LH, Hoffman HT, Funk GF, Buatti JM, Menck HR. The National Cancer Data Base report on squamous cell carcinoma of the base of tongue. Head Neck. 2004;26(8):660-674. doi:10.1002/hed.20064.

161. O'Connell D, Seikaly H, Murphy R, et al. Primary surgery versus chemoradiotherapy for advanced oropharyngeal cancers: a longitudinal population study. 2013;42(1):31. doi:10.1186/1916-0216-42-31.

162. Dahlstrom KR, Calzada G, Hanby JD, et al. An evolution in demographics, treatment, and outcomes of oropharyngeal cancer at a major cancer center: a staging system in need of repair. Cancer. 2013;119(1):81-89. doi:10.1002/cncr.27727.

163. Dayyani F, Etzel CJ, Liu M, Ho C-H, Lippman SM, Tsao AS. Meta-analysis of the impact of human papillomavirus (HPV) on cancer risk and overall survival in head and neck squamous cell carcinomas (HNSCC). Head & Neck Oncology 2010 2:1. 2010;2(1):1. doi:10.1186/1758-3284-2-15.

164. Adelstein DJ, Ridge JA, Gillison ML, et al. Head and neck squamous cell cancer and the human papillomavirus: Summary of a National Cancer Institute State of the Science Meeting, November 9–10, 2008, Washington, D.C. Head & Neck. 2009;31(11):1393-1422. doi:10.1002/hed.21269.

165. Chandarana SP, Lee JS, Chanowski EJP, et al. Prevalence and predictive role of p16 and epidermal growth factor receptor in surgically treated oropharyngeal and oral cavity cancer. Head & Neck. 2013;35(8):1083-1090. doi:10.1002/hed.23087.

166. Bristow RG, Benchimol S, Hill RP. The p53 gene as a modifier of intrinsic radiosensitivity: implications for radiotherapy. Radiotherapy and Oncology.

1996;40(3):197-223. doi:10.1016/0167-8140(96)01806-3.

167. Wang MB, Liu IY, Gornbein JA, Nguyen CT. HPV-Positive Oropharyngeal Carcinoma: A Systematic Review of Treatment and Prognosis. Otolaryngology -- Head and Neck Surgery. 2015;153(5):758-769. doi:10.1177/0194599815592157.

168. Ragin CCR, Taioli E. Survival of squamous cell carcinoma of the head and neck in relation to human papillomavirus infection: Review and meta-analysis. Int J Cancer. 2007;121(8):1813-1820. doi:10.1002/ijc.22851.

169. Brizel DM. Different Strokes for Different Folks: New Paradigms for Staging Oropharynx Cancer. Journal of Clinical Oncology. 2015;33(8):JCO.2014.60.1757-JCO.2014.60.1818. doi:10.1200/JCO.2014.60.1757.

170. Horne ZD, Glaser SM, Vargo JA, et al. Confirmation of proposed human papillomavirus risk-adapted staging according to AJCC/UICC TNM criteria for positive oropharyngeal carcinomas. Cancer. April 2016:1-10. doi:10.1002/cncr.30021.

171. Amin MB, Edge S, Greene FL, et al. AJCC Cancer Staging Manual. Springer; 2016.

172. Lydiatt WM, Patel SG, O'Sullivan B, et al. Head and Neck cancers-major changes in the American Joint Committee on cancer eighth edition cancer staging manual. CA: A Cancer Journal for Clinicians. 2017;67(2):122-137. doi:10.3322/caac.21389.

173. Teymoortash A, Werner JA. Current advances in diagnosis and surgical treatment of lymph node metastasis in head and neck cancer. GMS Curr Top Otorhinolaryngol Head Neck Surg. 2012;11:Doc04. doi:10.3205/cto000086.

174. Subramanian S, Chiesa F, Lyubaev V, Aidarbekova A. The Evolution of Surgery in the Management of Neck Metastases. Vol 26. Pacini Editore; 2006:309-316.

175. Parsons JT, Mendenhall WM, Stringer SP, et al. Squamous cell carcinoma of the oropharynx: surgery, radiation therapy, or both. Cancer. 2002;94(11):2967-2980. doi:10.1002/cncr.10567.

176. Parsons J, Mendenhall W, Million R, Stringer S, Cassisi N. The Management of Primary Cancers of the Oropharynx: Combined Treatment or Irradiation Alone? Semin Radiat Oncol. 1992;2(3):142-148. doi:10.1053/SRAO00200142.

177. Kumar B, Cipolla MJ, Old MO, et al. Surgical management of oropharyngeal

squamous cell carcinoma: Survival and functional outcomes. Head Neck. 2015;38(S1):E1794-E1802. doi:10.1002/hed.24319.

178. Stucken CL, de Almeida JR, Sikora AG, Tong CCL, Genden EM. Impact of human papillomavirus and smoking on survival outcomes after transoral robotic surgery. Head Neck. 2015;38(3):380-386. doi:10.1002/hed.23915.

179. Kass JI, Giraldez L, Gooding W, et al. Oncologic outcomes of surgically treated early-stage oropharyngeal squamous cell carcinoma. Head Neck. April 2016:1-5. doi:10.1002/hed.24456.

180. Camp AA, Fundakowski C, Petruzzelli GJ, Emami B. Functional and oncologic results following transoral laser microsurgical excision of base of tongue carcinoma. Otolaryngology -- Head and Neck Surgery. 2009;141(1):66-69. doi:10.1016/j.otohns.2009.02.028.

181. Smith RV, Schiff BA, Garg M, Haigentz M. The impact of transoral robotic surgery on the overall treatment of oropharyngeal cancer patients. Laryngoscope. 2015;125:S1-S15. doi:10.1002/lary.25534.

182. Monnier Y, Simon C. Surgery Versus Radiotherapy for Early Oropharyngeal Tumors: a Never-Ending Debate. Curr Treat Options in Oncol. 2015;16(9):42–13. doi:10.1007/s11864-015-0362-4.

183. Bernier J, Domenge C, Ozsahin M, et al. Postoperative irradiation with or without concomitant chemotherapy for locally advanced head and neck cancer. N Engl J Med. 2004;350(19):1945-1952. doi:10.1056/NEJMoa032641.

184. Bernier J, Cooper JS, Pajak TF, et al. Defining risk levels in locally advanced head and neck cancers: a comparative analysis of concurrent postoperative radiation plus chemotherapy trials of the EORTC (#22931) and RTOG (# 9501). Head Neck. 2005;27(10):843-850. doi:10.1002/hed.20279.

185. Cooper JS, Pajak TF, Forastiere AA, et al. Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck. N Engl J Med. 2004;350(19):1937-1944. doi:10.1056/NEJMoa032646.

186. Huang SH, Perez-Ordonez B, Liu F-F, et al. Atypical clinical behavior of p16-confirmed HPV-related oropharyngeal squamous cell carcinoma treated with radical radiotherapy. Int J Radiat Oncol Biol Phys. 2012;82(1):276-283. doi:10.1016/j.ijrobp.2010.08.031.

187. Langendijk JA, Doornaert P, Verdonck-de Leeuw IM, Leemans CR, Aaronson NK, Slotman BJ. Impact of late treatment-related toxicity on quality of life among patients with head and neck cancer treated with radiotherapy. J Clin Oncol. 2008;26(22):3770-3776.

doi:10.1200/JCO.2007.14.6647.

188. Sanguineti G, Sormani MP, Marur S, et al. Effect of radiotherapy and chemotherapy on the risk of mucositis during intensity-modulated radiation therapy for oropharyngeal cancer. Int J Radiat Oncol Biol Phys. 2012;83(1):235-242. doi:10.1016/j.ijrobp.2011.06.2000.

189. Adelstein DJ, Ridge JA, Brizel DM, et al. Transoral resection of pharyngeal cancer: Summary of a National Cancer Institute Head and Neck Cancer Steering Committee Clinical Trials Planning Meeting, November 6–7, 2011, Arlington, Virginia. Head & Neck. 2012;34(12):1681-1703. doi:10.1002/hed.23136.

190. Cooper T, Biron V, Adam B, Klimowicz AC, Puttagunta L, Seikaly H. Prognostic utility of basaloid differentiation in oropharyngeal cancer. 2013;42(1):57. doi:10.1186/1916-0216-42-57.

191. Barber BR, Biron VL, Klimowicz AC, Puttagunta L, Côté DWJ, Seikaly H. Molecular predictors of locoregional and distant metastases in oropharyngeal squamous cell carcinoma. 2013;42(1):53. doi:10.1186/1916-0216-42-53.

192. Forastiere AA, Goepfert H, Maor M, et al. Concurrent chemotherapy and radiotherapy for organ preservation in advanced laryngeal cancer. N Engl J Med. 2003;349(22):2091-2098. doi:10.1056/NEJMoa031317.

193. Lau HY, Brar S, Klimowicz AC, et al. Prognostic significance of p16 in locally advanced squamous cell carcinoma of the head and neck treated with concurrent cisplatin and radiotherapy. Head Neck. 2011;33(2):251-256. doi:10.1002/hed.21439.

194. Begum S, Gillison ML, Ansari-Lari MA, Shah K, Westra WH. Detection of human papillomavirus in cervical lymph nodes: a highly effective strategy for localizing site of tumor origin. Clin Cancer Res. 2003;9(17):6469-6475.

195. Liang C, Marsit CJ, McClean MD, et al. Biomarkers of HPV in Head and Neck Squamous Cell Carcinoma. Cancer Research. 2012;72(19):5004-5013. doi:10.1158/0008-5472.CAN-11-3277.

196. Clark TG, Bradburn MJ, Love SB, Altman DG. Survival analysis part I: basic concepts and first analyses. British Journal of Cancer. 2003;89(2):232-238. doi:10.1038/sj.bjc.6601118.

197. Bradburn MJ, Clark TG, Love SB, Altman DG. Survival analysis part II: multivariate data analysis--an introduction to concepts and methods. British Journal of Cancer. 2003;89(3):431-436. doi:10.1038/sj.bjc.6601119.

198. McDonald JT, Johnson-Obaseki S, Hwang E, Connell C, Corsten M. The relationship between survival and socio-economic status for head and neck

cancer in Canada. Journal of Otolaryngology - Head & Neck Surgery 2014 43:1. 2014;43(1):2. doi:10.1186/1916-0216-43-2.

199. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA: A Cancer Journal for Clinicians. 2016;66(1):7-30. doi:10.3322/caac.21332.

200. MD PBO, MD SHH, MSc JS, et al. Development and validation of a staging system for HPV-related oropharyngeal cancer by the International Collaboration on Oropharyngeal cancer Network for Staging (ICON-S): a multicentre cohort study. March 2016:1-12. doi:10.1016/S1470-2045(15)00560-4.

201. Horne ZD, Glaser SM, Vargo JA, et al. Confirmation of proposed human papillomavirus risk-adapted staging according to AJCC/UICC TNM criteria for positive oropharyngeal carcinomas. Cancer. April 2016:1-10. doi:10.1002/cncr.30021.

202. Wilkie MD, Upile NS, Lau AS, et al. Transoral laser microsurgery for oropharyngeal squamous cell carcinoma: A paradigm shift in therapeutic approach. Head & Neck. 2016;38(8):1263-1270. doi:10.1002/hed.24432.

203. Chaturvedi AK, Anderson WF, Lortet-Tieulent J, et al. Worldwide Trends in Incidence Rates for Oral Cavity and Oropharyngeal Cancers. Journal of Clinical Oncology. 2013;31(36):4550-4559. doi:10.1200/JCO.2013.50.3870.

204. Seikaly H, Biron VL, Zhang H, et al. Role of primary surgery in the treatment of advanced oropharyngeal cancer. Head Neck. 2015;38(S1):E571-E579. doi:10.1002/hed.24042.

Appendix

1. **Copyright figure 1.1 & 1.2**