Do Statins Improve Survival in Head and Neck Cancer Patients? An Investigation of Cancer Outcomes and Biologic Mechanisms.

by

Kayla R. Getz

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Epidemiological Science)
in The University of Michigan
2021

Doctoral Committee:

Associate Professor Alison M. Mondul, Chair
Associate Professor Laura S. Rozek
Associate Professor Jennifer A. Smith
Professor Jeremy M.G. Taylor
Professor Emeritus Gregory T. Wolf

Kayla R. Getz

kagetz@umich.edu

ORCID iD: 0000-0001-5552-044X

Dedication

I would like to dedicate this dissertation to my family, specifically my parents, Susan and Edward.

Acknowledgments

Through the process of preparing and writing this dissertation I have received support, guidance and assistance from many people. I would like to express my gratitude to Alison M. Mondul, PhD who is the chair of my dissertation committee. She has not only been an amazing mentor, but has been extremely supportive and helpful throughout this process.

I would also like to thank the other members of my committee Laura S. Rozek, PhD, Jennifer A. Smith, PhD, Jeremy M.G. Taylor, PhD, and Gregory T. Wolf, MD. I would like to thank Dr. Rozek for her advice throughout this process. I would also like to thank her for hiring me as a Masters student to work for the University of Michigan Head and Neck Cancer Specialized Program of Research Excellence (HNC SPORE), which gave me the opportunity to work with head and neck cancer patients and this research. Dr. Smith provided a lot of advice about genetics and support for Aim 3 of this dissertation. She also connected me with faculty who helped me with the development of the genetic instruments utilized in Aim 3.

Dr. Taylor lent his expertise in biostatistics and was always willing to provide advice on statistical analysis or recommend a better analytic method to answer the question. Through Dr. Taylor I have learned new techniques that I utilized in this dissertation and plan on using in my future research. Dr. Wolf not only provided guidance about the clinical and biological aspects of head and neck cancer, but he also allowed me to use his office space at the hospital so I could collect the medication data used throughout this dissertation. Without his kindness and expertise I would not have been able to complete this project.

In addition to the members of my dissertation committee I would like to thank all of the study subjects in the University of Michigan HNC SPORE as well as the participants in the

Michigan Genomics Initiative. Without their willingness to participate, complete surveys, and provide biospecimen I would not have been able to conduct this research. I would also like to thank some of the people who work with the HNC SPORE; Emily Bellile, Katie Zarins, Tingting Qin and Cailey Rullman. Emily, Katie and Tingting, thank you for providing me with some of the data for this project and lending your knowledge about the HNC SPORE cohort. I would also like to thank Cailey for working with me to collect the medication data from all of the patients in the SPORE 2 cohort.

I want to thank the Mondul/Pearce lab group, which consists of Dr. Alison Mondul, Dr. Leigh Pearce, Minh Tung Phung, Lilah Khoja, Aliya Alimujiang, Kate Brieger, Elizabeth Slocum, and Maxwell Salvatore for providing feedback on my project throughout the past few years. I would also like to thank the 2017 doctoral epidemiology cohort especially my friends Farah and Julia, for their advice through this process. Lastly, I am extremely grateful to my family for their support throughout my education and while writing and preparing this dissertation. I want to thank them for believing in me and helping to keep me motivated during the course of this doctoral program.

Table of Contents

List of Tables

List of Figures

Abbreviations

ACE-27	Adult Comorbidity Evaluation 27 Score
APOE	Apolipoprotein e
BMI	Body Mass Index
CD31	Cluster of Differentiation 31
CD4	Cluster of Differentiation 4
CD8	Cluster of Differentiation 8
CRP	C- reactive protein
DAG	Directed Acyclic Graph
EMERSE	Electronic Medical Record Search Engine
FoxP3	Forkhead Box P3
GED	General Education Development
GRO	Growth Related Oncogene
GWAS	Genome Wide Association Study
HDL	High-Density Lipoprotein
HGF	Hepatocyte Growth Factor
HMG-CoA	3-hydroxy-3 methylglutaryl coenzyme A
HNC SPORE	University of Michigan Head and Neck Cancer Specialized Program of Research Excellence
HNSCC	Head and Neck Squamous Cell Carcinoma
HPV	Human Papillomavirus
HR	Hazard Ratio
IFN-γ	Interferon Gamma
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
IL-17	Interleukin-17
IPW	Inverse Probability Weighting
LDL	Low-Density Lipoprotein
MGI	Michigan Genomics Initiative
MI	Multiple Imputation
MV-adj	Multivariable Adjusted
NDI	Social Security Death Index
OR	Odds Ratio
PCA	Principal Components Analysis
PCR	Polymerase Chain Reaction
REDCap	Research Electronic Data Capture
SD	Standard Deviation
SEER	Surveillance, Epidemiology and Ends Results Program
SES	Socioeconomic Status
SNP	Single nucleotide polymorphism
SPORE	Specialized Program of Research Excellence
TC	Total Cholesterol

TG	Triglycerides
TGF	Transforming Growth Factor
TIL	Tumor-Infiltrating Lymphocyte
TILws	Total Weighted Score
TNF-α	Tumor Necrosis Factor Alpha
VEGF	Vascular Endothelial Growth Factor

Abstract

Head and neck squamous cell carcinoma (HNSCC) is an especially debilitating cancer with unacceptably low survival among patients, which differs depending on the site and stage of disease. Statins possess anti-cancer properties that may inhibit disease development and progression through various mechanisms including, anti-inflammation, immunomodulation, and cholesterol-lowering. Although studies have investigated the association between statins and health outcomes among cancer patients with disease in various sites, research among HNSCC patients is limited and the mechanisms explaining the relationship are not clearly established.

Aim 1 of this dissertation investigates whether statin use influences HNSCC outcomes including, all-cause mortality, disease-specific mortality, and disease recurrence. Due to the differences in etiology and prognosis among patients with human papillomavirus (HPV) positive tumors and patients with HPV-negative tumors, HPV was assessed as an effect modifier. Statin use was found to be protective among all patients for all-cause mortality but only appeared to be protective for disease-specific mortality and disease recurrence among patients whose disease was HPV-positive. After the utilization of various analytic methods to address missing data, the protective associations did not change.

Aim 2 examined the association between statin use and both tumor-infiltrating lymphocytes (TILs) and circulating cytokines among HNSCC patients at diagnosis. There was a statistically significant positive association between statin use and TILs, particularly FoxP3, but similar to our findings in Aim 1, only among HPV-positive patients. We observed no association between statin use and circulating cytokines even after conducting a principal component analysis to reduce dimensionality among the highly correlated cytokine measures.

Aim 3 explored whether cholesterol may be the mechanism by which statins exert any observed influence on HNSCC risk or outcomes. Genetic data from the Michigan Genomics Initiative was utilized to conduct a case-control study of HNSCC risk and a survival analysis among HNSCC cases. Mendelian randomization analysis was conducted examining the association between instruments predicting hypercholesterolemia (total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides (TG)) and HNSCC risk as well as outcomes among HNSCC patients. Cholesterol-related instruments were not associated with HNSCC risk, but there was a positive association between the TC instrument and both all-cause mortality and disease recurrence among HNSCC patients.

The findings from this dissertation demonstrate that statins are protective against all-cause mortality among all HNSCC patients. Statins were protective for disease-specific mortality and disease recurrence, particularly among patients with HPV-positive tumors. Potential mechanisms that explain this association may be related to a synergistic relationship between statin use and the presence of HPV, leading to a more favorable immune response. Improving TC among HNSCC patients may improve all-cause mortality and disease recurrence, but future research is necessary to elucidate the impact of cholesterol-lowering on HNSCC outcomes. Larger and more diverse studies further investigating these observations are necessary to validate this research. Overall, this dissertation provides evidence to support the future development of an adjuvant clinical trial of statin therapy in HNSCC patients. If these findings are supported in larger, more diverse patient populations, statin use may be a relatively safe adjuvant tertiary treatment option for patients with HNSCC.

Chapter 1 : Background / Introduction

Head and Neck Squamous Cell Carcinoma (HNSCC)

Head and neck squamous cell carcinoma (HNSCC) is an especially debilitating and deadly form of cancer. HNSCC is a group of cancers arising in the aerodigestive tract starting at the nasal cavity and ending in the throat at the larynx, located in sites including the oral cavity, oropharynx, larynx, hypopharynx, and nasopharynx.[1–3] The incidence of HNSCC is 0.2-3.3 per 100,000, and the 5-year relative survival ranges from 31.9%-89.5% depending on the site of the cancer .[1] Recurrence is approximately 50% for patients with advanced disease.[1]

Although HNSCC may not have a high incidence rate compared to other cancers, the 5-year relative survival rate is unacceptably low and the morbidity of treatment can be extreme. Based on Surveillance, Epidemiology and Ends Results Program (SEER) data from 2008-2014, the percent of patients who survive 5 years who have cancer of the oral cavity or pharynx is approximately 64.8% and those with cancer of the larynx is on average 60.9%.[4,5] Other more common cancers have a much higher percentage of patients who survive 5 years after diagnosis based on SEER data from 2008-2014. 89.7% of breast cancer patients survive after 5 years and prostate cancer is about 98.2%.[6,7]

Although there have been advancements in treatment modalities specifically in surgery, chemotherapy and radiation therapy, prognosis has improved among HNSCC patients over time mainly due to the increase in the number of human papillomavirus (HPV) positive HNSCC patients, as HPV-positive tumors have a better prognosis.[1,2]

Risk Factors

HNSCC has two distinct etiologies of disease in the United States, patients whose tumors are HPV-positive, related to oral HPV infection, and patients whose tumors are HPV-negative, which is often the result of smoking tobacco and drinking alcohol.[1] In the past few decades, the incidence of HPV-positive HNSCC cancers has increased both in the US and globally.[8] The incidence of HNSCC that is not associated with HPV has drastically decreased, but the incidence of HPV-positive associated disease has increased, particularly among younger men.[9] This etiological shift may be related to a decrease in smoking and tobacco use in the US population.[10] HPV-positive HNSCC patients have better overall and disease-specific survival compared to HNSCC patients who are HPV-negative.[11] The superior prognosis may be related to the differing patient characteristics of HPV-positive and negative HNSCC patients. HPV-positive patients are often younger, white males who are less likely to have used tobacco or alcohol than patients who are HPV-negative.[1,9] It is also important to keep in mind HPV-positive and negative tumors are different at the molecular level.[12,13] They elicit a different immune response with HPV-positive tumors often promoting a stronger immune reaction and as a result are often more responsive to treatment.[14] Genetic mutations that lead to the development of HNSCC are also different between patients with HPV-positive and negative disease. HPV-positive tumors often have mutations in the PI3K pathways whereas, patients with HPV-negative HNSCC are more likely to experience mutations in TP53 and CDKN2A.[15] Information about the differences in incidence, survival, risk factors, and patient characteristics between patients with HPV-positive and HPV-negative disease are presented in Table 1.1.

Site of disease is often related to the risk factors that may have caused the disease. HPV-positive disease is often found in the oropharynx site, and smoking and drinking are associated with the other sites in the head and neck.[1] The stronger immune response observed among HPV-positive disease may be related to the site of disease since it is located in the

oropharynx, including the tonsils, which are lymphatic tissue.[14] There are currently no evidence-based recommendations for changes that a HNSCC patient can make after onset of disease to improve or prevent mortality.

Although genetics are often identified as a risk factor for developing certain types of cancers, genetics do not appear to be a strong risk factor for developing HNSCC.[16] There does appear to be an association with certain genetic mutations, for example, P53 and other multiple mutations and HNSCC but the relationship is not clear.[16] Since the biology and treatment of HNSCC is different by site of disease further research is necessary to elucidate the genetic markers that are associated with each site and identify the treatment that would most benefit each patient profile.[16]

Statins

Statins are medications that help lower cholesterol levels, specifically LDL cholesterol and triglycerides.[17] They are often administered to adults who have high cholesterol or are at risk for cardiovascular disease.[17] Statins are a very commonly prescribed drug in the United States. Guidelines that were implemented in 2013 estimate that approximately “1 in 3 American adults” will be prescribed a statin regardless of cholesterol levels or if they are suffering from cardiovascular disease.[18] The US Preventative Services Task Force also supports the use of statins in adults who may have risk factors for cardiovascular disease other than high cholesterol.[19] It is abundantly clear that statins are associated with reducing the risk of cardiovascular disease and overall mortality.[20] Although this relationship is clearly established, the association between statins and disease-specific survival or statins and recurrence among HNSCC patients has not been thoroughly investigated.

Cholesterol-lowering

Statins lower cholesterol by blocking HMG-CoA reductase (3-hydroxy-3 methylglutaryl coenzyme A) activity, which is a necessary step in the mevalonate pathway.[21] By blocking this pathway, a chain reaction occurs because the production of mevalonic acid, a product of HMG-CoA, is obstructed.[22] The mevalonate pathway stimulates the production of sterols, which are the foundation of cholesterol.[22] Since this is reduced or blocked by the introduction of a statin, LDL decreases and plaque formation diminishes in the arteries, which explains why statins are so protective for cardiovascular disease.[22]

The association between cholesterol levels and risk of developing cancer or experiencing cancer-associated outcomes is not clear. The majority of the literature investigating this relationship is quite dated. It is possible that the association between cholesterol levels and risk of cancer or mortality is different depending on the site of the disease.[23] A study from the 1980s found an inverse association between cholesterol levels and risk of developing cancers associated with tobacco use.[24] It has also been suggested that the reduction of circulating cholesterol may reduce the risk of developing certain cancers, specifically prostate cancer.[25] Although cholesterol is necessary for cells to function, it is possible that too much cholesterol may influence carcinogenesis through various processes.[26] The impact of cholesterol on cancer development may be different by cancer subtype because the molecular makeup of the tumor cells differs between cancers.[27] It has also been identified that statins have many biologic actions in addition to cholesterol-lowering that may be protective for cancer. In the paper, *Hallmarks of Cancer: The Next Generation*, the authors clearly explain the common biologic mechanisms that underlie cancer development and progression.[28] These hallmarks are displayed in Table 1.2. Many of these mechanisms may be obstructed when statins are present in the body, including inflammation and immunomodulation, apoptosis and angiogenesis effects.[29] These beneficial effects of statins will be explained in more detail below.

Immunomodulatory and Anti-inflammatory Effects

When cancer develops, the body elicits an immune response and a strong immune response has been shown to have favorable results among cancer patients.[28] Inflammatory markers are often present when cancer arises due to initiation by the immune system.[28] Inflammation is described as an "enabling characteristic", and the response of the immune system is described as an "emerging hallmark", in the Hallmarks of Cancer paper and is displayed in Table 1.2.[28] The inflammatory and immunomodulatory effects of cancer are not clearly directional by cell type. It often depends on the type of cancer, whether the presence of inflammatory markers and what combination of these markers are beneficial or harmful to cancer prognosis.[30]

There have been studies that investigated the relationship between circulating pro-inflammatory cytokines and the use of statins in populations of patients with hypercholesterolemia and in the general population. These studies found that statins reduce the presence of certain cytokines, but the cytokines that were reduced were not consistent across studies.[31–34] In the studies that investigated the association of statin use and cytokines in participants with hypercholesterolemia, two of the studies found a reduction in interleukin-6 (IL-6) and two of the studies found a reduction in tumor necrosis factor (TNF) for participants on a statin.[31,32] In the study that investigated the association among a general population (random sample) of Swiss adults they only found lower C- reactive protein (CRP) concentrations among participants using a statin compared to those who were not.[34]

Although this relationship does not appear to have been studied among HNSCC patients, it has been investigated among patients with a diagnosis of colorectal cancer. Malicki et al. investigated this relationship in tissue as well as serum and found an inverse association between statin use and certain pro-inflammatory cytokines.[35] It is possible that this association may also be present for patients with HNSCC because an increase in the presence of pro-

inflammatory cytokines in a HNSCC patient is often not beneficial, and may promote the development of new disease or the metastasis of disease already present through multiple biological mechanisms.[36,37] The pro-inflammatory cytokine, IL-6 has been found to be positively associated with recurrence in patients with HNSCC.[38]

Statins are also associated with the increased production of T-cells; this has been observed particularly *in vitro* among cancer cell lines and lung cancer cells in mice.[39] The literature also demonstrates that the presence of T-cells are often associated with better outcomes among HNSCC patients.[30] The association between tumor-infiltrating lymphocytes (TILs) and HNSCC outcomes appears to be inverse; patients who have a higher number of TILs are less likely to die or experience recurrence than HNSCC patients who have a lower number of TILs.[40,41] Although the association between statin use and tumor-infiltrating lymphocytes has not been investigated among HNSCC patients; this relationship has been observed among patients with colorectal cancer. Al-Husein et al. identified that participants with colorectal cancer who had taken a statin had more immune cell infiltration compared to those who did not take a statin.[42] This association appeared to be modified by stage of disease.[42] It is possible the pathway by which statins improve HNSCC disease-specific mortality and recurrence is through their immune and anti-inflammatory properties.

It is important to investigate the possible modifying effect of HPV on the association between statin use and inflammatory biomarkers in HNSCC patients. HPV-positive associated disease and HPV-negative disease are quite different in a plethora of ways, including immune response. Patients with HPV-positive tumors often elicit a stronger immune response than patients who have HPV-negative disease.[43,44] This may be related to the disease site associated with HPV-positive disease, which is often located in the oropharynx including the tonsils. Since the tonsils are made up of lymphatic tissue it is possible the immune response would be stronger in this location due to the tissue type.[14,44] It is possible that the strong immune

response of HNSCC patients with HPV-positive disease works synergistically with the anti-inflammatory and immunomodulatory actions of statins to improve cancer-related outcomes.

Pro-Apoptosis

When non-cancerous cells no longer serve their purpose in the body or become old, they are programmed to die through apoptosis, paving the way for new cells to replace them.[28] Malignant cells proliferate and resist death. They continue to grow into cancerous masses that can become invasive and spread through the lymphatic system and migrate to other organ systems other than where it originated.[28] One of the hallmarks of cancer (as discussed above and displayed in Table 1.2) is avoiding apoptosis.[28] Statins have properties that promote apoptosis. As mentioned above, statins block the production of mevalonic acid through obstructing the HMG-CoA pathway (displayed in Figure 1.1). Through blocking this pathway, statins inhibit the production of cholesterol [22] and, downstream of this, they also block protein prenylation (Figure 1.1).[45] Protein prenylation is a post-translational modification that allows the protein to be more active.[45] A study conducted by Tsubaki et al. looked at the effect of Fluvastatin and Simvastatin on HNSCC cell lines with the goal of understanding the mechanism by which these statins promote apoptosis.[46] They found that these statins did promote apoptosis in HNSCC cell lines through blocking Ras pathways.[46] Ras is a proto-oncogene that promotes the growth of new cells and, when mutated into an oncogene, promotes overgrowth and avoidance of apoptosis and is associated with many different types of cancer.[47,48] Prenylation is required for Ras proteins to be maximally active.[49] Therefore, if statins block the prenylation of Ras proteins that are the product of a mutated Ras oncogene, it is possible that apoptosis will mitigate or stop the overgrowth of cells.[48]

Angiogenesis

Angiogenesis refers to the development/growth of new blood vessels, which is associated with the growth of cancer by allowing more blood flow to feed the growing tumor, and is displayed as a hallmark of cancer in Table 1.2.[28] There is evidence of poorer outcomes for HNSCC patients with an increase in angiogenesis.[50,51] There is also evidence that statins influence angiogenesis in animal studies. However, the directionality remains unclear. Several of these studies found that the relationship is associated with the dose of statin administered; with low doses of statins promoting angiogenesis and high doses inhibiting it.[52–55] It is possible that the different types of statins have different effects on the development of angiogenesis.[55] It is not evident if the same pattern observed in animals will be observed in HNSCC patients since, to our knowledge, no human studies investigated the association between statins and angiogenesis in HNSCC patients. Al-Husein investigated the association between statin use and certain biomarkers that may denote angiogenesis among patients with colorectal cancer and found that those who used a statin had a reduction in cluster of differentiation (CD31), which may be related to a decrease in angiogenesis.[42]

Statins and Cancer

Given that statins impact important biologic functions related to cancer, we hypothesize that the effect of statins on HNSCC outcomes can be observed in a large study of human subjects.

Few previous studies have investigated the relationship between statin use and disease-specific survival among HNSCC patients *in vivo*. Studies have investigated the relationship between statin exposure and HNSCC *in vitro*, specifically looking at the impact of statin or cholesterol-lowering medication on HNSCC tumor cell lines.[56] These studies have demonstrated that statins may induce apoptosis, and they may be cytotoxic to cancer cells.[56]

A case-control study investigated the association between HNSCC incidence and statin use in a Taiwanese population.[57] This study found statins to be significantly protective against the development of HNSCC.[57] To our knowledge, only two studies have examined the association between statin use and disease-specific outcomes in patients diagnosed with HNSCC. Both studies reported that statins were statistically significantly protective for overall mortality and disease-specific mortality.[58,59] The Lebo et al. study identified a statistically significant protective association between statin use and disease-specific mortality but the Gupta et al. manuscript only observed a statistically significant association between statin use and disease-specific mortality when comparing those who were non-statin users without high cholesterol to those who were statin users with high cholesterol.[59] Although these studies produced promising results there were a few methodological limitations and questions that need to be addressed.

Both studies were missing information on essential variables that may confound the relationship between statin use and HNSCC outcomes, such as smoking and drinking behaviors as well as clinical factors that may influence HNSCC outcomes such as body mass index (BMI).[58,59] BMI may influence the association between statin use and HNSCC outcomes as a positive confounder, which may lead to a more protective observed association than the association that would truly be expected between statin use and HNSCC outcomes. Higher BMI is often related to improved survival among patients with HNSCC.[60–62] Statin use is often recommended as a preventive treatment for cardiovascular disease among patients who are obese because obesity is a risk factor for cardiovascular disease development.[63] This may lead to patients with a higher BMI to be more likely to use statin drugs.

Both studies did not investigate how HPV status may modify the association between statin use and HNSCC outcomes. The Lebo et al. study excluded patients who had HNSCC at sites that are associated with HPV-positive disease because they did not have information about HPV status (oropharynx and oral cavity). [58] Although the oral cavity is not predominately

known as being the primary site for HPV-positive tumors, authors were concerned for misclassification of the oropharynx site diagnosis as oral cavity.[58] Excluding subjects with HPV-positive associated disease sites is an issue because HPV related HNSCC is steadily increasing over time and represents a large proportion of HNSCC cases. It is possible that their findings are not very applicable to the current development and epidemiological landscape of disease and cannot be generalized to HNSCC patients as a whole. Lastly, although they looked at overall survival and disease-specific survival they did not investigate the relationship between statins and recurrence of disease, which is important in HNSCC because recurrence is quite common among patients with advanced disease.[1,58] Findings and limitations from both studies are presented in Table 1.3.

The Gupta et al. paper also did not have information on HPV status but did not exclude any disease site, they also limited their analysis to subjects over the age of 65.[59] This is exclusion criteria is limiting their study sample leading to potentially non-generalizable results. Excluding younger participants will limit the number of subjects with HPV-positive disease. HNSCC patients with HPV-positive disease are often younger than those with HPV-negative disease.[1] The Gupta et al. paper also did not investigate the relationship between statins and recurrence of disease, which as mentioned above is an important outcome to investigate among HNSCC patients.[1,58,59]

In addition to *in vitro* and observational studies there has been one phase I trial investigating statins as a potential tertiary prevention strategy for HNSCC and cervical cancer patients. This trial administered Lovastatin among squamous cell carcinoma patients of the head and neck and cervix with advanced disease but the sample size was quite small, making it difficult to identify the impact of the drug.[64] However, as this was a phase I trial, the goal of the study was to identify the maximum dose of statin that can be tolerated among patients, rather than its impact on outcomes.[64] This study concluded that statins could be tolerated at high doses among HNSCC patients, but this study only represents a small number of patients and is

not generalizable to all patients especially those without advanced disease. Therefore, although the literature on human population-based studies investigating the relationship between statins and HNSCC survival is scarce, there have been studies investigating the relationship between statin use and survival among other cancers with protective results, and phase I trials support that statins may be a safe adjuvant treatment for these patients.[64,65]

Randomized controlled trials investigating the association between statin use and cancer outcomes or risk of developing cancer at other sites have mixed results. A randomized control trial investigating the use of statins and survival among patients with hepatocellular cancer found a protective association between Pravastatin use and survival; patients on Pravastatin lived on average 9 months longer than the participants who were not in the treatment group.[66] A meta-analysis exploring the association between statin use and breast cancer risk combining results from multiple randomized control trials and observational studies did not find an association; the results were null between statin use and breast cancer risk.[67] Findings were also null for an analysis on the association between risk of developing cancer and statin use based on a meta-analysis of 35 randomized control trials.[68] A large randomized control trial exploring the association between Pravastatin used concurrently with traditional treatment and mortality among lung cancer patients found null results.[69] It is possible statin drugs do not reduce risk of developing cancer, but may be beneficial in improving cancer-related outcomes and is dependent on site of disease.

Although, as described above, the literature on statins and HNSCC outcomes is sparse, and information about the association between statin use and cancer-related outcomes identified through randomized control trials are limited. There is a plethora of information and research investigating the association between using statins and cancer outcomes for cancers at various other sites through observational studies. A study investigating the association between statins and cancer using the “Women’s Health Initiative” data found statins may improve survival among cancer patients who have used statins at higher doses or for prolonged

periods of time across different cancer subgroups, but this study did not look at HNSCC as its own subgroup.[65]

A systematic review and meta-analysis conducted by Zhong et al. compiled observational studies that investigated the relationship between statins and cancer outcomes through 2015.[70] Although their analysis contained studies on over ten types of cancer, including breast, colorectal, gastric, hepatocellular, melanoma, lymphoma, and cancers of various disease sites in the male and female reproductive organs, they did not include any studies on HNSCC.[70] This meta-analysis found that statins were protective for overall survival and disease-specific survival, but they did not investigate recurrence of disease.[70] A more recently published meta-analyses have been conducted with similar findings supporting the protective association between statin use and most cancer-related outcomes.[71,72] Based on the potential for statin use to improve outcomes among HNSCC patients and the limited tertiary care options for these patients other than conventional or emerging oncological treatment options, it is necessary to conduct additional research to identify the potential benefits of statin drugs for HNSCC outcomes.

Remaining Questions:

Given the gaps in the current literature, it is necessary to conduct further research to identify how statin use will impact HNSCC outcomes and the possible mechanisms in which statins influence these outcomes.

1. How does statin use influence health outcomes among patients with HNSCC? Does it improve overall and disease-specific survival, and impede disease recurrence? How does HPV status modify the association between statin use and HNSCC outcomes?
2. How are commonly investigated inflammatory and immunomodulatory biomarkers associated with statin use among HNSCC patients? Does HPV status modify the association between these biomarkers and statin use?
3. What is the influence of cholesterol levels (total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglycerides) on risk of developing HNSCC and outcomes among HNSCC patients? Are circulating cholesterol levels the mechanism behind cancer development?

Specific Aims

Aim 1: Investigate whether statin use at or post-diagnosis influences HNSCC outcomes including overall survival, disease-specific survival, and recurrence free survival. (Chapter 2)

Aim 1 Hypothesis: Patients who were using a statin at diagnosis will have better HNSCC outcomes compared to those who were not (i.e. longer overall, disease-specific and recurrence free survival). Patients who start a statin at or after diagnosis will have better HNSCC outcomes than patients who never used a statin, but we hypothesize it will not be as effective as if it were identified as being taken at diagnosis.

Aim 2: Examine the influence of statin use at diagnosis on tumor-level and systemic inflammation by investigating pre-treatment tumor-infiltrating lymphocytes (TILs) in tumor tissue and circulating cytokines in baseline blood samples among HNSCC patients who were using a statin at diagnosis compared to those who were not. (Chapter 3)

Aim 2 Hypothesis: Statins are anti-inflammatory, so use of these medications will be associated with less inflammation and a more favorable immune and inflammatory profile in both tumor tissue (higher number of TILs) and systemically (lower number of pro-inflammatory cytokines) compared to non-use.

Aim 3: Explore whether lower cholesterol may be the mechanism by which statins exert any observed influence on HNSCC risk, or outcomes among HNSCC patients using genetic data from the Michigan Genomics Initiative (MGI). We will conduct a Mendelian randomization analysis examining the association between genetic variants/genetic instruments that predict hypercholesterolemia (HDL, LDL and triglycerides) and risk of developing HNSCC, and HNSCC outcomes among HNSCC patients. (Chapter 4)

Aim 3 Hypothesis: Patients who have genetic variants that predispose them to hypercholesterolemia (higher LDL and triglyceride, and lower HDL) will be more likely to have HNSCC compared to participants who do not have these genetic variants (lower risk scores for LDL and triglycerides and higher HDL) and the same associations will be observed for HNSCC patients and outcomes.

References

1. Rettig EM, D'Souza G. Epidemiology of head and neck cancer. *Surg Oncol Clin N Am*. 2015;24(3):379-396. doi:10.1016/j.soc.2015.03.001
2. Argiris A, Karamouzis M V, Raben D, Ferris RL. Head and neck cancer. *Lancet*. 2008;371(9625):1695-1709. doi:10.1016/S0140-6736(08)60728-X
3. Forastiere A, Koch W, Trotti A, Sidransky D. Head and neck cancer. *N Engl J Med*. 2001;345(26):1890-1900. doi:10.1056/NEJMra001375
4. National Cancer Institute. Cancer Stat Facts: Laryngeal Cancer. https://seer.cancer.gov/statfacts/html/laryn.html. Accessed March 15, 2019.
5. National Cancer Institute. Cancer Stat Facts: Oral Cavity and Pharynx Cancer. https://seer.cancer.gov/statfacts/html/oralcav.html. Accessed March 15, 2019.
6. National Cancer Institute. Cancer Stat Facts: Female Breast Cancer. https://seer.cancer.gov/statfacts/html/breast.html. Accessed March 15, 2019.
7. National Cancer Institute. Cancer Stat Facts: Prostate Cancer. https://seer.cancer.gov/statfacts/html/prost.html. Accessed March 15, 2019.
8. 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
9. Marur S, D'Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. *Lancet Oncol*. 2010;11(8):781-789. doi:10.1016/S1470-2045(10)70017-6
10. Sturgis EM, Cinciripini PM. Trends in head and neck cancer incidence in relation to smoking prevalence: an emerging epidemic of human papillomavirus-associated cancers? *Cancer*. 2007;110(7):1429-1435. doi:10.1002/cncr.22963
11. O'Rorke MA, Ellison MV, Murray LJ, Moran M, James J, Anderson LA. Human papillomavirus related head and neck cancer survival: a systematic review and meta-analysis. *Oral Oncol*. 2012;48(12):1191-1201. doi:10.1016/j.oraloncology.2012.06.019
12. Lajer CB, Buchwald C V. The role of human papillomavirus in head and neck cancer. *Apmis*. 2010;118(6-7):510-519. doi:10.1111/j.1600-0463.2010.02624.x
13. 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
14. Chakravarthy A, Henderson S, Thirdborough SM, et al. Human papillomavirus drives tumor development throughout the head and neck: improved prognosis is associated with an immune response largely restricted to the oropharynx. *J Clin Oncol*. 2016;34(34):4132-4141. doi:10.1200/JCO.2016.68.2955
15. Farah CS. Molecular landscape of head and neck cancer and implications for therapy. *Ann Transl Med*. 2021;9(10). doi:10.21037/atm-20-6264
16. Leemans CR, Snijders PJF, Brakenhoff RH. The molecular landscape of head and neck cancer. *Nat Rev Cancer*. 2018;18(5):269-282. doi:10.1038/nrc.2018.11
17. Maron DJ, Fazio S, Linton MF. Current perspectives on statins. *Circulation*. 2000;101(2):207-213. doi:10.1161/01.CIR.101.2.207

18. Ridker PM, Cook NR. Statins: new American guidelines for prevention of cardiovascular disease. *Lancet*. 2013;382(9907):1762-1765. doi:10.1016/S0140-6736(13)62388-0

19. Bibbins-Domingo K, Grossman DC, Curry SJ, et al. Statin use for the primary prevention of cardiovascular disease in adults. *Jama*. 2016;316(19):1997-2007. doi:10.1001/jama.2016.15450

20. Brugts JJ, Yetgin T, Hoeks SE, et al. The benefits of statins in people without established cardiovascular disease but with cardiovascular risk factors: meta-analysis of randomised controlled trials. *BMJ*. 2009;338:b2376. doi:10.1136/bmj.b2376

21. Takemoto M, Liao JK. Pleiotropic effects of 3-hydroxy-2-methylglutaryl coenzyme a reductase inhibitors. *Arter Thromb Vas Biol*. 2001;21(11):1712-1719. doi:10.1161/hq1101.098486

22. Buhaescu I, Izzedine H. Mevalonate pathway: a review of clinical and therapeutical implications. *Clin Biochem*. 2007;40(9-10):575-584. doi:10.1016/j.clinbiochem.2007.03.016

23. Kuzu OF, Noory MA, Robertson GP. The role of cholesterol in cancer. *Cancer Res*. 2016;76(8):2063-2070. doi:10.1158/0008-5472.CAN-15-2613

24. Schatzkin A, Hoover R, Taylor P, et al. Site-specific analysis of total serum cholesterol and incident cancer in the NHANES I epidemiologic follow-up study. *Cancer Res*. 1988;48(2):452-458.

25. Solomon KR, Freeman MR. Do the cholesterol-lowering properties of statins affect cancer risk? *Trends Endocrinol Metab*. 2008;19(4):113-121. doi:10.1016/j.tem.2007.12.004

26. Brown AJ. Cholesterol, statins and cancer. *Clin Exp Pharmacol Physiol*. 2007;34(3):135-141. doi:10.1111/j.1440-1681.2007.04565.x

27. Chimento A, Casaburi I, Avena P, et al. Cholesterol and its metabolites in tumor growth: therapeutic potential of statins in cancer treatment. *Front Endocrinol (Lausanne)*. 2019;9:807. doi:10.3389/fendo.2018.00807

28. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646-674. doi:10.1016/j.cell.2011.02.013

29. Demierre M-F, Higgins PDR, Gruber SB, Hawk E, Lippman SM. Statins and cancer prevention. *Nat Rev Cancer*. 2005;5(12):930-942. doi:10.1038/nrc1751

30. Fridman WH, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer*. 2012;12(4):298-306. doi:10.1038/nrc3245

31. Rezaie-Majd A, Maca T, Bucek RA, et al. Simvastatin reduces expression of cytokines interleukin-6, interleukin-8, and monocyte chemoattractant protein-1 in circulating monocytes from hypercholesterolemic patients. *Arterioscler Thromb Vasc Biol*. 2002;22(7):1194-1199. doi:10.1161/01.ATV.0000022694.16328.CC

32. Ascer E, Bertolami MC, Venturinelli ML, et al. Atorvastatin reduces proinflammatory markers in hypercholesterolemic patients. *Atherosclerosis*. 2004;177(1):161-166. doi:10.1016/j.atherosclerosis.2004.07.003

33. Ferro D, Parrotto S, Basili S, Alessandri C, Violi F. Simvastatin inhibits the monocyte expression of proinflammatory cytokines in patients with hypercholesterolemia. *J Am Coll Cardiol*. 2000;36(2):427-431. doi:10.1016/S0735-1097(00)00771-3

34. Lyngdoh T, Vollenweider P, Waeber G, Marques-Vidal P. Association of statins with inflammatory cytokines: a population-based Colaus study. *Atherosclerosis*. 2011;219(1):253-258. doi:10.1016/j.atherosclerosis.2011.07.117

35. Malicki S, Winiarski M, Matlok M, Kostarczyk W, Guzdek A, Konturek P. Il-6 and il-8 responses of colorectal cancer in vivo and in vitro cancer cells subjected to simvastatin. *J Physiol pharamacology*. 2009;60(4):141-146.

36. Pries R, Wollenberg B. Cytokines in head and neck cancer. *Cytokine Growth Factor Rev*. 2006;17(3):141-146. doi:10.1016/j.cytogfr.2006.02.001

37. Pries R, Nitsch S, Wollenberg B. Role of cytokines in head and neck squamous cell carcinoma. *Expert Rev Anticancer Ther*. 2006;6(9):1195-1203. doi:10.1586/14737140.6.9.1195

38. Duffy SA, Taylor JMG, Terrell JE, et al. Interleukin-6 predicts recurrence and survival among head and neck cancer patients. *Cancer*. 2008;113(4):750-757. doi:10.1002/cncr.23615

39. Lee KJ, Moon JY, Choi HK, et al. Immune regulatory effects of simvastatin on regulatory t cell-mediated tumour immune tolerance. *Clin Exp Immunol*. 2010;161(2):298-305. doi:10.1111/j.1365-2249.2010.04170.x

40. Nguyen N, Bellile E, Thomas D, et al. Tumor infiltrating lymphocytes and survival in patients with head and neck squamous cell carcinoma (HNSCC). *Head Neck*. 2016;38(7):1074-1084. doi:10.1002/hed.24406

41. Spector ME, Bellile E, Amlani L, et al. Prognostic value of tumor-infiltrating lymphocytes in head and neck squamous cell carcinoma. *JAMA Otolaryngol Head Neck Surg*. 2019;145(11):1012-1019. doi:10.1001/jamaoto.2019.2427

42. Al-Husein BA, Dawah B, Bani-Hani S, Al Bashir SM, Al-Sawalmeh KM, Ayoub NM. Immunomodulatory effect of statins on regulatory t lymphocytes in human colorectal cancer is determined by the stage of disease. *Oncotarget*. 2018;9(87):35752-35761. doi:10.18632/oncotarget.26293

43. Mandal R, Şenbabaoğlu Y, Desrichard A, et al. The head and neck cancer immune landscape and its immunotherapeutic implications. *JCI Insight*. 2016;1(17):1-18. doi:10.1172/jci.insight.89829

44. Andersen AS, Solling ASK, Ovesen T, Rusan M. The interplay between HPV and host immunity in head and neck squamous cell carcinoma. *Int J Cancer*. 2014;134(12):2755-2763. doi:10.1002/ijc.28411

45. Hindler K, Cleeland CS, Rivera E, Collard CD. The role of statins in cancer therapy. *Oncologist*. 2006;11(3):306-315. doi:10.1634/theoncologist.11-3-306

46. Tsubaki M, Fujiwara D, Takeda T, et al. The sensitivity of head and neck carcinoma cells to statins is related to the expression of their Ras expression status, and statin-induced apoptosis is mediated via suppression of the Ras/ERK and Ras/mTOR pathways. *Clin Exp Pharmacol Physiol*. 2017;44(2):222-234. doi:10.1111/1440-1681.12690

47. Bos JL. ras oncogenes in human cancer: a review. *Cancer Res*. 1989;49(17):4682-4689. doi:10.1158/0008-5472.can-08-0755

48. Malumbres M, Barbacid M. RAS oncogenes: the first 30 years. *Nat Rev Cancer*. 2003;3(6):459-465. doi:10.1038/nrc1097

49. Berndt N, Hamilton AD, Sebti SM. Targeting protein prenylation for cancer therapy. *Nat*

Rev Cancer. 2011;11(11):775-791. doi:10.1038/nrc3151

50. Seiwert TY, Cohen EEW. Targeting angiogenesis in head and neck cancer. *Semin Oncol*. 2008;35(3):274-285.

51. Vassilakopoulou M, Psyrri A, Argiris A. Targeting angiogenesis in head and neck cancer. *Oral Oncol*. 2015;51(5):409-415. doi:10.1016/j.oraloncology.2015.01.006

52. Skaletz-Rorowski A, Walsh K. Statin therapy and angiogenesis. *Curr Opin Lipidol*. 2003;14(6):599-603. doi:10.1097/00041433-200312000-00008

53. Weis M, Heeschen C, Glassford AJ, Cooke JP. Statins have biphasic effects on angiogenesis. *Circulation*. 2002;105(6):739-745. doi:10.1161/hc0602.103393

54. Kureishi Y, Luo Z, Shiojima I, et al. The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals. *Nat Med*. 2000;6(8):1004-1010. doi:10.1038/79510

55. Dulak J, Jozkowicz A. Anti-angiogenic and anti-inflammatory effects of statins: relevance to anti-cancer therapy. *Curr Cancer Drug Targets*. 2005;5(8):579-594. doi:10.2174/156800905774932824

56. Pavan LMC, Rêgo DF, Elias ST, De Luca Canto G, Guerra ENS. In vitro anti-tumor effects of statins on head and neck squamous cell carcinoma: a systematic review. *PLoS One*. 2015;10(6):e0130476. doi:10.1371/journal.pone.0130476

57. Kao L-T, Hung S-H, Kao P-F, Liu J-C, Lin H-C. Inverse association between statin use and head and neck cancer: population-based case-control study in Han population. *Head Neck*. 2019;41(5):1193-1198. doi:10.1002/hed.25501

58. Lebo NL, Griffiths R, Hall S, Dimitroulakos J, Johnson-Obaseki S. Effect of statin use on oncologic outcomes in head and neck squamous cell carcinoma. *Head Neck*. 2018;40(8):1697-1706. doi:10.1002/hed.25152

59. Gupta A, Stokes W, Eguchi M, et al. Statin use associated with improved overall and cancer specific survival in patients with head and neck cancer. *Oral Oncol*. 2019;90:54-66. doi:10.1016/j.oraloncology.2019.01.019

60. Arthur AE, Peterson KE, Rozek LS, et al. Pretreatment dietary patterns, weight status, and head and neck squamous cell carcinoma prognosis. *Am J Clin Nutr*. 2013;97(2):360-368. doi:10.3945/ajcn.112.044859

61. Gama RR, Song Y, Zhang Q, et al. Body mass index and prognosis in patients with head and neck cancer. *Head Neck*. 2017;39(6):1226-1233. doi:10.1002/HED.24760

62. den Hollander D, Kampman E, van Herpen CML. Pretreatment body mass index and head and neck cancer outcome: a review of the literature. *Crit Rev Oncol / Hematol*. 2015;96(2):328-338. doi:10.1016/j.critrevonc.2015.06.002

63. Arnett DK, Blumenthal RS, Albert MA, et al. 2019 ACC / AHA guideline on the primary prevention of cardiovascular disease. *J Am Coll Cardiol*. 2019;74(10):1376-1414. doi:10.1016/j.jacc.2019.03.010

64. Knox JJ, Siu LL, Chen E, et al. A phase I trial of prolonged administration of lovastatin in patients with recurrent or metastatic squamous cell carcinoma of the head and neck or of the cervix. *Eur J Cancer*. 2005;41(4):523-530. doi:10.1016/j.ejca.2004.12.013

65. Wang A, Aragaki AK, Tang JY, et al. Statin use and all-cancer survival : prospective results from the women's health initiative. *Br J Cancer*. 2016;115(1):129-135.

doi:10.1038/bjc.2016.149

66. Kawata S, Yamasaki E, Nagase T, et al. Effect of pravastatin on survival in patients with advanced hepatocellular carcinoma . A randomized controlled trial. *Br J Cancer*. 2001;84(7):886-891. doi:10.1054/ bjoc.2001.1716

67. Bonovas S, Filioussi K, Tsavaris N, Sitaras NM. Use of statins and breast cancer : a meta-analysis of seven randomized clinical trials and nine observational studies. *J Clin Oncol*. 2005;23(34):8606-8612. doi:10.1200/JCO.2005.02.7045

68. Bonovas S, Filioussi K, Tsavaris N, Sitaras NM. Statins and cancer risk : a literature-based meta-analysis and meta-regression analysis of 35 randomized controlled trials. *J Clin Oncol*. 2006;24(30):4808-4817. doi:10.1200/JCO.2006.06.3560

69. Seckl MJ, Ottensmeier CH, Cullen M, et al. Multicenter, phase III, randomized, double-blind, placebo-controlled trial of pravastatin added to first-line standard chemotherapy in small-cell lung cancer (LUNGSTAR). *J Clin Oncol*. 2017;35(14):1506-1514. doi:10.1200/JCO.2016.69.7391

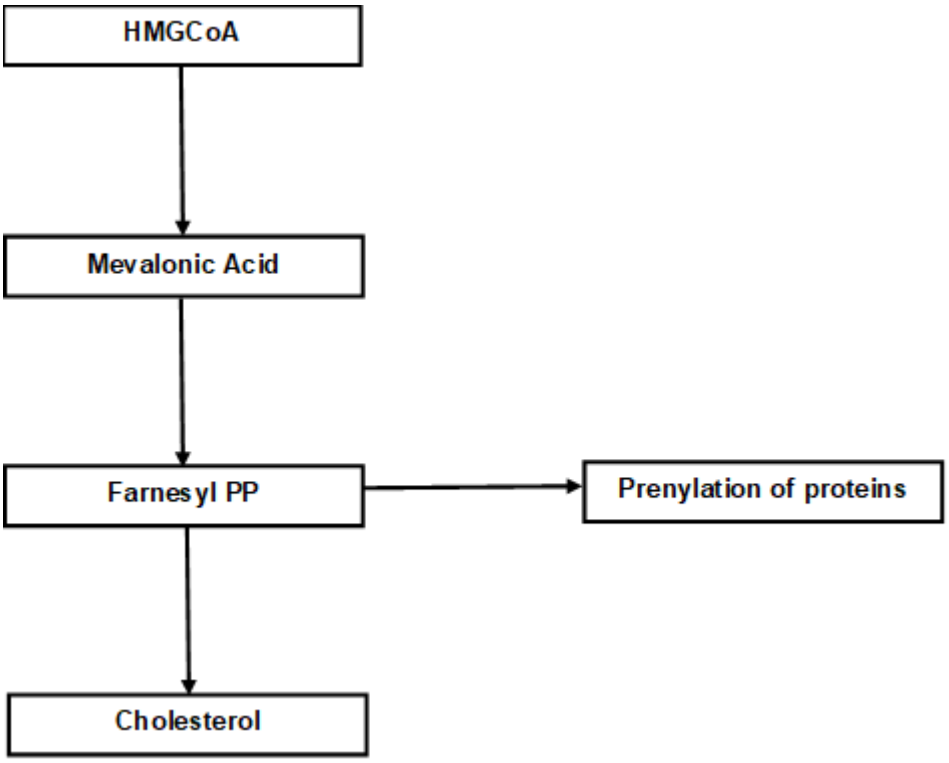
70. Zhong S, Zhang X, Chen L, Ma T, Tang J, Zhao J. Statin use and mortality in cancer patients: systematic review and meta-analysis of observational studies. *Cancer Treat Rev*. 2015;41(6):554-567. doi:10.1016/j.ctrv.2015.04.005

71. Mei Z, Liang M, Li L, Zhang Y, Wang Q, Yang W. Effects of statins on cancer mortality and progression: a systematic review and meta-analysis of 95 cohorts including 1,111,407 individuals. *Int J Cancer*. 2017;140(5):1068-1081. doi:10.1002/ijc.30526

72. Yang J, Li C, Shen Y, et al. Impact of statin use on cancer-specific mortality and recurrence: a meta-analysis of 60 observational studies. *Medicine (Baltimore)*. 2020;99(14):1-10.

Chapter 1: Figures/Tables

Figure 1.1: Mevalonate Pathway



Adpated from Figure 1 in : Buhaescu I, Izzedine H. Mevalonate pathway: a review of clinical and therapeutical implications. *Clin Biochem*. 2007;40(9-10):575-584. doi:10.1016/j.clinbiochem.2007.03.

Table 1.1: HNSCC by HPV Status

	Human Papillomavirus (HPV) Positive Patients	Human Papillomavirus (HPV) Negative Patients
Incidence*	• Increasing in the USA • 4.62 per 100,000 (oropharynx) • 0.62 per 100,000 (non-oropharynx disease sites)	• Decreasing in the USA • 1.82 per 100,000 (oropharynx) • 1.38 per 100,000 (non-oropharynx disease sites)
Survival	• 60%-90% (5 year, oropharynx)	• 20%-25% (5 year, oropharynx) • 65% (5 year, non-oropharynx disease sites)
Recurrence	• 10%-15% (5 year, oropharynx)	• 50% (5 year, oropharynx) • 50% (5 year, non-oropharynx disease sites)
Risk Factors	• Oral HPV infection	• Tobacco • Alcohol
Patient Characteristics	• Young (<60 years old) • White • Male • Higher socioeconomic status • Less likely to use tobacco	• Older (55+ years old) • White • Male • Lower socioeconomic status • More likely to use tobacco
Disease Site	• Oropharynx	• Nasal cavity • Oral Cavity • Oropharynx • Hypopharynx • Larynx
Immune Response	• Strong, possibly due to site of disease including tonsil (lymphatic tissue)	• Not as strong as HPV-positive associated HNSCC

Adpated from Table 1 in: Rettig EM, D'Souza G. Epidemiology of head and neck cancer. *Surg Oncol Clin NA*. 2015;24(3):379-396. doi:10.1016/j.soc.2015.03.001

* Mahal BA, Catalano PJ, Haddad RI, et al. Incidence and demographic burden of HPV-associated oropharyngeal head and neck cancers in the United States. *Cancer Epidemiol Biomarkers Prev*. 2019;28(10):1660-1668. doi:10.1158/1055-9965.EPI-19-0038

Table 1.2: Hallmarks of Cancer

Hallmarks of Cancer	Emerging Hallmarks and Enabling Characteristics
Resisting Cell Death	Deregulating cellular energetics
Inducing Angiogenesis	Avoiding immune destruction
Sustaining proliferative signaling	Genome instability and mutation
Enabling replicative immortality	Tumor promoting inflammation
Evading growth suppressors	
Activating invasion and metastasis	

Adapted from Figure 1 and Figure 3 in: Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-674. doi:10.1016/j.cell.2011.02.013

Table 1.3: Comparison of Statin and HNSCC Outcomes Studies

Study	Exposure	Overall Mortality	Cancer Specific Mortality	Limitations
Lebo et al.	Never Statins	**1.0 (ref)**	**1.0 (ref)**	• Missing information on recurrence and other important confounders • Possible incorrect staging information • Missing HPV status
	Ever Statins	**0.76 (0.64-0.90)**	**0.69 (0.54-0.89)**	
Gupta et al.	H[a], Statin Users	**1.0 (ref)**	**1.0 (ref)**	• Missing information on recurrence and other important confounders • Included only patients on Medicare (65 years+) • Excluded patients distant metastatic disease
	No H[a], Non-Statin User	**1.64 (1.29-2.07)**	**1.56 (1.1-2.21)**	
	H[a], Non-Statin User	**1.40 (1.12-1.74)**	**1.37 (0.99-1.89)**	

a. H= hypercholesterolemia

Lebo NL, Griffiths R, Hall S, Dimitroulakos J, Johnson-Obaseki S. Effect of statin use on oncologic outcomes in head and neck squamous cell carcinoma. *Head Neck*. 2018;40(8):1697-1706. doi:10.1002/hed.25152

Gupta A, Stokes W, Eguchi M, et al. Statin use associated with improved overall and cancer specific survival in patients with head and neck cancer. *Oral Oncol*. 2019;90:54-66. doi:10.1016/j.oraloncology.2019.01.019

Chapter 2 : Statin Use and Head and Neck Squamous Cell Carcinoma Outcomes

(**Article citation:** Getz KR, Bellile E, Zarins KR, et al. Statin use and head and neck squamous cell carcinoma outcomes. *Int J Cancer*. 2021;148(10):2440-2448. doi:10.1002/ijc.33441)

Authors: Kayla R. Getz[1], Emily Bellile[2], Katie R. Zarins[3], Cailey Rullman[1], Steven B. Chinn[4], Jeremy M.G. Taylor[2], Laura S. Rozek[3, 4], Gregory T. Wolf[4], Alison M. Mondul[1]

1. Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, Michigan
2. Department of Biostatistics, University of Michigan School of Public Health, Ann Arbor, Michigan
3. Department of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, Michigan
4. Department of Otolaryngology-Head and Neck Surgery, University of Michigan, Ann Arbor Michigan

Abstract

Background: Head and neck squamous cell carcinoma (HNSCC) is a morbid cancer with poor outcomes. Statins have been identified as having anti-cancer properties such as immunomodulatory and anti-inflammatory effects. The objective of this study is to identify the association between statin use among previously untreated HNSCC patients and health outcomes, specifically overall death, disease-specific death and recurrence.

Methods: Incident HNSCC patients were recruited through the University of Michigan Rogel Cancer Center to participate in the University of Michigan Head and Neck Cancer Specialized Program of Research Excellence (SPORE) from 2003-2014. Statin use data were collected through medical record review. Participants were considered a statin user if they used a statin at or after diagnosis. Outcome data were collected through medical record review, social security death index or LexisNexis. Our analytic cohort included 1,638 participants who had information

on statin use and HNSCC outcomes. Cox proportional hazard models were used to estimate the association between ever statin use and HNSCC outcomes.

Results: Statin use was seen in 36.0% of participants. We observed a statistically significant inverse association between ever using a statin and both overall death (HR=0.75, 95%CI=0.63-0.88) and HNSCC-specific death (HR=0.79, 95%CI=0.63-0.99) and a non-statistically significant inverse association for recurrence (HR=0.85, 95%CI=0.70-1.04). When investigating the association between statin use and HNSCC outcomes utilizing interaction terms between statin use and human papillomavirus (HPV), a statistically significant interaction for HNSCC-specific death and recurrence were identified (HNSCC-specific death: HPV-positive HR=0.41, 95%CI=0.21-0.84; HPV-negative HR=1.04, 95%CI=0.71-1.51; p-int-0.02; recurrence: HPV-positive HR=0.49, 95%CI=0.29-0.84; HPV-negative HR=1.03, 95%CI=0.74-1.43; p-int-0.02).

Conclusions*:* Statin use may be protective for adverse outcomes in HNSCC patients, particularly those with HPV-positive disease. If true, these findings could have a meaningful impact on tertiary prevention for this cancer.

Background

Head and neck squamous cell carcinoma (HNSCC) is a debilitating disease with high morbidity and a 5-year relative survival rate ranging from 31.9%-89.5% depending on the site of disease.[1] HNSCC arises from the mucosal lining of the aerodigestive tract starting in the nasal cavity and ending in the throat at the larynx, with other sites including the oral cavity, oropharynx, and hypopharynx.[1–3] The epidemiology of HNSCC has changed over the past decades in the United States due to a shift in the major risk factors associated with certain disease sites. There has been a decrease in the number of patients with HNSCC of the oral cavity and larynx in the United States which may be explained by the large decrease in smoking over time, the main risk factor for HNSCC in these sites.[4,5] Human papillomavirus (HPV) positive HNSCC, which is predominantly found in the oropharynx, has been increasing in the United States over the past decades.[6] HPV-positive disease has better prognosis than HPV-negative HNSCC and appears to be more responsive to treatment.[7] This may be related to the difference in patient characteristics between patients with HPV-positive tumors and patients with HPV-negative HNSCC.[6,7]

Although treatment options have improved and expanded over the decades, there are still no evidence-based tertiary prevention strategies for HNSCC other than tobacco cessation. Statins are a class of commonly prescribed medications that are primarily used to lower cholesterol.[8] In addition, statins exhibit anti-cancer effects including, anti-inflammatory and immunomodulatory properties.[9] Thus, it is hypothesized that they may prevent the development or progression of cancer through these mechanisms or through cholesterol-lowering.[9] Cholesterol may be an essential factor in cancer development or progression because it is involved in various pathways associated with carcinogenesis.[10]

Numerous studies have investigated the association between statin use and cancer outcomes among different cancer sites and have found protective results, but the literature is

limited when investigating this association among HNSCC patients.[11,12] The few studies that have investigated this association found a protective association between statin use and the development of HNSCC, as well as a protective association between the use of statins and overall death and HNSCC-specific death.[13–15] Although these analyses provide promising results they had some important limitations. First, they did not adjust for some important confounding variables in their models, notably characteristics such as body mass index (BMI). Further, none of these studies examined HPV-positive and HPV-negative disease separately other than through the elimination of certain disease sites from analysis.[14] This is important because HPV-positive HSNCC is increasing in the US and have different pathogeneses and immune/inflammatory response modulation.[1] They also did not investigate the association between statin use and disease recurrence as an outcome.

The objective of this study is to examine the association between statin use and HNSCC survival outcomes in a large prospective cohort of previously untreated HNSCC patients, with thorough adjustment for potential confounding factors and examining HPV-positive and negative disease separately.

Material and Methods

Study Population

This study includes incident cases of HNSCC who participated in the University of Michigan Head and Neck Cancer Specialized Program of Research Excellence 1 and 2 (SPORE 1 and SPORE 2). Participants in SPORE 1 were diagnosed and recruited from 2003-2008, and recruitment for SPORE 2 participants started in 2008 and ended in 2014. Subjects were eligible for participation if they were over the age of 18 years old, newly diagnosed with disease, and had not previously received treatment for their cancer. A more in-depth explanation of the study recruitment and procedures has been published previously.[16,17] There

were 1,648 patients who were recruited through the University of Michigan in SPORE 1 (N=606) and SPORE 2 (N=1,042). Of the patients who participated in the Head and Neck Cancer SPORE, 1,638 participants had complete information on statin use.

Exposure

Information on use of selected medications, including statins, at any point during follow-up, was previously collected for SPORE 1 participants from the medical record. For the current analysis, medication information for SPORE 2 participants was newly collected through medical record abstraction. The following statins were used among SPORE 2 participants: Atorvastatin (Lipitor, Caduet)= 23.7%, Lovastatin (Mevacor)=9.9%, Pravastatin (Pravachol)=11.7%, Rosuvastatin (Crestor)=9%, Simvastatin (Simcor, Zocor, Vytorin)=46.5%; the total does not equal 100% because categories were not mutually exclusive, 3 participants reported taking two different statins. Comprehensive information on medication use was routinely collected by physicians prior to initiating treatment. Patients were asked if they were currently using any medications. If yes, the name of the medication was recorded in the medical record. The initial visit related to the patient's HNSCC diagnosis at the University of Michigan Rogel Cancer Center was identified in the electronic medical record, and all medications reported were abstracted from that encounter, if available. If this information was not available during that encounter, the information was abstracted from the closest encounter to the initial encounter date but prior to treatment initiation. To identify if participants started a statin after diagnosis, electronic medical record search engine (EMERSE) was used to search for statin medication names throughout the medical record.[18] If a participant was identified as using a statin at or after diagnosis, they were classified as ever using a statin.

In addition to investigating the exposure as ever statin use, we also investigated the association between statin use at diagnosis and HNSCC outcomes among the SPORE 2 participants for whom pre- and post-diagnosis use could be differentiated. Trained research

personnel collected the exposure information from all participants if available. The inter-rater reliability was quite high between abstractors with a Kappa value of approximately 95%. Patient records that were not concordant were reviewed by both abstractors and reconciled. All data were stored in a Research Electronic Data Capture (REDCap) database.[19]

Outcome

Patients seen at the University of Michigan were annually monitored for overall death, disease-specific death, and recurrence through medical record review and patient follow-up. Recurrence of disease was defined as patients who were identified as never being free of disease, as well as those who experienced distant, local or regional recurrence of disease. Beyond this surveillance, linkage with the national social security death index (NDI) to ascertain participant survival was conducted annually. After study follow-up was complete, a final update of the participants' survival status was conducted through LexisNexis. If a participant was in the NDI, or if a family member informed us that they had died, medical records or resources through LexisNexis were reviewed by trained study personnel to determine the date of death and whether the death was due to HNSCC or another cause. Death or recurrence information was reviewed by physicians for accuracy. Participants' outcome information was collected and updated through April 2016.

Confounding Variables

Information on potential confounding variables were collected through various data sources. Confounders related to behaviors such as smoking and drinking were collected through health surveys that were completed yearly by patients. Demographic and clinically related confounders such as race, gender, age, BMI, and Adult Comorbidity Evaluation 27 score (ACE-27) were collected through the patient's medical record. There were several effect modifiers that we assessed; HPV status, disease site, and stage at diagnosis. HPV status was

identified through the following testing; polymerase chain reaction (PCR) testing and in situ hybridization, both of which have high sensitivity and specificity (>80%).[20] If tissue was not tested for HPV status from a previously conducted sub-study of SPORE 1 or SPORE 2, it was obtained through the patient's medical record or pathology reports.

All models were adjusted for age at diagnosis as a continuous variable. The following factors that are hypothesized or known to be associated with either HNSCC outcomes or statin use were considered as potential confounding variables: gender, race, ACE-27, smoking, BMI, education and stage. Certain clinical variables thought to be associated with HNSCC survival that are often adjusted for in survival analyses, such as site of disease and HPV status, were not independently associated with the use of statins in our data. Factors such as age, gender and race may be on the path by which these variables are associated with statin use, therefore by adjusting for these variables should block all backdoor paths that may have been present because these variables occur temporally before HPV status. Directed acyclic graphs displaying the relationships between the exposure and outcome variables of interest as well as other variables that may influence the association as confounders or mediators are displayed in Figures 2.1 and 2.2. Each potential confounder was entered into the age-adjusted model to evaluate whether the point estimates for statin use changed by at least 10%. The variables that impacted the association between statin use and HNSCC outcomes the most were age, ACE-27, and BMI. Other potential confounders were added to the model individually and cumulatively, but their inclusion resulted in a less than 10% magnitude change in the point estimates.

The most parsimonious and final model includes only age, ACE-27, and BMI as confounders. We also presented results from a fully adjusted model (adjusted for, age (continuous), BMI (<25, 25-<30, 30+ kg/m^2, missing), ACE-27 (none, mild, moderate, severe), gender (female, male), education (less than high school, high school or General Education

Development (GED), some college, 4-year degree, more than 4-year degree, missing), race (white, not white, missing), smoking status (never, former, current, missing), drinking status (never, former, current, missing), and stage of disease (0 or 1, 2, 3, 4). Only 7 participants were missing information for ACE-27, so these individuals were included in the most common category (Mild) for adjustment, as there were too few for a separate missing category.

Statistical Analysis

Descriptive and bivariate analysis was conducted to compare demographic, behavioral, and clinical characteristics between participants who were considered ever statin users compared to those who did not use a statin at or after diagnosis. Statistical significance was determined through chi-square tests for categorical variables and t-tests for continuous variables.

Cox proportional hazard models were used to estimate the association between ever statin use and overall and disease-specific death as well as disease recurrence. For recurrence, we began follow-up at the time of diagnosis. Patients who experienced recurrence ended follow-up at the date of documented recurrence. Participants who did not experience recurrence were censored at last follow-up. Similarly, for progression to disease-specific or overall death, follow-up began at the time of diagnosis. For disease-specific death, participants who did not die due to malignancy were censored at the time of death due to other causes or last follow-up. Follow-up ended for participants who did die from malignancy at the time of death. For overall death, participants who did not die were censored at the end of follow-up. Follow-up ended for participants who died at the time of death. We estimated the hazard ratio (HR) of recurrence, overall death, and disease-specific death for ever statin use (versus non-use). We also estimated the HR of recurrence, overall death, and disease-specific death for statin use at diagnosis (versus non-use) among SPORE 2 participants, as explained above. The

proportional hazards assumption was confirmed through conducting interaction models with time.

In this study, we investigated if effect modification was present across HPV status, disease site, and stage of disease. Subgroup analyses were conducted stratifying by disease site (HPV-positive associated disease sites: oropharynx vs. HPV-negative associated disease sites: oral cavity, larynx, hypopharynx and other), stage of disease (stage 4 vs. stages 0-3), and HPV status (HPV-positive, HPV-negative, HPV status invalid/missing). Statistical interaction was evaluated using the likelihood ratio test. HPV status was missing for approximately half of the participants (49.45%). Thus, in order to identify if missing HPV status biased the point estimates, we conducted various sensitivity analyses. We kept the participants with missing HPV status as a separate category, dropped the participants with HPV status missing, and used stabilized inverse probability weighting (IPW) to weigh participants to emulate the population if no one was missing HPV status. In order to calculate stabilized inverse probability weights logistic regression models were conducted to calculate the probability of not having HPV status missing. The predictors in this model included: year enrolled in the study, age, gender, smoking status, drinking status, marital status, stage of disease, ACE-27 scores, disease site and BMI. Observations with values missing for the predictors in the model were dropped from the analysis and are missing probabilities. Probabilities generated from this model were the denominators of the weights. An intercept only model for the probability of not having HPV status missing was conducted. The probabilities calculated from the intercept only model were the numerator of the weights and were used instead of the value 1 to standardize the distribution of the weights, which allows provides a more normal distribution. Participants who have similar predictors to those who are missing HPV status will have higher weights in the final model and participants missing HPV status were dropped.

Causal Inference Method

To address the issue of missing observations across various potential confounding variables, I conducted multiple imputation (MI) using the MICE package in R generating 20 datasets. In order to efficiently address potential confounding of the association between statin use and HNSCC outcomes, I created IPWs for all participants. The combination of utilizing MI for missing values and IPW for addressing confounding is an optimal approach to identify the association between statin use and HNSCC outcomes through minimizing bias due to missingness and confounding.[21]

After MI was complete, probabilities were predicted for statin use for all participants. A logistic regression model was conducted in each of the 20 imputed datasets. Variables included in the logistic regression to predict statin use were age, gender, race, smoking status, education, ACE-27, BMI, and diagnosis of myocardial infarction, angina/coronary artery disease, congestive heart failure and diabetes mellitus. In order to identify if the probability of statin use was balanced between identified statin users vs. non-users, balance plots were examined. Figures 2.3 and 2.4 display that there appears to be a relatively similar distribution of the probability of statin use among non-statin users and statin users, although there are much more non-statin users in the study than statin users and non-statin users are more likely to have a lower probability of statin use, there appears to be an overlap of probabilities between users and non-users. IPWs were calculated as follows; 1/ (probability of statin use), for participants who were considered statin users in the study and 1/ (1-probability of statin use), for participants who were not considered statin users. In the event of imbalance, additional truncated weights were created which excluded participants who had a weight larger than 13.

Marginal structural models using Cox proportional hazard models were conducted to calculate hazard ratios (HRs) and 95% confidence intervals for all of the outcomes (overall mortality, disease-specific mortality and disease recurrence) weighted with the inverse

probability weights across the 20 imputed datasets. Marginal structural models were also conducted using the truncated IPWs. In addition to conducting marginal models, models that additionally adjusted for age, age and BMI, and age, BMI and ACE-27 were also conducted to determine if additionally adjusting for these strong confounders helped to eliminate potential residual confounding.

In addition to analyzing the association between statin use and the listed HNSCC outcomes, I also conducted interaction models to identify if HPV status remained an effect modifier after addressing the missing values through MI and adjusting for confounding using the IPWs mentioned above. Point estimates and 95% confidence intervals across the 20 datasets were pooled using Rubin's Rule.[22]

All analyses were conducted using SAS version 9.4 (Cary, NC). All tests were two-sided, and results were considered statistically significant if $p<0.05$.

Results

Descriptive statistics of statin use across baseline demographic and clinical characteristics are displayed in Table 2.1. In the study cohort, 36.0% of participants ever used a statin. Participants who used a statin were on average older, were more likely to be white, overweight or obese, or former smokers and drinkers, to have mild ACE-27 scores, and were more highly educated.

Over the follow-up period (657) 40.1% of participants in the study died due to any cause and (379) 23.1% died from HNSCC. Disease recurrence occurred in 30.2% (495) of participants. We observed a statistically significant inverse association between ever statin use and the rate of overall [multivariable adjusted (MV)-adj HR= 0.75; 95% CI= (0.63, 0.88)] and disease-specific [MV-adj HR= 0.79; 95% CI= (0.63, 0.99)] death among HNSCC patients in this study (Table 2.2). Similarly, we observed a suggestive inverse association between ever statin

use and disease recurrence [MV-adj HR= 0.85; 95% CI= (0.70, 1.04), Table 2.2]. Results from the fully adjusted model were similar (Table 2.2). Additional analyses were conducted to identify if the association between statin use and HNSCC outcomes was still present after excluding participants who had distant metastatic disease at diagnosis (N=44) as well as excluding participants who died within 6 months of diagnosis (N=104) (Table 2.4); results from these additional analyses are consistent with the findings presented in Table 2.2.

When we examined participants with HPV-positive and HPV-negative tumors separately, excluding participants who were missing HPV status, we observed a protective association between statin use and overall death for those in the HPV-positive [MV-adj HR= 0.52; 95% CI= (0.31, 0.86)], and HPV-negative [MV-adj HR= 0.76; 95% CI= (0.57, 1.02)] groups. However, when we examined the association between statin use and rate of disease-specific death we observed a significant interaction such that this inverse association was only observed for patients who were HPV-positive [HNSCC-specific death: MV-adj HR= 0.41; 95% CI= (0.21, 0.84)] and null for patients who were HPV-negative, HNSCC-specific death: [MV-adj HR= 1.04; 95% CI= (0.71, 1.51)], p for interaction = 0.02 (Table 2.3). This protective relationship was also observed for HPV-positive patients' rate of recurrence: [MV-adj HR= 0.49; 95% CI= (0.29, 0.84)] while a null association for patients whose tumor HPV status was negative was observed: [MV-adj HR= 1.03; 95% CI= (0.74, 1.43)], p for interaction=0.02. Results were very similar when we excluded participants who had distant metastatic disease (Table 2.5) as well as when we included a missing category for HPV status or when IPW was used to address missingness (Table 2.6). Results remain inverse for HPV-positive patients when conducting the analysis among participants in SPORE 2 for statin use at baseline.

When excluding participants who were missing HPV status, we observed a protective association for the relationship between statin use at diagnosis and overall death among participants who were HPV-positive and who were HPV-negative but neither these results nor

the interaction term were statistically significant. The association between statin use at diagnosis and HNSCC-specific death as well as recurrence were similar to the association that was displayed above between ever statin use and HNSCC-specific death and recurrence. Results for the HNSCC-specific death associations were, HPV-positive: [MV-adj HR= 0.53; 95% CI= (0.21, 1.31)], HPV-negative: [MV-adj HR= 1.19; 95% CI= (0.76, 1.85)], (p for interaction= 0.09) and for recurrence HPV-positive: [MV-adj HR= 0.51; 95% CI= (0.25, 1.03)], HPV-negative: [MV-adj HR= 1.14; 95% CI= (0.77, 1.69)], (p for interaction=0.04).

We found a statistically significant inverse association between ever statin use and recurrence for the stratified analysis among participants whose site of disease was located in the oropharynx compared to those who never took a statin (Table 2.3), but these results were not as strong as those observed in the HPV stratified analysis. We observed no statistically significant interaction by stage (Table 2.3).

Estimates calculated using the combined MI and IPW technique were similar to the point estimates calculated in the adjusted analyses explained above. Missing values and balance of covariates did not appear to bias the estimates by a meaningful amount. Results for all models conducting using this combined MI and IPW approach are presented in Tables 2.7 and 2.8.

Discussion

In this large prospective study of HNSCC patients, we observed a protective association between statin use and disease-specific death and recurrence that was restricted to HPV-positive patients. However, statins were protective for overall death in all patients. To our knowledge, this is the first paper to investigate the association between statin use and HNSCC outcomes stratified by HPV status and with comprehensive adjustment for potential confounding factors (Table 2.2).

Two previous studies observed a protective association for both HNSCC overall death and disease-specific death. One of these studies only included HNSCC disease sites that are not associated with HPV-positive disease and the other study did not consider HPV status in their analyses.[14,15] To our knowledge, the present analyses are the first to consider the role of HPV status in the statin-HNSCC outcome association. Importantly, neither of these previous studies adjusted for BMI in their analyses.[14,15] Higher BMI has been found to be protective against adverse HNSCC outcomes.[23–25] Further, according to the American College of Cardiology and the American Heart Association, obesity is a risk factor for developing heart disease and statin use is recommended for people who are at "borderline or intermediate risk" for cardiovascular disease, making statin use more common among individuals with a higher BMI.[26] Thus, BMI would be a positive confounder of the statin-HNSCC outcome association, and failing to adjust for this confounder could have biased their results toward a more protective association than is actually present. In fact, in the present analysis, our results were markedly attenuated when we adjusted for BMI. This may explain the apparently discrepant findings between previous studies that found a protective association even among patients who were likely HPV-negative.

Our findings are consistent with the potential biological impact of statin drugs on cancer cells. Statins possess anti-inflammatory and immunomodulatory effects and these effects may influence cancer.[9,27] There have been studies to suggest the presence of certain inflammatory markers may improve HNSCC outcomes such as tumor-infiltrating lymphocytes, or make HNSCC outcomes worse such as the pro-inflammatory cytokine, IL-6.[17,28,29] Although there do not appear to be any studies about the production of inflammatory markers and statin use among patients with HNSCC, there are a few studies that have investigated the use of statins and the presence of inflammatory biomarkers among the general population and among patients with hypercholesterolemia. Some studies found a reduction in the number of circulating

pro-inflammatory cytokines among statin users.[30–33] Statins have also been associated with the increased production of T-cells in mice and lung tumor cell lines, and as mentioned above the presence of T-cells in tumors are often associated with better outcomes among HNSCC patients.[34] Thus, anti-inflammation and immune modulation are plausible mechanisms by which statins may provide protection against adverse outcomes in HNSCC patients specifically among patients whose disease is HPV-positive. This association may be related to the known pro-inflammatory and active immune response seen predominantly in HPV-positive HNSCC, however, further research is needed to establish the biologic mechanism by which statins are protective.[28,29,35–39]

Strengths and limitations

The strengths of the study are a large sample size representing various sites of HNSCC, excellent survival ascertainment, and information on many clinical tumor characteristics, potential confounders, and effect modifiers. One possible limitation is that, although the data available at diagnosis for patients was collected systematically, the data collected post-diagnosis was not consistently available for all participants depending upon whether they attended the University of Michigan for routine follow-up care. This may lead to misclassifying participants as never statin users who may have started a statin after diagnosis but were lost to follow-up. If statins are truly protective against cancer outcomes, as the literature suggests, this would bias our estimates toward the null, meaning the true association may be even stronger than what we observed. It is possible that participants who are very ill prior to study recruitment or HNSCC diagnosis may discontinue medication/statin use. We may potentially misclassify those participants as never statin users, which may lead to observing a stronger protective association than what is expected. To mitigate this issue we conducted a sensitivity analysis that excluded participants who had distant metastatic disease and those who passed away within 6 months of diagnosis (Table 2.4). After excluding participants with distant metastatic

disease we still observed a statistically significant interaction such that we observed an inverse association between statin use and both disease-specific death and recurrence only among HPV-positive patients (Table 2.5). Using an ever statin use variable may lead to immortal time bias, which leads to possible misclassification of the exposure variable because participants may start statins after diagnosis or discontinue statin use at any point in the study. This would lead to misclassifying participants as statin users during periods of time in which they were not using a statin. However, when we considered use of statins at baseline only among the SPORE 2 participants, our results for the HPV-positive patients remained inverse, although they were no longer statistically significant, likely due to the reduction in sample size. It should be noted that among SPORE 2 participants, 32.2% were statin users at diagnosis and only a small minority of participants initiated statin use after diagnosis (7.1%), making this source of bias likely to be minimal. Additional studies with detailed information on statin use are needed to fully address this possible source of bias.

Lastly, we were unable to investigate the dose-response relationship between statin use and HNSCC outcomes; this information was not available for all participants through their medical record. Future research is necessary to investigate if there is a dose-response relationship between statin use and HNSCC outcomes as well as duration of statin use and HNSCC outcomes.

Conclusion

Our findings from this large, prospective study demonstrate that statin use may be protective for adverse outcomes in HNSCC patients, particularly those with HPV-positive disease. If true, these findings could have a meaningful impact on tertiary prevention for this cancer, which lacks evidence-based recommendations.

Acknowledgments

This research was funded through the National Cancer Institute of the National Institutes of Health Award number R01CA129102. Research reported in this publication was also supported by the National Cancer Institute of the National Institutes of Health under Award Number P30CA046592 by the use of the following Cancer Center Shared Resource(s): Cancer Data Science.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Ethics Statement: Patients recruited through SPORE 1 and SPORE 2 provided written informed consent, and the medical record review and SPORE studies were approved through the University of Michigan Medical School's Institutional Review Board.

References

1. Rettig EM, D'Souza G. Epidemiology of head and neck cancer. *Surg Oncol Clin N Am*. 2015;24(3):379-396. doi:10.1016/j.soc.2015.03.001

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

3. Forastiere A, Koch W, Trotti A, Sidransky D. Head and neck cancer. *N Engl J Med*. 2001;345(26):1890-1900. doi:10.1056/NEJMra001375

4. Sturgis EM, Cinciripini PM. Trends in head and neck cancer incidence in relation to smoking prevalence: an emerging epidemic of human papillomavirus-associated cancers? *Cancer*. 2007;110(7):1429-1435. doi:10.1002/cncr.22963

5. McDermott JD, Bowles DW. Epidemiology of head and neck squamous cell carcinomas: impact on staging and prevention strategies. *Curr Treat Options Oncol*. 2019;20(5):1-13. doi:10.1007/s11864-019-0650-5

6. Marur S, D'Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. *Lancet Oncol*. 2010;11(8):781-789. doi:10.1016/S1470-2045(10)70017-6

7. O'Rorke MA, Ellison MV, Murray LJ, Moran M, James J, Anderson LA. Human papillomavirus related head and neck cancer survival: a systematic review and meta-analysis. *Oral Oncol*. 2012;48(12):1191-1201. doi:10.1016/j.oraloncology.2012.06.019

8. Maron DJ, Fazio S, Linton MF. Current perspectives on statins. *Circulation*. 2000;101(2):207-213. doi:10.1161/01.CIR.101.2.207

9. Demierre MF, Higgins PDR, Gruber SB, Hawk E, Lippman SM. Statins and cancer prevention. *Nat Rev Cancer*. 2005;5(12):930-942. doi:10.1038/nrc1751

10. Brown AJ. Cholesterol, statins and cancer. *Clin Exp Pharmacol Physiol*. 2007;34(3):135-141. doi:10.1111/j.1440-1681.2007.04565.x

11. Zhong S, Zhang X, Chen L, Ma T, Tang J, Zhao J. Statin use and mortality in cancer patients: systematic review and meta-analysis of observational studies. *Cancer Treat Rev*. 2015;41(6):554-567. doi:10.1016/j.ctrv.2015.04.005

12. Mei Z, Liang M, Li L, Zhang Y, Wang Q, Yang W. Effects of statins on cancer mortality and progression: a systematic review and meta-analysis of 95 cohorts including 1,111,407 individuals. *Int J Cancer*. 2017;140(5):1068-1081. doi:10.1002/ijc.30526

13. Kao L-T, Hung S-H, Kao P-F, Liu J-C, Lin H-C. Inverse association between statin use and head and neck cancer: population-based case-control study in Han population. *Head Neck*. 2019;41(5):1193-1198. doi:10.1002/hed.25501

14. Lebo NL, Griffiths R, Hall S, Dimitroulakos J, Johnson-Obaseki S. Effect of statin use on oncologic outcomes in head and neck squamous cell carcinoma. *Head Neck*. 2018;40(8):1697-1706. doi:10.1002/hed.25152

15. Gupta A, Stokes W, Eguchi M, et al. Statin use associated with improved overall and cancer specific survival in patients with head and neck cancer. *Oral Oncol*. 2019;90:54-66. doi:10.1016/j.oraloncology.2019.01.019

16. Peterson LA, Bellile EL, Wolf GT, et al. Cigarette use, comorbidities and prognosis in a prospective head and neck squamous cell carcinoma population. *Head Neck*. 2016;38(12):1810-1820. doi:10.1002/hed.24515

17. Duffy SA, Taylor JMG, Terrell JE, et al. Interleukin-6 predicts recurrence and survival among head and neck cancer patients. *Cancer*. 2008;113(4):750-757. doi:10.1002/cncr.23615

18. Hanauer DA, Mei Q, Law J, Khanna R, Zheng K. Supporting information retrieval from electronic health records: a report of University of Michigan's nine-year experience in developing and using the electronic medical record search engine (EMERSE). *J Biomed Inform*. 2015;55:290-300. doi:10.1016/j.jbi.2015.05.003

19. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-381. doi:10.1016/j.jbi.2008.08.010

20. 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

21. Leyrat C, Seaman SR, White IR, et al. Propensity score analysis with partially observed covariates: how should multiple imputation be used? *Stat Methods Med Res*. 2019;28(1):3-19. doi:10.1177/0962280217713032

22. Heymans MW, Eekhout I. *Applied Missing Data Analysis with SPSS and (R)Studio*. 1st ed. Amsterdam; 2019. https://bookdown.org/mwheymans/bookmi/.

23. Arthur AE, Peterson KE, Rozek LS, et al. Pretreatment dietary patterns, weight status, and head and neck squamous cell carcinoma prognosis. *Am J Clin Nutr*. 2013;97(2):360-368. doi:10.3945/ajcn.112.044859

24. den Hollander D, Kampman E, van Herpen CML. Pretreatment body mass index and head and neck cancer outcome: a review of the literature. *Crit Rev Oncol / Hematol*. 2015;96(2):328-338. doi:10.1016/j.critrevonc.2015.06.002

25. Gama RR, Song Y, Zhang Q, et al. Body mass index and prognosis in patients with head and neck cancer. *Head Neck*. 2017;39(6):1226-1233. doi:10.1002/HED.24760

26. Arnett DK, Blumenthal RS, Albert MA, et al. 2019 ACC / AHA guideline on the primary prevention of cardiovascular disease. *J Am Coll Cardiol*. 2019;74(10):1376-1414. doi:10.1016/j.jacc.2019.03.010

27. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646-674. doi:10.1016/j.cell.2011.02.013

28. Spector ME, Bellile E, Amlani L, et al. Prognostic value of tumor-infiltrating lymphocytes in head and neck squamous cell carcinoma. *JAMA Otolaryngol Head Neck Surg*. 2019;145(11):1012-1019. doi:10.1001/jamaoto.2019.2427

29. Nguyen N, Bellile E, Thomas D, et al. Tumor infiltrating lymphocytes and survival in patients with head and neck squamous cell carcinoma (HNSCC). *Head Neck*. 2016;38(7):1074-1084. doi:10.1002/hed.24406

30. Rezaie-Majd A, Maca T, Bucek RA, et al. Simvastatin reduces expression of cytokines interleukin-6, interleukin-8, and monocyte chemoattractant protein-1 in circulating monocytes from hypercholesterolemic patients. *Arterioscler Thromb Vasc Biol*. 2002;22(7):1194-1199. doi:10.1161/01.ATV.0000022694.16328.CC

31. Ascer E, Bertolami MC, Venturinelli ML, et al. Atorvastatin reduces proinflammatory markers in hypercholesterolemic patients. *Atherosclerosis*. 2004;177(1):161-166. doi:10.1016/j.atherosclerosis.2004.07.003

32. Ferro D, Parrotto S, Basili S, Alessandri C, Violi F. Simvastatin inhibits the monocyte expression of proinflammatory cytokines in patients with hypercholesterolemia. *J Am Coll Cardiol*. 2000;36(2):427-431. doi:10.1016/S0735-1097(00)00771-3

33. Lyngdoh T, Vollenweider P, Waeber G, Marques-Vidal P. Association of statins with inflammatory cytokines: a population-based Colaus study. *Atherosclerosis*. 2011;219(1):253-258. doi:10.1016/j.atherosclerosis.2011.07.117

34. Lee KJ, Moon JY, Choi HK, et al. Immune regulatory effects of simvastatin on regulatory t cell-mediated tumour immune tolerance. *Clin Exp Immunol*. 2010;161(2):298-305. doi:10.1111/j.1365-2249.2010.04170.x

35. Nasman A, Romanitan M, Nordfors C, et al. Tumor infiltrating CD8+ and foxp3+ lymphocytes correlate to clinical outcome and human papillomavirus (HPV) status in tonsillar cancer. *PLoS One*. 2012;7(6):e38711. doi:10.1371/journal.pone.0038711

36. Wansom D, Light E, Thomas D, et al. Infiltrating lymphocytes and human papillomavirus-16 – associated oropharyngeal cancer. *Laryngoscope*. 2012;122(1):121-127. doi:10.1002/lary.22133

37. Andersen AS, Solling ASK, Ovesen T, Rusan M. The interplay between HPV and host immunity in head and neck squamous cell carcinoma. *Int J Cancer*. 2014;134(12):2755-2763. doi:10.1002/ijc.28411

38. Heusinkveld M, Goedemans R, Briet RJP, et al. Systemic and local human papillomavirus 16-specific t-cell immunity in patients with head and neck cancer. *Int J Cancer*. 2012;131(2):E74-E85. doi:10.1002/ijc.26497

39. Lechien JR, Seminerio I, Descamps G, et al. Impact of HPV infection on the immune system in oropharyngeal and non-oropharyngeal squamous cell carcinoma: a systematic review. *Cells*. 2019;8(9):1061. doi:10.3390/cells8091061

Chapter 2: Figures/Tables

Figure 2.1: Directed Acyclic Graph of Association between Statin Use and Overall/Disease-Specific Mortality

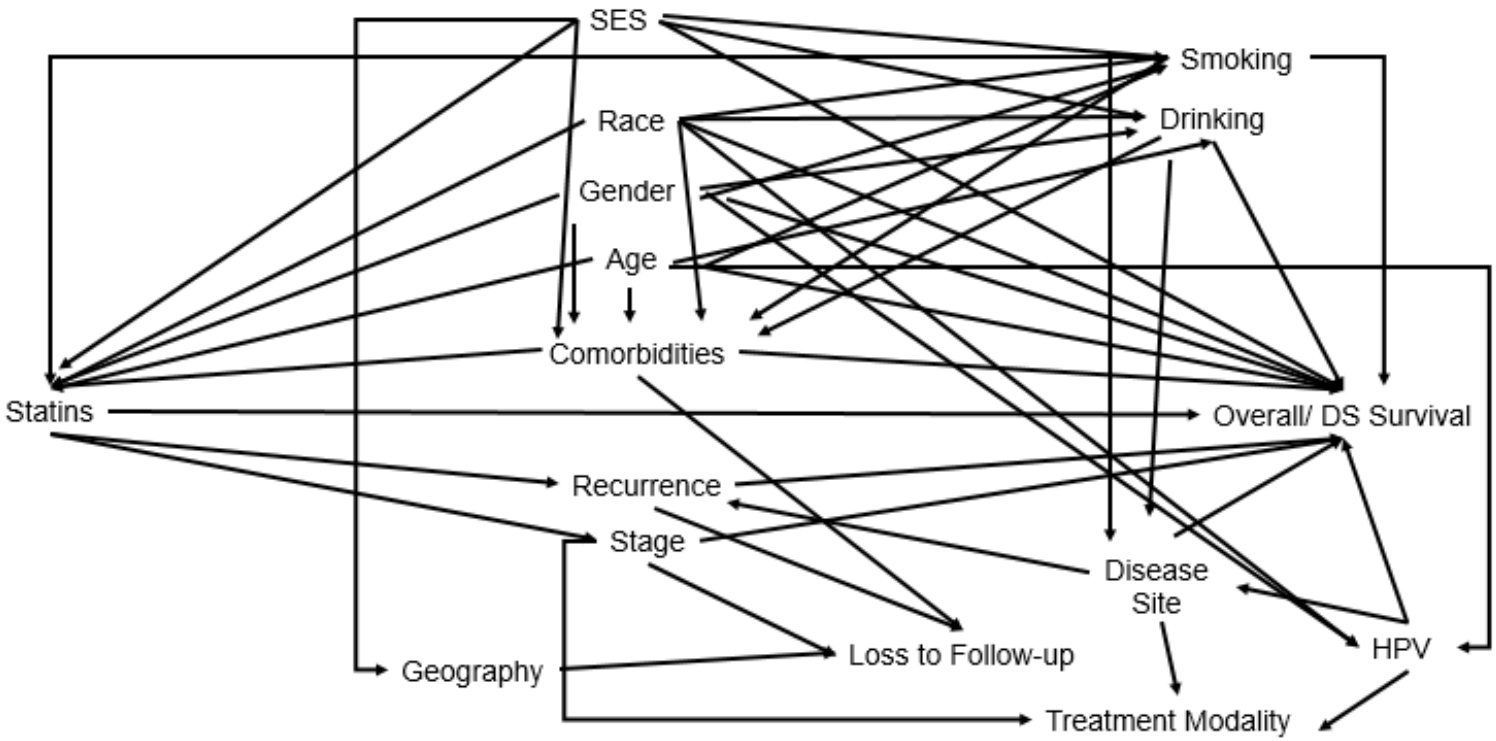


Figure 2.2: Directed Acyclic Graph of Association between Statin Use and Disease Recurrence

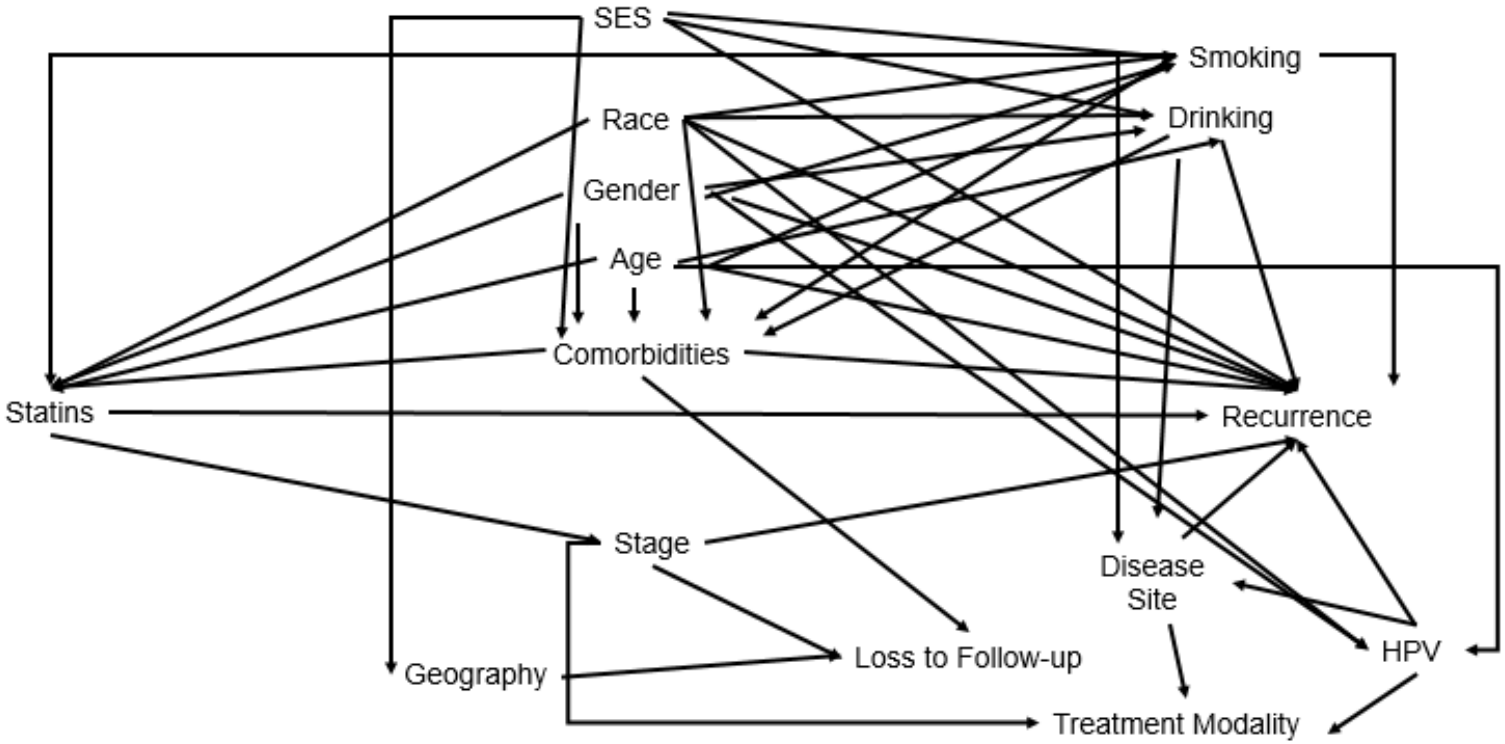


Figure 2.3: Balance Plots; Probability of Statin Use (Statin Users vs. Non-Statin Users), Imputed Datasets (1-10)

Figure 2.4: Balance Plots; Probability of Statin Use (Statin Users vs. Non-Statin Users), Imputed Datasets (11-20)

Table 2.1: Demographic and Clinical Characteristics by Statin Use

	Ever Statin User (N=589, 35.96%)	Never Statin User (N=1049, 64.04%)	P-value
Age at Diagnosis, years	63.49	57.79	<.0001
Sex (Male)	73.17%	74.55%	0.54
Race			0.006
White	93.89%	91.80%	
Not white	3.40%	6.67%	
Missing	2.72%	1.53%	
Disease Site			0.78
Larynx	17.83%	18.40%	
Oral Cavity	33.28%	31.74%	
Oropharynx	36.16%	38.42%	
Hypopharynx	3.74%	3.81%	
Other	9.00%	7.63%	
Stage at Diagnosis			0.47
0	2.04%	1.53%	
1	12.05%	10.87%	
2	11.54%	9.91%	
3	14.26%	13.16%	
4	60.10%	64.54%	
Treatment			0.27
Surgery alone	21.39%	18.78%	
Surgery + adjuvant radiation	11.21%	10.10%	
Surgery + adjuvant chemoradiation	8.66%	10.20%	
Radiation alone	6.96%	6.01%	
Chemoradiation alone	40.07%	39.18%	
Chemotherapy alone	2.55%	3.34%	
Palliative, unknown	9.17%	12.39%	
HPV status			0.47
Negative	29.54%	26.79%	
Positive	22.58%	22.88%	
Invalid/Missing	47.88%	50.33%	
ACE Score[a]			<.0001
None	13.41%	33.75%	
Mild	46.86%	41.94%	
Moderate	26.99%	16.97%	
Severe	11.88%	7.15%	
Missing	0.85%	0.19%	
Highest Education			0.008
Less than high school	4.75%	9.44%	
High school/GED	24.28%	24.79%	
Some College	28.52%	25.83%	
4 yr degree	8.32%	8.96%	
More than 4 year degree	12.05%	8.87%	
Missing	22.07%	22.12%	
BMI			<.0001
Underweight/Normal Weight	25.64%	43.76%	
Overweight/Obese 1	57.05%	46.04%	
Obese 2/Obese 3	13.75%	8.10%	
Missing	3.57%	2.10%	
Smoking Status			<.0001

Current	32.77%	47.95%	
Former	40.24%	25.55%	
Never	23.94%	24.79%	
Missing	3.06%	1.72%	
Drinking Status			<.0001
Current	55.86%	68.06%	
Former	29.71%	21.16%	
Never	11.04%	9.06%	
Missing	3.40%	1.72%	

Table 2.2: Multivariable Cox Proportional Hazards Models of Ever Use of Statins and HNSCC Outcomes

	No. of Events	Person-Months	HR (95% CI)[a]	HR (95% CI)[b]	HR (95% CI)[c]	HR (95% CI)[d]
Overall Death	657					
Statin Use						
No	432	55627.60	1	1	1	1
Yes	225	29847.95	0.95 (0.81, 1.11)	0.78 (0.66, 0.92)	0.75 (0.63, 0.88)	0.74 (0.62, 0.88)
HNSCC Death	379					
Statin Use						
No	250	55627.60	1	1	1	1
Yes	129	29847.95	0.91 (0.74, 1.13)	0.80 (0.64, 0.99)	0.79 (0.63, 0.99)	0.78 (0.61, 0.98)
Recurrence	495					
Statin Use						
No	318	34653.14	1	1	1	1
Yes	177	19442.40	0.97 (0.81, 1.17)	0.86 (0.71, 1.04)	0.85 (0.70, 1.04)	0.84 (0.69, 1.02)

a. Unadjusted analysis
b. Age-adjusted analysis
c. Adjusted for age, BMI and ACE-27
d. Adjusted for age, BMI, ACE-27, gender, education, race, smoking status, drinking status and stage of disease (0 or 1, 2, 3, 4)

Table 2.3: HPV, Stage and Disease Site Stratified Analysis

	Never Statin User			Ever Statin user		
Effect Modifiers	**No. of Events**	**Person Months**	**HR (95% CI)[a]**	**No. of Events**	**Person Months**	**HR (95% CI)[a]**
HPV status						
Overall Death						
HPV-Positive	61	12943.93	1 (REF)	21	7660.39	0.52 (0.31, 0.86)
HPV-Negative	131	12916.80	1 (REF)	80	7560.38	0.76 (0.57, 1.02)
P for interaction	0.18					
Disease-Specific Death						
HPV-Positive	38	12943.93	1 (REF)	10	7660.39	0.41 (0.21, 0.84)
HPV-Negative	71	12916.80	1 (REF)	52	7560.38	1.04 (0.71, 1.51)
P for interaction	0.02					
Recurrence						
HPV-Positive	55	8814.55	1 (REF)	18	5798.21	0.49 (0.29, 0.84)
HPV-Negative	95	8996.63	1 (REF)	68	5028.99	1.03 (0.74, 1.43)
P for interaction	0.02					
Stage						
Overall Death						
Stage 4	313	34729.56	1 (REF)	155	17522	0.68 (0.56, 0.83)
Stage 0-3	119	20898.04	1 (REF)	70	12325.95	0.82 (0.61, 1.10)
P for interaction	0.32					
Disease-Specific Death						
Stage 4	195	34729.56	1 (REF)	97	17522	0.74 (0.58, 0.96)
Stage 0-3	55	20898.04	1 (REF)	32	12325.95	0.83 (0.54, 1.29)
P for interaction	0.67					
Recurrence						
Stage 4	237	21384.74	1 (REF)	120	11211.86	0.78 (0.62, 0.99)
Stage 0-3	81	13268.40	1 (REF)	57	8230.54	0.99 (0.70, 1.39)
P for interaction	0.26					
Disease Site						
Overall Death						
Oropharynx	144	23395.71	1 (REF)	64	12503.82	0.64 (0.48, 0.87)
Other site	288	32231.89	1 (REF)	161	17344.13	0.80 (0.66, 0.98)

P for interaction	0.22					
Disease-Specific Death						
Oropharynx	87	23395.71	1 (REF)	35	12503.82	0.64 (0.43, 0.96)
Other site	163	32231.89	1 (REF)	94	17344.13	0.87 (0.67, 1.13)
P for interaction	0.21					
Recurrence						
Oropharynx	111	14423.62	1 (REF)	44	8470.44	0.62 (0.43, 0.88)
Other site	207	20229.52	1 (REF)	133	10971.96	0.99 (0.79, 1.24)
P for interaction	0.02					

a. Adjusted for Age at diagnosis, BMI and ACE-27

Table 2.4: Multivariable Cox Proportional Hazards Models of Ever Use of Statins and HNSCC Outcomes (No Distant Metastatic Disease and No Participants Who Died Within 6 Months)

	No. of Events [a]	Person-Months [a]	HR (95% CI) [a]	No. of Events [b]	Person-Months [b]	HR (95% CI) [b]
Overall Death	621			553		
Statin Use						
No	405	54725.72	1	366	55409.12	1
Yes	216	29669.65	0.75 (0.63, 0.89)	187	29719.52	0.75 (0.62, 0.90)
HNSCC Death	349			318		
Statin Use						
No	228	54725.72	1	209	55409.12	1
Yes	121	29669.65	0.79 (0.63, 1.00)	109	29719.52	0.80 (0.63, 1.03)
Recurrence	461			419		
Statin Use						
No	291	34379.93	1	264	34621.70	1
Yes	170	19378.56	0.88 (0.72, 1.08)	155	19391.87	0.92 (0.74, 1.13)

a. No patients with distant metastatic disease, adjusted for age, BMI and ACE-27
b. Excluding patients who died within 6 months of follow-up, adjusted for age, BMI and ACE-27

Table 2.5: HPV Stratified Analysis Excluding Patients with Distant Metastatic Disease

	Never Statin User			Ever Statin user		
Outcomes	**No. of Events**	**Person Months**	**HR (95% CI)[a]**	**No. of Events**	**Person Months**	**HR (95% CI)[a,]**
Overall Death						
HPV status						
HPV-Positive	57	12550.70	1	19	7614.03	0.49 (0.29, 0.83)
HPV-Negative	124	12763.47	1	80	7560.38	0.78 (0.58, 1.05)
P for interaction	0.11					
Disease-Specific Death						
HPV status						
HPV-Positive	34	12550.70	1	9	7614.03	0.40 (0.19,0.84)
HPV-Negative	65	12763.47	1	52	7560.38	1.08 (0.74, 1.59)
P for interaction	0.01					
Recurrence						
HPV status						
HPV-Positive	50	8600.18	1	17	5782.70	0.49 (0.28, 0.86)
HPV-Negative	87	8996.37	1	68	5028.99	1.10 (0.79, 1.54)
P for interaction	0.01					

a. Adjusted for Age at diagnosis, BMI and ACE-27

Table 2.6: HPV Stratified Analysis Using Various Strategies to Account for Missing Data on HPV Status

	Never Statin User			Ever Statin user		
Outcomes	**No. of Events**	**Person Months**	**HR (95% CI)[a]**	**No. of Events**	**Person Months**	**HR (95% CI)[a,b]**
Overall Death						
HPV status with missing						
HPV-Positive	61	12943.93	1 (REF)	21	7660.39	0.51 (0.31, 0.85)
HPV-Negative	131	12916.80	1 (REF)	80	7560.38	0.76 (0.57, 1.01)
HPV-Missing/Invalid	240	29766.87	1 (REF)	124	14627.19	0.81 (0.64, 1.01)
P for interaction	0.25					
HPV status IPW						
HPV-Positive	57	12437.75	1 (REF)	20	7522.4	0.55 (0.32, 0.95)
HPV-Negative	126	12641.97	1 (REF)	76	7317.68	0.80 (0.55, 1.18)
P for interaction	0.23					
Disease-Specific Death						
HPV status with missing						
HPV-Positive	38	12943.93	1 (REF)	10	7660.39	0.43 (0.21, 0.86)
HPV-Negative	71	12916.80	1 (REF)	52	7560.38	1.02 (0.70, 1.47)
HPV-Missing/Invalid	141	29766.87	1 (REF)	67	14627.19	0.77 (0.57, 1.04)
P for interaction	0.07					
HPV status IPW						
HPV-Positive	36	12437.75	1 (REF)	10	7522.4	0.47 (0.22, 0.99)
HPV-Negative	69	12641.97	1 (REF)	49	7317.68	0.99 (0.61, 1.62)
P for interaction	0.07					
Recurrence						
HPV status with missing						
HPV-Positive	55	8814.55	1 (REF)	18	5798.21	0.51 (0.30, 0.87)
HPV-Negative	95	8996.63	1 (REF)	68	5028.99	1.03 (0.75, 1.42)
HPV-Missing/Invalid	168	16841.95	1 (REF)	91	8615.2	0.89 (0.68, 1.15)
P for interaction	0.07					
HPV status IPW						
HPV-Positive	52	8429.93	1 (REF)	17	5725.8	0.53 (0.29, 0.95)
HPV-Negative	90	8845.34	1 (REF)	64	4899.45	1.10 (0.73, 1.65)
P for interaction	0.03					

a. Adjusted for Age at diagnosis, BMI and ACE-27
b. Stabilized inverse probability weighted model

Table 2.7: Pooled Multivariable Cox Proportional Hazards Models of Ever Use of Statins and HNSCC Outcomes, Utilizing Multiple Imputation for Missing Data

	No. of Events	Person-Months	HR (95% CI)[a]	HR (95% CI)[b]	HR (95% CI)[c]	HR (95% CI)[d]	HR (95% CI)[e]
Overall Death	657						
Statin Use							
No	432	55627.60	1	1	1	1	1
Yes	225	29847.95	0.75 (0.61,0.93)	0.75 (0.61, 0.93)	0.76 (0.61, 0.94)	0.74 (0.60, 0.92)	0.73 (0.60, 0.89)
HNSCC Death	379						
Statin Use							
No	250	55627.60	1	1	1	1	1
Yes	129	29847.95	0.75 (0.55, 1.02)	0.74 (0.55, 1.01)	0.75 (0.55, 1.02)	0.75 (0.55, 1.02)	0.70 (0.54, 0.91)
Recurrence	495						
Statin Use							
No	318	34653.14	1	1	1	1	1
Yes	177	19442.40	0.84 (0.65, 1.08)	0.84 (0.65, 1.07)	0.84 (0.65, 1.07)	0.83 (0.65, 1.07)	0.81 (0.65, 1.01)

a. IPW
b. IPW (Adjusted for age)
c. IPW, (Adjusted for age and BMI)
d. IPW, (Adjusted for age, BMI and ACE-27)
e. IPW, truncated weight

Table 2.8: Pooled HPV, Stratified Analysis

	Never Statin User	Ever Statin user
	HR (95% CI)	HR (95% CI)
IPW		
Overall Death		
HPV-Positive	1 (REF)	0.67 (0.45, 1.00)
HPV-Negative	1 (REF)	0.77 (0.59, 1.02)
Disease-Specific Death		
HPV-Positive	1 (REF)	0.58 (0.32, 1.06)
HPV-Negative	1 (REF)	0.80 (0.54, 1.18)
Recurrence		
HPV-Positive	1 (REF)	0.67 (0.40, 1.14)
HPV-Negative	1 (REF)	0.90 (0.64, 1.27)
IPW, Age-Adjusted		
Overall Death		
HPV-Positive	1 (REF)	0.66 (0.45, 0.97)
HPV-Negative	1 (REF)	0.78 (0.59, 1.03)
Disease-Specific Death		
HPV-Positive	1 (REF)	0.57 (0.32, 1.04)
HPV-Negative	1 (REF)	0.80 (0.55, 1.17)
Recurrence		
HPV-Positive	1 (REF)	0.67 (0.42, 1.08)
HPV-Negative	1 (REF)	0.92 (0.66, 1.27)
IPW, Age & BMI Adjusted		
Overall Death		
HPV-Positive	1 (REF)	0.67 (0.46, 0.97)
HPV-Negative	1 (REF)	0.79 (0.60, 1.04)
Disease-Specific Death		
HPV-Positive	1 (REF)	0.58 (0.32, 1.04)
HPV-Negative	1 (REF)	0.81 (0.55, 1.18)
Recurrence		
HPV-Positive	1 (REF)	0.67 (0.42, 1.07)
HPV-Negative	1 (REF)	0.92 (0.66, 1.28)
IPW, Age, BMI & ACE-27 Adjusted		
Overall Death		
HPV-Positive	1 (REF)	0.67 (0.46, 0.98)
HPV-Negative	1 (REF)	0.77 (0.58, 1.02)
Disease-Specific Death		
HPV-Positive	1 (REF)	0.58 (0.32, 1.04)
HPV-Negative	1 (REF)	0.81 (0.55, 1.18)
Recurrence		
HPV-Positive	1 (REF)	0.68 (0.43, 1.08)
HPV-Negative	1 (REF)	0.91 (0.65, 1.26)
IPW Truncated		
Overall Death		
HPV-Positive	1 (REF)	0.64 (0.44, 0.93)
HPV-Negative	1 (REF)	0.77 (0.59, 0.99)
Disease-Specific Death		
HPV-Positive	1 (REF)	0.49 (0.28, 0.85)
HPV-Negative	1 (REF)	0.78 (0.57, 1.08)
Recurrence		
HPV-Positive	1 (REF)	0.63 (0.40, 0.996)
HPV-Negative	1 (REF)	0.90 (0.67, 1.21)

Chapter 3 : The Association between Inflammatory Biomarkers and Statin Use among Head and Neck Squamous Cell Carcinoma Patients

Abstract

Background: Inflammatory markers such as tumor-infiltrating lymphocytes (TILs) and circulating cytokines are found within tumor tissue and blood, respectively. It is well-established that strong lymphocyte infiltration is associated with better prognosis, and pro-inflammatory cytokines are associated with poorer prognosis for many cancer sites, including head and neck squamous cell carcinoma (HNSCC). Statins are a class of cholesterol-lowering drugs that may protect against cancer at multiple sites, including HNSCC, but the mechanism of their action remains unclear. There is limited literature investigating the association between statin use and lymphocyte infiltration in tumors as well as circulating cytokines among cancer patients, however, statins' upregulation of regulatory T-cell activity in other contexts is well-documented. Thus we examined the association between statin use and inflammatory biomarkers in a cohort of HNSCC patients.

Methods: A large HNSCC cohort conducted from 2008-2014 collected TILs from previously untreated patients from tumor biopsy and circulating cytokines from baseline blood samples. Statin use was collected through a retrospective medical record review from the closest medical encounter to the patients' HNSCC diagnosis. TILs (cluster of differentiation 4 (CD4), cluster of differentiation 8 (CD8), and forkhead box P3 (FoxP3)) were counted within tumor parenchyma on tissue microarrays and presented as number of cells per millimeters squared; in addition, a total weighted score (TILws) was created across the three types of TILs. Individual TIL

measures as well as the TILws were categorized as low or high (i.e., < median or ≥ median) and also analyzed continuously.

Circulating cytokine measures including IFN-γ, IL-6, IL-8, IL-10, IL-17, GRO, HGF, TNF-α, VEGF, and TGF were operationalized similarly. Logistic regression models were conducted to estimate the odds of high inflammatory biomarker levels (TILs or circulating cytokines) comparing patients who used a statin at diagnosis to those who did not, and linear regression models explored the association between log-transformed biomarker measures and statin use at diagnosis. Multivariable-adjusted (MV-adj) models adjusted for the following variables that may confound the association between statin use and inflammatory biomarkers: age, smoking status, and ACE-27.

Results: The total cohort was comprised of 1,042 patients, of which 475 patients had all three TILs, and 205 patients had all circulating cytokines measured. There was a suggestive positive association between statin use and the TILws, although this association was not statistically significant (MV-adj OR=1.15; 95%CI=0.74-1.78). We found similar non-significant positive associations for CD4, CD8 and FoxP3 individually. However, we observed a positive association between statin use and the TILws among HPV-positive patients (MV-adj OR=2.80; 95%CI=1.03-7.61). When individual TIL subsets were examined, this positive association with the score appeared to be largely driven by an association with FoxP3 (OR=4.15; 95%CI=1.55-11.14). There did not appear to be a clear association between circulating cytokines and statin use among HNSCC patients.

Conclusion: Our findings suggest that one mechanism by which statins may influence prognosis in HNSCC patients whose tumors are HPV-positive is through an effect on TILs, particularly FoxP3 levels. Further studies are needed to examine other immune and inflammatory markers that predict HNSCC outcomes to further elucidate this potential mechanism by which statins may be protecting against poor outcomes in HNSCC patients.

Background

Head and neck squamous cell carcinoma (HNSCC) is a debilitating cancer that can be found in the mucosal lining of the aerodigestive tract with prominent sites including the nasopharynx, oral cavity, oropharynx, hypopharynx, and larynx.[1–3] The main risk factors associated with HNSCC development can be delineated into two subgroups: patients with human papillomavirus (HPV)-positive tumors and those with HPV-negative tumors, whose risk for disease is often attributed to smoking and alcohol drinking. [1] Etiology of disease, underlying tumorigenesis, patient characteristics, site of disease, treatment, and prognosis often differ between patients with HPV-positive versus HPV-negative tumors.[4]

Statins are a class of cholesterol-lowering medications that are often utilized to prevent the development or progression of heart disease.[5] In addition to their cholesterol-lowering attributes, statins possess anti-inflammatory and immunomodulatory actions that may inhibit the development or progression of cancer.[6] Research has established a protective association between the use of statin drugs and the incidence and mortality of cancer at numerous sites, [7,8] including HNSCC.[9–11] Our recently published study similarly found that statins were protective for HNSCC outcomes, but this protective association was observed only among patients whose tumors were HPV-positive.[12] Although the relationship between statin use, cancer development, and cancer-related outcomes has been investigated across various cancer sites with promising findings, the potential mechanisms by which statins may be exerting their protective effect remains unclear, particularly for HNSCC. However, their effects on inflammation and immunomodulation that may be responsible for factors that influence the progression and development of cancer are largely unknown.[13]

Recent research findings suggest that there is an inverse association between the number of tumor-infiltrating lymphocytes (TILs) and HNSCC death and recurrence.[14,15] Another inflammatory marker that potentially influences HSNCC outcomes is the level of circulating

cytokines. Previous research has identified that the pro-inflammatory cytokine IL-6 has been found to be positively associated with recurrence and death among patients with HNSCC.[16]

Given the established relationship between HNSCC outcomes and both TILs and circulating cytokines, as well as the known effects of statins on inflammation and immune modulation, this study aimed to identify the association between statin use and these inflammatory biomarkers. Through this research, we determined whether statins may be influencing HNSCC outcomes through these inflammatory processes. Because patients with HPV-positive tumors may have a different immune response and are different etiologically than HPV-negative tumors, we explored whether HPV status may modify the association between statin use and the presence and quantity of TILs and circulating cytokines.

Methods

Study Population

Subjects in this study were recruited to participate in the University of Michigan Head and Neck Cancer Specialized Program of Research Excellence 2 (SPORE 2). The SPORE 2 cohort consists of incident HNSCC patients who were diagnosed and/or treated at the University of Michigan Rogel Cancer Center from 2008-2014. In order to be eligible to participate in the study, patients had to be 18 years or older, their cancer could not have been previously treated, and their disease could not be a recurrence of disease (i.e., they had to be free of disease for 5 years prior to their current diagnosis). A description of the cohort, including study recruitment and procedures, has been published previously.[16,17] The SPORE 2 cohort consists of 1,042 participants; complete TILs and statin use information was available for 475 participants, and circulating cytokines were measured in 205 participants.

Inflammatory Marker Measurements

Tumor-infiltrating lymphocytes (TILs)

Tumor tissue was collected from previously untreated patients who had tissue available from biopsies. TILs (cluster of differentiation 4 (CD4), cluster of differentiation 8 (CD8), and forkhead box P3 (FoxP3)) were manually counted within tumor parenchyma on tissue microarrays and presented as number of cells per millimeters squared; in addition, a total weighted score (TILws) was created combining data across the three cell types of TILs.[15] A more in-depth explanation about the TILs measurement procedure and creation of variables has been previously published.[14,15] TILs were measured for CD4 (N=481), CD8 (N=481), FoxP3 (N=485) and TILws (N=475).

Circulating Cytokines

Circulating cytokines were measured from blood samples collected from participants at diagnosis prior to treatment. Cytokines were measured at the University of Michigan Cancer Center Immune Monitoring Core using an ELISA kit. A detailed explanation of the procedure to measure the circulating cytokines was previously published.[16,18] The cytokines measured were interferon-gamma (IFN-γ), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-17 (IL-17), growth-related oncogene (GRO), hepatocyte growth factor (HGF), tumor necrosis factor-alpha (TNF-α), vascular endothelial growth factor (VEGF) and transforming growth factor (TGF). Blinded duplicates of study samples for two participants were included as quality control samples (coefficients of variation for each circulating cytokine measure are displayed in Table 3.1). Samples that exceeded the limit of detection were assigned the maximum value.

Confounding Variables

Variables that may confound the association between statin use and biomarkers were collected in various ways. Clinical variables such as age (continuous) and comorbidities (none, mild, moderate, or severe) measured through the Adult Comorbidity Evaluation 27 score (ACE-27) were collected through medical record review, whereas potential confounders associated with behavior such as smoking status (never, former, current) were collected through a baseline health survey. Individuals that were missing the listed potential confounders, specifically smoking status (N=20) and ACE-27 (N=4) were assigned to the most common category for the TILs analysis; no individuals were missing confounding variables for the cytokines analyses.

Statin Measurement

Statin use was collected through a retrospective medical record review by trained research personnel. Study personnel identified the patient's initial encounter at the University of Michigan hospital for the patient's HNSCC diagnosis, and medications were recorded from that encounter. If the patient did not have medications recorded at the initial encounter, the next closest encounter was checked. Medications were recorded from the closest encounter to initial diagnosis prior to treatment initiation. If a participant was identified as using a statin at diagnosis, s/he was considered a statin user. Data were collected by two reviewers and achieved an inter-rater reliability coefficient, Kappa of 95%. Information that was not concordant across reviewers was reconciled after comparison. Data was stored in a Research Electronic Data Capture (REDCap) database.[19]

Statistical Analysis

Basic descriptive statistics were calculated to identify if demographic and clinical characteristics were different for statin users compared to non-statin users at diagnosis. The relationship between statin use and inflammatory biomarkers if displayed in Figure 3.1. TIL and

cytokine variables were operationalized continuously and categorically. Due to the highly skewed distribution of the data toward zero, TILs and cytokine values were log-transformed to achieve a more normal distribution. In addition to analyzing this association linearly, based on the highly skewed distribution, individual TIL measures and cytokine measures were categorized as low or high (i.e. < median or ≥ median). Since TILs and cytokines were examined continuously and dichotomously (< median vs. ≥ median), linear and logistic regression models were conducted. Multivariable-adjusted (MV-adj) models included the following variables that may confound the association between statin use and TILs/cytokines; age, smoking status, and ACE-27. Because statin use appeared to be associated with HPV status and disease site for participants who had cytokines measured, we conducted models adjusting for those variables as well.

Based on our previous research, we observed an association between statin use and HNSCC outcomes, but only among patients whose disease was HPV-positive.[12] We, therefore, wanted to assess if effect modification by HPV status was present (HPV-positive, HPV-negative, HPV status invalid/missing) for the statin-inflammatory marker associations. Statistical interaction was evaluated using the likelihood ratio test.

HPV status was missing for 125 participants who had at least 1 of the TILs biomarkers measured and 30 participants who had the circulating cytokines measured. In order to identify if these missing values influenced the association of statin use and the TIL measures, we utilized various methods to analyze the HPV status as a potential effect modifier. Initially, participants who had HPV status missing were excluded from the analysis. We also conducted the analysis by including the participants with HPV missing as a separate category. Lastly, we utilized inverse probability weighting (IPW) by generating weights to emulate a population where no participants are missing HPV status. We created the weights by calculating the probability of having HPV status missing by conducting a logistic regression model consisting of variables (

year enrolled in the study, age at diagnosis, gender, smoking status, marital status, stage of disease at diagnosis, disease site, ACE-27, drinking status and body mass index (BMI)); this is the denominator of the weight. Participants with missing values for the predictors in the logistic regression model were dropped from the analysis. The weights were then applied to a logit model with a binomial distribution and robust 95% confidence intervals, excluding participants who were missing HPV status. This approach was also utilized to assess the potential effect modification of HPV status on the association between statin use and the circulating cytokine measures.

Principal Components Analysis (PCA)

Correlations were assessed for both the TIL values (Figure 3.2) and the cytokine measures (Figure 3.3). Due to the potential for high correlation between the different cytokine measures, we conducted a principal components analysis (PCA) to reduce the number of variables (dimensionality) in the models and maintain as much variability as possible.[20] Utilizing PCA will identify patterns that may allow us to identify cytokines that may exhibit analogous behavior and impact the data similarly. Due to the highly skewed nature of the data, the circulating cytokine values were log-transformed and standardized. A PCA analysis was conducted using an orthogonal rotation which generated eigenvalues and a scree plot (Table 3.2 and Figure 3.4, respectively). We assessed this information to identify the optimal number of factors to explain the most variability. We included 3 factors because the first 3 factors explained 86% of the variability in the data and had an eigenvalue of 0.74.

All analyses were conducted using SAS version 9.4 (Cary, NC). All tests were two-sided, and results were considered statistically significant if $p<0.05$.

Results

Descriptive statistics

Presented in Tables 3.3 and 3.4 are the distribution of baseline demographic characteristics by statin use for the participants included in the TILs and circulating cytokines analyses, respectively. For the TILs analysis, participants who were taking a statin at diagnosis (29.32%) appear to be older, more likely to have a higher BMI, higher ACE-27, and are less likely to be current smokers compared to non-statin users at baseline. The distribution of characteristics among statin users (36.10%) and non-users were slightly different for participants who had circulating cytokines measured at baseline. Statin users were older, less likely to have larynx or oral cavity as their primary site of disease, more likely to have HPV status missing, higher ACE- 27, and less likely to be current smokers compared to participants who were not using a statin at diagnosis.

Multivariable analysis

When assessing the relationship between statin use and TILs, operationalizing the outcome as high vs. low TIL counts, there does not appear to be a clear association between statin use and TILs. After adjusting for confounders: age, smoking status and ACE-27, those who were taking a statin had a slightly higher odds of having higher TILs but this association was not statistically significant for any of the measured TIL values (TILws: [MV-adj OR=1.15; 95% CI= (0.74, 1.78)]; FoxP3: [MV-adj OR=1.13; 95% CI= (0.74, 1.73)] ; CD4: [MV-adj OR=1.23; 95% CI= (0.80, 1.90)]; CD8: [MV-adj OR=1.10; 95% CI= (0.72, 1.69)]. Similar null findings were observed when TILs were log-transformed and examined as a continuous variable (Table 3.5). The association between statin use and circulating cytokines was also null (Table 3.6).

Effect modification

We observed a statistically significant interaction with HPV status such that there was a statistically significant positive association between statin use and having a high number of FoxP3 (HPV-positive: [MV-adj OR=4.15; 95% CI= (1.55, 11.14)]; HPV-negative: [MV-adj HR= 0.81; 95% CI= (0.43, 1.52)], p for interaction = 0.003 (Table 3.7)). There was a marginally significant association observed for TILws (above the median) among statin users whose tumors were HPV-positive (TILws: HPV-positive [MV-adj OR=2.80; 95% CI= (1.03, 7.61)]; HPV-negative [MV-adj HR= 1.07; 95% CI= (0.57, 2.02)], p for interaction = 0.1 (Table 3.7)), which may be driven by FoxP3. Interactions were suggestive, but not statistically significant for the association between statin use and CD4 and CD8.

When we examined the association between statin use and circulating cytokines, we observed no statistically significant interaction between statin use at diagnosis and HPV status for any of the individual circulating cytokines except HGF. Patients who were HPV-positive and on a statin at diagnosis had higher odds of having a high level of HGF compared to those who had HPV-positive tumors and were not taking a statin, whereas those who were HPV-negative and were taking a statin appear to have an inverse relationship with HGF. However, neither of the stratum-specific associations were statistically significant (HPV-positive: [MV-adj OR=2.27; 95% CI= (0.82, 6.27)]; HPV-negative: [MV-adj HR= 0.47; 95% CI= (0.17, 1.31)], p for interaction = 0.03 (Table 3.8)).

Similar findings were observed when the HPV status missing category was included, and when using the IPW method; although the associations observed using the IPW method were slightly stronger they did not appear to be meaningfully different for both the individual TIL and circulating cytokine measures (Tables 3.9 & 3.10).

PCA Results

Due to the high correlation between the individual circulating cytokines, we conducted a principal component analysis to reduce the dimensionality (number of variables measured) and identify which circulating cytokines would group together. Utilizing an orthogonal rotation, there appeared to be 3 factors that explained the majority of the cumulative variance (86%), with the lowest eigenvalue 0.74 (Table 3.2). Factor patterns are displayed in Table 3.11, where high factor loadings are highlighted in green, medium in yellow, and low in red. When using an orthogonal rotation, it appears as though factor 1 has high loadings for IL-6, IL-8, IL-17, HGF and TNF-α; factor 2 has high loadings for IFN-γ, IL-10 and GRO; lastly, factor 3 only displays high loadings for VEGF. There does not appear to be a clear pattern or biological explanation by which these circulating cytokines are grouped together.

As displayed in Table 3.12, communality factor loadings are quite high; this demonstrates that a large proportion of the variance experienced can be explained by the presented factors. In order to assess the association between these factors and statin use, we operationalized the factors dichotomously at the median and conducted logistic regression analysis. There does not appear to be a statistically significant association between the statin use and any of the factors presented when they are operationalized utilizing a median split (Table 3.13) or when looking at the factor loadings as a continuous measure (Table 3.14). This supports the original findings presented above, demonstrating that there does not seem to be an association between statin use and circulating cytokines.

When assessing if HPV status is an effect modifier of the association between statin use and the median operationalized PCA factor loadings, there only appeared to be a statistically significant association between having high factor loadings for factor 3, which represents the high VEGF and statin use at diagnosis among HPV-positive patients (HPV-positive: [MV-adj OR=4.55; 95% CI= (1.60, 12.96)]; HPV-negative: [MV-adj HR= 0.51; 95% CI= (0.19, 1.38)], p

for interaction = 0.02 (Table 3.15)). These findings are stronger than the point estimates that were observed for this association when looking at VEGF alone. When investigating these associations taking the HPV missing category into account or when using the IPW method the point estimates do not meaningfully change (Table 3.16).

Discussion

In this study, we observed that HNSCC patients taking a statin at the time of diagnosis had higher lymphocyte infiltration in their tumors than non-users, but only for HPV-positive patients. The strongest association was observed for FoxP3. These findings support that the inverse association between statin use and HPV-positive HNSCC previously reported by our group may be due to an effect of statins on TILs in patients with HPV-positive tumors.[12]

The inflammatory and immunomodulatory effects of cancer are not clearly directional by cell type. This effect often depends on the type of cancer, whether there is a presence of inflammatory markers and what combination of these markers are beneficial or harmful to cancer prognosis.[21] Research has established that FoxP3 influences cancer prognosis but the directionality of this association differs by cancer type, with certain cancers such as breast, cervical, pancreatic and melanoma observing a positive association between FoxP3 infiltration and death whereas other cancer sites such as HNSCC, colorectal and esophageal cancers observe and inverse association.[14,15,22] FoxP3 infiltration appears to have varying impacts on cell development and proliferation but the explanation behind why its influences differ by cancer sites is not clearly established.[23]

Although, to our knowledge, this is the first study to investigate an association between statin use and TILs among HNSCC patients, a study investigating this association among patients with colorectal cancer identified similar results. Al-Husein et al. identified a positive association between statin use and FoxP3 among patients with colorectal cancer and

determined this association was modified by stage of disease.[24] Another study by Lee et al. found that statins were associated with the increased production of T-cells ("FoxP3 transcription factor") in mice and lung tumor cell lines.[25]

Our finding that the statin-TIL association may be limited to patients with HPV-positive tumors is plausible given that patients with HPV-positive tumors often have a different immune response and may be less immunosuppressed than HNSCC patients with HPV-negative disease.[26,27] HPV-positive HNSCC has been shown to have a stronger immune response, particularly stronger T-cell infiltration, than patients with HPV-negative disease.[28,29] One possible explanation is that HPV-positive HNSCC is usually found in the oropharynx, (specifically the tonsils).[29,30] Tonsils are made of lymphatic tissue, which is rich in various immunological processes.[31] Therefore, one possible explanation for our finding of a statin-TIL association only among HPV-positive patients is that the stronger immune response of HNSCC patients with HPV-positive disease works synergistically with the anti-inflammatory and immunomodulatory actions of statins. Statins may, therefore, improve cancer-related outcomes, specifically in HPV-positive patients.

Although our findings were relatively null for the association between statin use and circulating cytokines, other studies have reported statin-cytokine associations, although the specific cytokines reported to be associated with statin use were not consistent across studies. One previous study identified an inverse association between pro-inflammatory cytokines in tissue and serum among patients with colorectal cancer.[32] Two studies investigated this association in participants with hypercholesterolemia finding a reduction in the pro-inflammatory cytokines IL-6 and TNF-α for participants on a statin.[33,34] In a study that investigated the association among a random sample of Swiss adults, the authors reported lower C- reactive protein (CRP) concentrations among participants using a statin.[35] To our knowledge, our study is the first to investigate this association in HNSCC patients. It is possible our findings differ

from those reported previously because cytokine levels may not be as affected by statin use in HNSCC patients. It is also possible that the results are different due to the design of the studies. The studies that found an association between statin use and circulating cytokine levels utilized a more experimental design in which they administered statins to participants and then measured circulating cytokine levels pre and post medication administration. Our study uses an observational approach which may make it more difficult to identify the true effect of statins on circulating cytokines, particularly if the effect is modest.

Strengths and Limitations

To our knowledge, this study is the first to assess the association between inflammatory biomarkers and statin use among patients with HNSCC. A strength of this study is the amount of data we have for each patient and the opportunity we have to integrate biomarker data with behavioral, epidemiological and clinical data. This allowed us to test and identify confounders and should mitigate bias that may arise due to lack of information from participants.

Although this study has many strengths, there are some notable limitations that should be addressed in future studies. The sample size for this study is quite small. Not all participants within the SPORE 2 cohort had tumor tissue from biopsy available to measure TILs or provided a blood sample at baseline to measure circulating cytokines. This may lead to a reduction in power, especially when investigating the interaction between statin use and HPV status. Since the effect size of the association, particularly for FoxP3, was relatively large, this may not be an issue, but as noted by the wide confidence interval, the point estimate may not be precise. Future studies investigating this association among a larger study population is necessary. This may also help to explain why there did not appear to be a clear relationship between circulating cytokines and statin use among HPV-positive patients.

There is the potential for selection bias as well. Since not all participants provided specimen for biomarker measurement, it is possible that the patients who provided specimen were different from those who did not, with regard to the relationship between statin use and these biomarker measures. This does not appear to be an issue for the analytic sample who have TILs measured. The frequency of participants who were using a statin at diagnosis and distribution of the demographic and clinical characteristics between statin users and non-users was very similar to what was observed in the entire study population. There did appear to be some differences between the sample of participants who had baseline circulating cytokines measured compared to the complete study population. There appeared to be slightly more males and patients with higher stages of disease, but other factors that may bias the associations observed are similarly distributed in the total study population and the analytic sample who have baseline circulating cytokines measured. We additionally used IPW to emulate a population had no SPORE participants had TILs or circulating cytokines missing. This would provide participants who are similar to those who are missing to have larger weights. After including these weights, the association between TILs and statin use at diagnosis does not meaningfully change (Table 3.17). The point estimates for the association between circulating cytokines and statin use do slightly change but the findings still remain relatively null (Table 3.18). It is possible that selection bias may be an issue for the circulating cytokine measures.

Another limitation of this study is that the data is cross-sectional. Both the medication information and the inflammatory markers were measured at diagnosis prior to cancer treatment. This can possibly lead to reverse causation, specifically with the cytokines measures. If inflammation and high cholesterol are associated, we may observe a positive association between inflammation and statin use if those who have higher levels of pro-inflammatory cytokines were taking a statin because of risk factors associated with high cholesterol such as coronary heart disease and obesity, but this association is not clearly defined.[36–38] It would be

very difficult to identify this bias because we do not have information on when statins were initiated. We also do not have biomarker information from patients (blood and tumor tissue samples) prior to their HNSCC diagnosis. Since we did not appear to observe an association between statin use and circulating cytokines or an interaction between HPV status and statin use for the majority of the studied cytokines, it is possible this limitation did not affect our study. This limitation should not be an issue with TILs because this measurement is based on inflammatory markers that are found within the tumor tissue. We would assume that TILs would not be influenced by other comorbidities that the patient may have at diagnosis.

Conclusion

Our findings suggest that one mechanism by which statins may influence prognosis in HNSCC patients is through an effect on TILs, particularly FoxP3. This association appears to be restricted to HPV-positive patients. Future research investigating this association may shed light on the role of type and dose of statin and duration of use with TILs in HNSCC tumors. Additional studies are needed to examine other immune and inflammatory markers that predict HNSCC outcomes to further elucidate this potential mechanism by which statins may be protecting against poor outcomes in HPV-positive HNSCC patients.

Acknowledgments

This research was funded through the National Cancer Institute of the National Institutes of Health Award number R01CA129102. Research reported in this publication was also supported by the National Cancer Institute of the National Institutes of Health under Award Number P30CA046592 by the use of the following Cancer Center Shared Resource(s): Cancer Data Science and Immune Monitoring.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Ethics Statement: Patients recruited through SPORE 2 provided written informed consent, and the medical record review and SPORE 2 study was approved through the University of Michigan Medical School's Institutional Review Board.

References

1. Rettig EM, D'Souza G. Epidemiology of head and neck cancer. *Surg Oncol Clin N Am*. 2015;24(3):379-396. doi:10.1016/j.soc.2015.03.001
2. Argiris A, Karamouzis M V, Raben D, Ferris RL. Head and neck cancer. *Lancet*. 2008;371(9625):1695-1709. doi:10.1016/S0140-6736(08)60728-X
3. Forastiere A, Koch W, Trotti A, Sidransky D. Head and neck cancer. *N Engl J Med*. 2001;345(26):1890-1900. doi:10.1056/NEJMra001375
4. Marur S, D'Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. *Lancet Oncol*. 2010;11(8):781-789. doi:10.1016/S1470-2045(10)70017-6
5. Maron DJ, Fazio S, Linton MF. Current perspectives on statins. *Circulation*. 2000;101(2):207-213. doi:10.1161/01.CIR.101.2.207
6. Demierre MF, Higgins PDR, Gruber SB, Hawk E, Lippman SM. Statins and cancer prevention. *Nat Rev Cancer*. 2005;5(12):930-942. doi:10.1038/nrc1751
7. Zhong S, Zhang X, Chen L, Ma T, Tang J, Zhao J. Statin use and mortality in cancer patients: systematic review and meta-analysis of observational studies. *Cancer Treat Rev*. 2015;41(6):554-567. doi:10.1016/j.ctrv.2015.04.005
8. Mei Z, Liang M, Li L, Zhang Y, Wang Q, Yang W. Effects of statins on cancer mortality and progression: a systematic review and meta-analysis of 95 cohorts including 1,111,407 individuals. *Int J Cancer*. 2017;140(5):1068-1081. doi:10.1002/ijc.30526
9. Kao L-T, Hung S-H, Kao P-F, Liu J-C, Lin H-C. Inverse association between statin use and head and neck cancer: population-based case-control study in Han population. *Head Neck*. 2019;41(5):1193-1198. doi:10.1002/hed.25501
10. Gupta A, Stokes W, Eguchi M, et al. Statin use associated with improved overall and cancer specific survival in patients with head and neck cancer. *Oral Oncol*. 2019;90:54-66. doi:10.1016/j.oraloncology.2019.01.019
11. Lebo NL, Griffiths R, Hall S, Dimitroulakos J, Johnson-Obaseki S. Effect of statin use on oncologic outcomes in head and neck squamous cell carcinoma. *Head Neck*. 2018;40(8):1697-1706. doi:10.1002/hed.25152
12. Getz KR, Bellile E, Zarins KR, et al. Statin use and head and neck squamous cell carcinoma outcomes. *Int J Cancer*. 2021;148(10):2440-2448. doi:10.1002/ijc.33441
13. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646-674. doi:10.1016/j.cell.2011.02.013
14. Nguyen N, Bellile E, Thomas D, et al. Tumor infiltrating lymphocytes and survival in patients with head and neck squamous cell carcinoma (HNSCC). *Head Neck*. 2016;38(7):1074-1084. doi:10.1002/hed.24406
15. Spector ME, Bellile E, Amlani L, et al. Prognostic value of tumor-infiltrating lymphocytes in head and neck squamous cell carcinoma. *JAMA Otolaryngol Head Neck Surg*. 2019;145(11):1012-1019. doi:10.1001/jamaoto.2019.2427
16. Duffy SA, Taylor JMG, Terrell JE, et al. Interleukin-6 predicts recurrence and survival among head and neck cancer patients. *Cancer*. 2008;113(4):750-757. doi:10.1002/cncr.23615

17. Peterson LA, Bellile EL, Wolf GT, et al. Cigarette use, comorbidities and prognosis in a prospective head and neck squamous cell carcinoma population. *Head Neck*. 2016;38(12):1810-1820. doi:10.1002/hed.24515

18. Arthur AE, Peterson KE, Shen J, et al. Diet and proinflammatory cytokine levels in head and neck squamous cell carcinoma. *Cancer*. 2014;120(17):2704-2712. doi:10.1002/cncr.28778

19. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-381. doi:10.1016/j.jbi.2008.08.010

20. Ringnér M. What is principal component analysis? *Nat Biotechnol*. 2008;26(3):303-304.

21. Fridman WH, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer*. 2012;12(4):298-306. doi:10.1038/nrc3245

22. Shang B, Liu Y, Jiang S, Liu Y. Prognostic value of tumor- infiltrating foxp3+ regulatory t cells in cancers: a systematic review and meta-analysis. *Sci Rep*. 2015;5(1):1-9. doi:10.1038/srep15179

23. Jia H, Qi H, Gong Z, et al. The expression of foxp3 and its role in human cancers. *Biochim Biophys Acta (BBA)- Rev Cancer*. 2019;1871(1):170-178. doi:10.1016/j.bbcan.2018.12.004

24. Al-Husein BA, Dawah B, Bani-Hani S, Al Bashir SM, Al-Sawalmeh KM, Ayoub NM. Immunomodulatory effect of statins on regulatory t lymphocytes in human colorectal cancer is determined by the stage of disease. *Oncotarget*. 2018;9(87):35752-35761. doi:10.18632/oncotarget.26293

25. Lee KJ, Moon JY, Choi HK, et al. Immune regulatory effects of simvastatin on regulatory t cell-mediated tumour immune tolerance. *Clin Exp Immunol*. 2010;161(2):298-305. doi:10.1111/j.1365-2249.2010.04170.x

26. Heusinkveld M, Goedemans R, Briet RJP, et al. Systemic and local human papillomavirus 16-specific t-cell immunity in patients with head and neck cancer. *Int J Cancer*. 2012;131(2):E74-E85. doi:10.1002/ijc.26497

27. Lechien JR, Seminerio I, Descamps G, et al. Impact of HPV infection on the immune system in oropharyngeal and non-oropharyngeal squamous cell carcinoma: a systematic review. *Cells*. 2019;8(9):1061. doi:10.3390/cells8091061

28. Mandal R, Şenbabaoğlu Y, Desrichard A, et al. The head and neck cancer immune landscape and its immunotherapeutic implications. *JCI Insight*. 2016;1(17):1-18. doi:10.1172/jci.insight.89829

29. Andersen AS, Solling ASK, Ovesen T, Rusan M. The interplay between HPV and host immunity in head and neck squamous cell carcinoma. *Int J Cancer*. 2014;134(12):2755-2763. doi:10.1002/ijc.28411

30. Chakravarthy A, Henderson S, Thirdborough SM, et al. Human papillomavirus drives tumor development throughout the head and neck: improved prognosis is associated with an immune response largely restricted to the oropharynx. *J Clin Oncol*. 2016;34(34):4132-4141. doi:10.1200/JCO.2016.68.2955

31. Perry M, Whyte A. Immunology of the tonsils. *Immunol Today*. 1998;19(9):414-421.

doi:10.1016/S0167-5699(98)01307-3

32. Malicki S, Winiarski M, Matlok M, Kostarczyk W, Guzdek A, Konturek P. Il-6 and il-8 responses of colorectal cancer in vivo and in vitro cancer cells subjected to simvastatin. *J Physiol pharamacology*. 2009;60(4):141-146.

33. Rezaie-Majd A, Maca T, Bucek RA, et al. Simvastatin reduces expression of cytokines interleukin-6, interleukin-8, and monocyte chemoattractant protein-1 in circulating monocytes from hypercholesterolemic patients. *Arterioscler Thromb Vasc Biol*. 2002;22(7):1194-1199. doi:10.1161/01.ATV.0000022694.16328.CC

34. Ascer E, Bertolami MC, Venturinelli ML, et al. Atorvastatin reduces proinflammatory markers in hypercholesterolemic patients. *Atherosclerosis*. 2004;177(1):161-166. doi:10.1016/j.atherosclerosis.2004.07.003

35. Lyngdoh T, Vollenweider P, Waeber G, Marques-Vidal P. Association of statins with inflammatory cytokines: a population-based Colaus study. *Atherosclerosis*. 2011;219(1):253-258. doi:10.1016/j.atherosclerosis.2011.07.117

36. Danesh J, Kaptoge S, Mann AG, et al. Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. *PLoS Med*. 2008;5(4):e78. doi:10.1371/journal.pmed.0050078

37. Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis*. 2000;148(2):209-214. doi:10.1016/S0021-9150(99)00463-3

38. Lowe GDO. Circulating inflammatory markers and risks of cardiovascular and non-cardiovascular disease. *J Thromb Haemost*. 2005;3(8):1618-1627. doi:10.1111/j.1538-7836.2005.01416.x

Chapter 3: Figures/Tables

Figure 3.1: Directed Acyclic Graph of the Association between Statins and Inflammatory Biomarkers

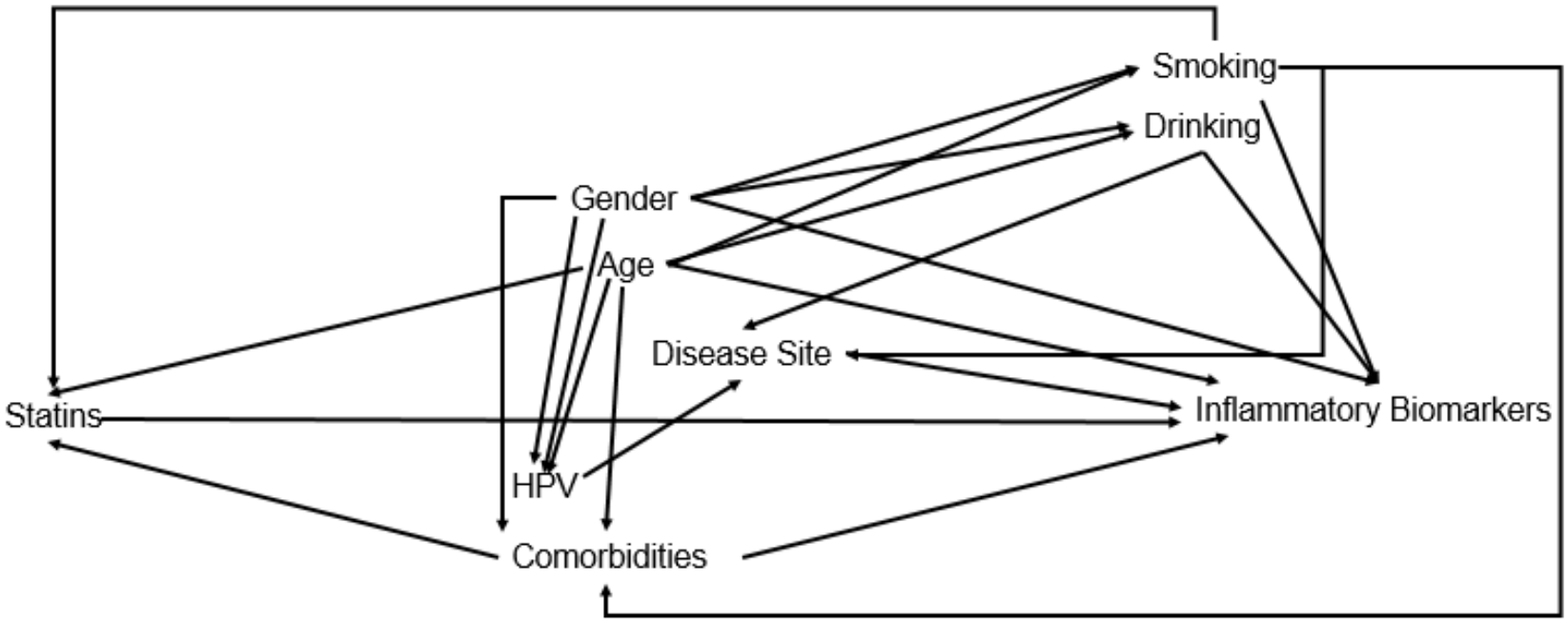


Figure 3.2: Correlations of TIL Values

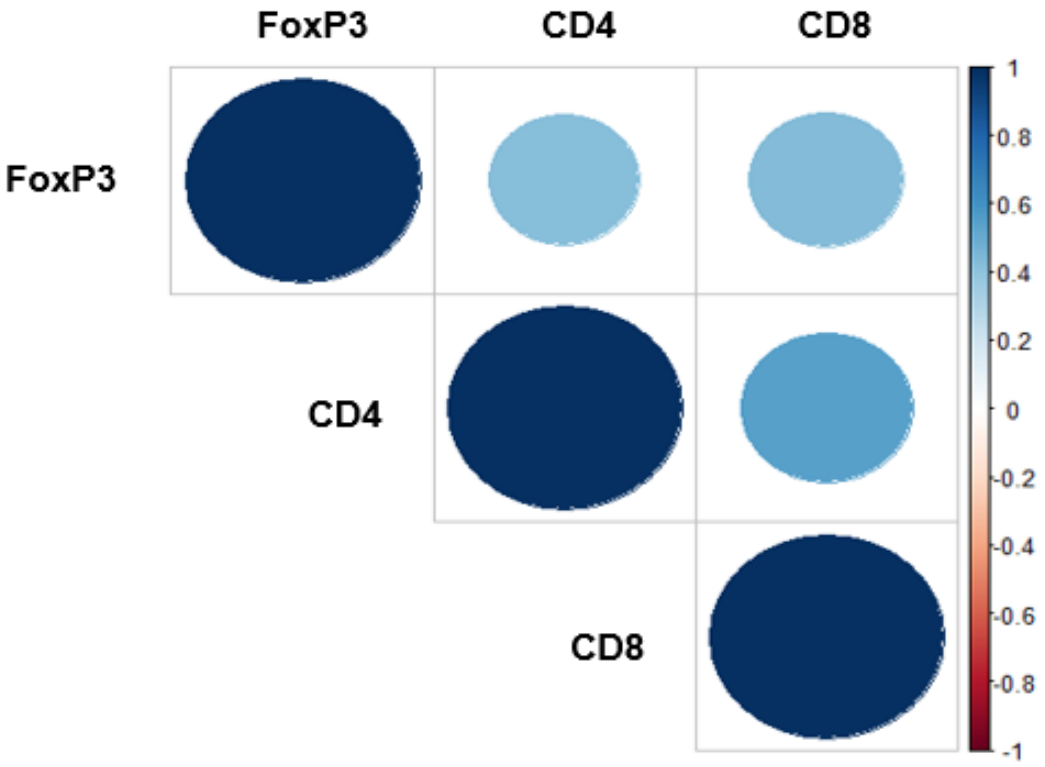


Figure 3.3: Correlations of Circulating Cytokine Values

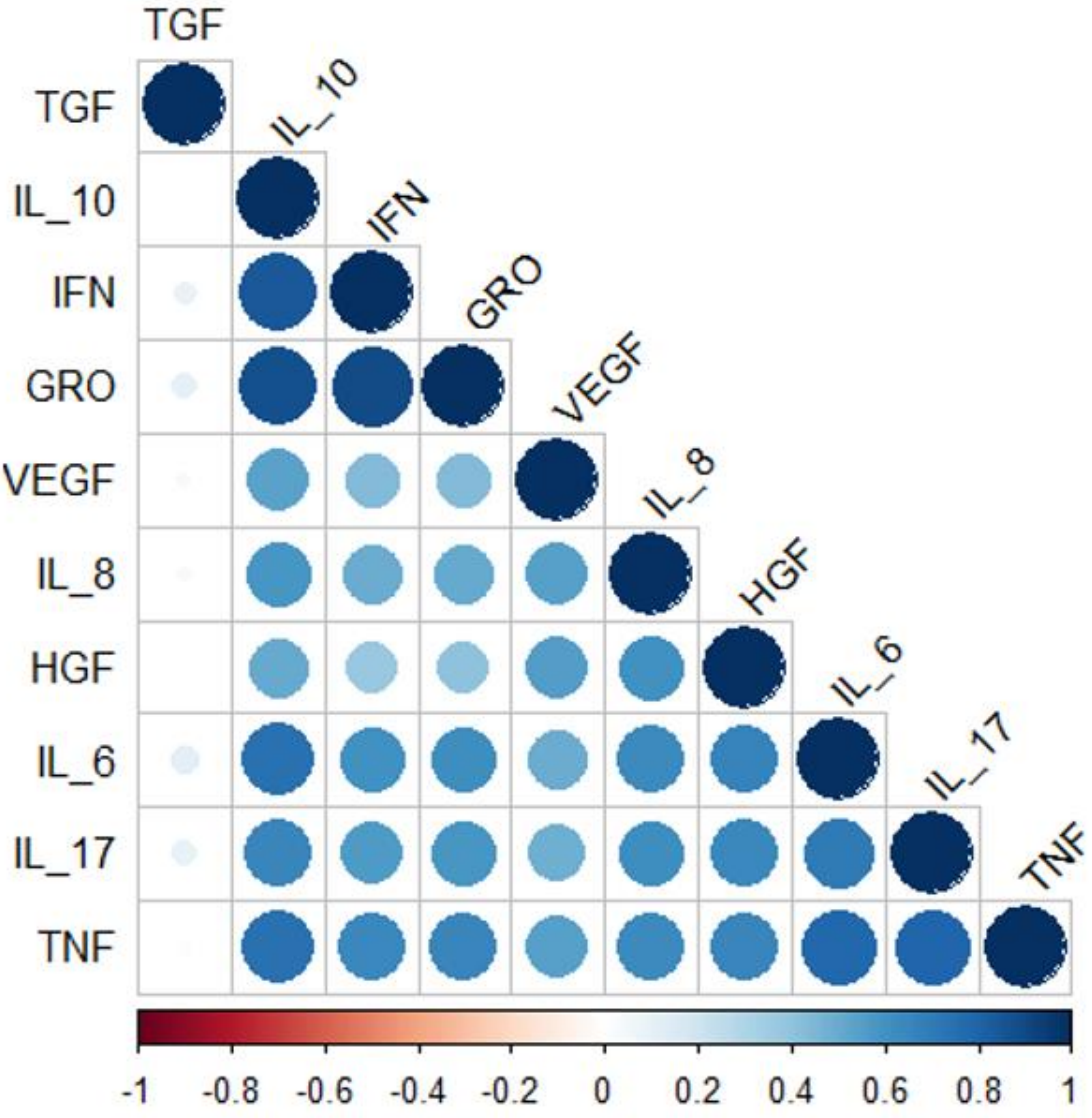


Figure 3.4: PCA: Scree Plot

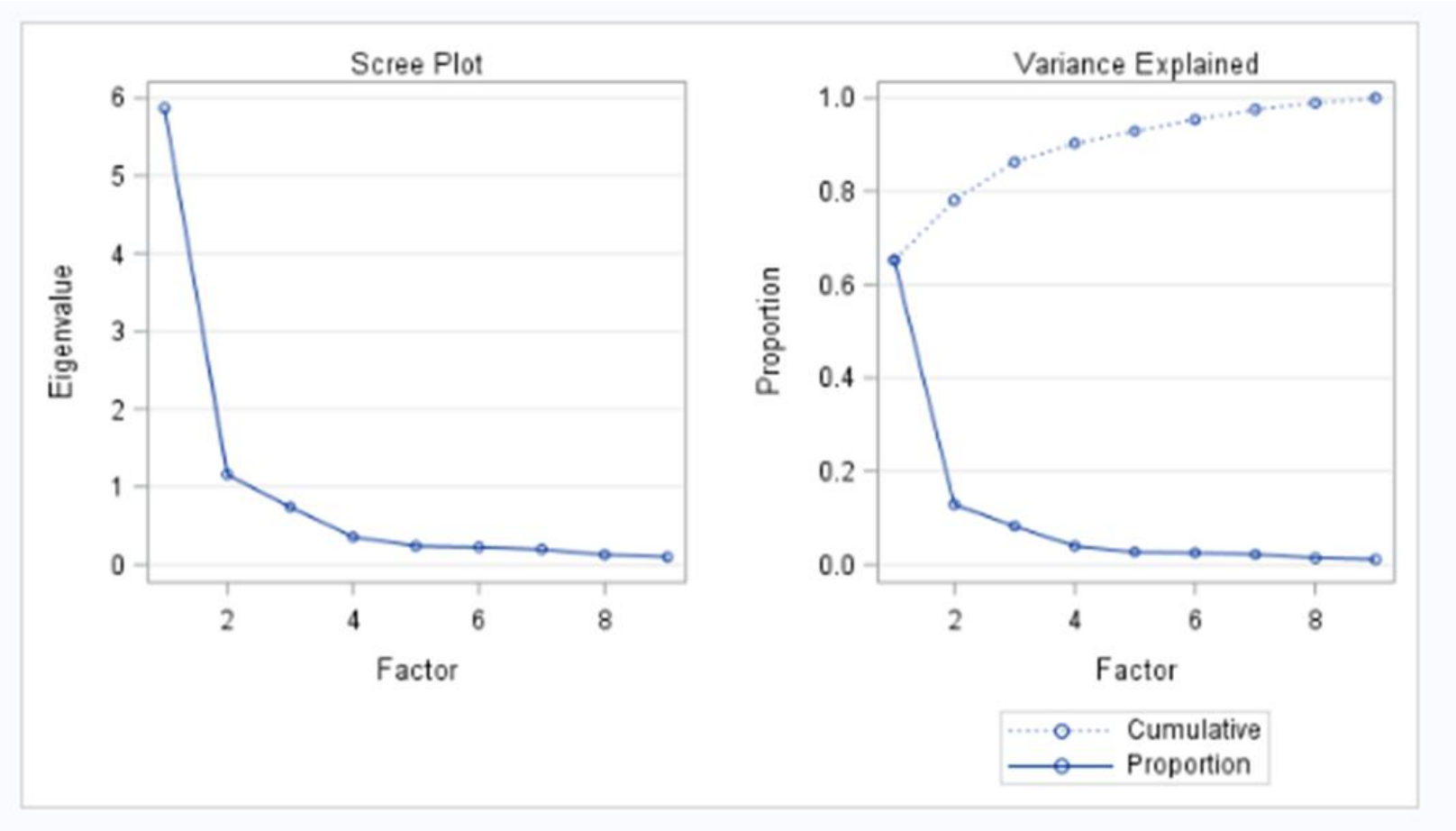


Table 3.1: Cytokines Coefficient of Variation

Cytokines	Coefficient of Variation	
	Subject 1	***Subject 2***
IFN-γ	116.22	12.76
IL-6	14.02	15.55
IL-8	2.10	4.48
IL-10	7.21	1.74
IL-17	7.63	7.29
GRO	11.04	0
HGF	8.53	2.46
TNF-α	8.55	15.84
VEGF	0.37	8.80
TGF	45.49	12.22

Table 3.2: PCA: Eigenvalues

	Eigenvalue	Proportion	Cumulative
1	5.87	0.65	0.65
2	1.15	0.13	0.78
3	0.74	0.08	0.86
4	0.35	0.04	0.90
5	0.24	0.03	0.93
6	0.22	0.02	0.95
7	0.19	0.02	0.98
8	0.13	0.01	0.99
9	0.1	0.01	1.00

Table 3.3: Demographic and Clinical Characteristics by Statin Use (TILs Participants)

	Statin User (N=156, 29.32%)	Non-Statin User (N=376, 70.68%)	P-value
Age at Diagnosis, years	66.89	58.91	<.0001
Sex (Male)	67.95%	71.81%	0.37
Race			0.45
White	92.95%	91.49%	
Not white	3.21%	5.59%	
Missing	3.85%	2.93%	
Disease Site			0.78
Larynx	14.10%	15.16%	
Oral Cavity	47.44%	49.20%	
Oropharynx	30.13%	28.99%	
Hypopharynx	3.21%	3.72%	
Other	5.13%	2.93%	
Stage at Diagnosis			0.997
0 and 1	13.46%	13.30%	
2	14.74%	15.16%	
3	14.74%	14.10%	
4	57.05%	57.45%	
HPV status			
Negative	48.08%	48.94%	0.73
Positive	26.28%	28.46%	
Invalid/Missing	25.64%	22.61%	
ACE Score*			
None	11.54%	29.26%	<.0001
Mild	50.00%	47.07%	
Moderate	21.15%	17.55%	
Severe	15.38%	5.85%	
Missing	1.92%	0.27%	
Highest Education			
Less than high school	3.21%	6.65%	0.56
High school/GED	22.44%	24.20%	
Some College	25.64%	23.67%	
4 yr degree	8.97%	9.04%	
More than 4 year degree	10.90%	7.71%	
Missing	28.85%	28.72%	
BMI			
Underweight/Normal Weight	24.36%	39.36%	0.01
Overweight/Obese 1	58.33%	47.07%	
Obese 2/Obese 3	12.82%	9.84%	
Missing	4.49%	3.72%	
Smoking Status			
Current	26.28%	48.67%	<.0001
Former	46.79%	25.80%	
Never	21.79%	22.34%	
Missing	5.13%	3.19%	
Drinking Status			
Current	53.21%	64.10%	0.13
Former	28.85%	21.81%	
Never	12.82%	10.64%	
Missing	5.13%	3.46%	

* Fisher's exact test p-value, for variables that had cell sizes smaller than 5

Table 3.4: Demographic and Clinical Characteristics by Statin Use (Cytokines Participants)

	Statin User (N=74, 36.10%)	Non-Statin User (N=131, 63.90%)	P-value
Age at Diagnosis, years	63.34	57.55	<0.0001
Sex (Male)	78.38%	82.44%	0.48
Race			0.75*
White	95.95%	93.89%	
Not white	4.05%	6.11%	
Disease Site			0.05*
Larynx	16.22%	22.90%	
Oral Cavity	24.32%	31.30%	
Oropharynx	44.59%	40.46%	
Hypopharynx	1.35%	2.29%	
Other	13.51%	3.05%	
Stage at Diagnosis			0.97
0 and 1	8.11%	6.87%	
2	8.11%	6.87%	
3	16.22%	16.03%	
4	67.57%	70.23%	
HPV status			0.0015
Negative	35.14%	53.44%	
Positive	39.19%	38.17%	
Invalid/Missing	25.68%	8.40%	
ACE Score			0.0005*
None	13.51%	34.35%	
Mild	51.35%	41.98%	
Moderate	20.27%	20.61%	
Severe	14.86%	3.05%	
Highest Education			0.53*
Less than high school	4.05%	6.87%	
High school/GED	25.68%	32.06%	
Some College	27.03%	28.24%	
4 yr degree	14.86%	7.63%	
More than 4 year degree	14.86%	15.27%	
Missing	13.51%	9.92%	
BMI			0.77
Underweight/Normal Weight	29.73%	33.59%	
Overweight/Obese 1	59.46%	58.02%	
Obese 2/Obese 3	10.81%	8.40%	
Smoking Status			0.08
Current	29.73%	45.80%	
Former	43.24%	32.82%	
Never	27.03%	21.37%	
Drinking Status			0.63
Current	64.86%	70.99%	
Former	27.03%	21.37%	
Never	8.11%	7.63%	

* Fisher's exact test p-value, for variables that had cell sizes smaller than 5

Table 3.5: Multivariable Models-TILs

	Crude Model Median TILs	Adjusted Model* Median TILs	Crude Model (Log Transformed TILs)	P	Adjusted Model* (Log Transformed TILs)	P
TILws (N=475)			0.06 (0.14)	0.67	0.09 (0.14)	0.54
Non-Statin User	1	1				
Statin User	1.20 (0.80, 1.78)	1.15 (0.74, 1.78)				
FoxP3 (N=485)			0.0095 (0.16)	0.95	0.09 (0.17)	0.59
Non-Statin User	1	1				
Statin User	1.01 (0.69, 1.50)	1.13 (0.74, 1.73)				
CD4 (N=481)			0.058 (0.19)	0.76	0.14 (0.20)	0.50
Non-Statin User	1	1				
Statin User	1.08 (0.73, 1.59)	1.23 (0.80, 1.90)				
CD8 (N=481)			0.19 (0.18)	0.31	0.12 (0.19)	0.55
Non-Statin User	1	1				
Statin User	1.19 (0.80, 1.77)	1.10 (0.72, 1.69)				

* Adjusted for Age at diagnosis, Smoking status and ACE-27

Table 3.6: Multivariable Models-Cytokines

N=205	Crude Model Median Cytokines	Adjusted Model* Median Cytokines	Fully Adjusted Model[a] Median Cytokines	Crude Model (Log Transformed Cytokines)	P	Adjusted Model* (Log Transformed Cytokines)	P	Fully Adjusted Model[a] (Log Transformed Cytokines)	P
IFN-γ				0.12 (0.46)	0.79	0.09 (0.51)	0.85	0.07 (0.54)	0.89
Non-Statin User	1	1	1						
Statin User	1.17 (0.66, 2.06)	1.19 (0.64, 2.22)	1.15 (0.60, 2.22)						
IL-6[c]				0.11 (0.32)	0.73	0.17 (0.35)	0.63	0.08 (0.37)	0.82
Non-Statin User	1	1	1						
Statin User	0.79 (0.45, 1.39)	0.79 (0.43, 1.48)	0.76 (0.39, 1.46)						
IL-8				0.21 (0.18)	0.25	0.21 (0.20)	0.30	0.15 (0.21)	0.47
Non-Statin User	1	1	1						
Statin User	1.74 (0.97, 3.09)	1.62 (0.87, 3.03)	1.46 (0.75, 2.83)						
IL-10 [c]				0.20 (0.39)	0.61	0.32 (0.42)	0.44	0.18 (0.44)	0.68
Non-Statin User	1	1	1						
Statin User	0.93 (0.53, 1.65)	1.01 (0.54, 1.88)	0.97 (0.50, 1.88)						
IL-17[b]				0.77 (0.42)	0.07	0.87 (0.45)	0.06	0.67 (0.48)	0.16
Non-Statin User	1	1	1						
Statin User	1.71 (0.89, 3.30)	1.91 (0.91, 3.97)	1.64 (0.76, 3.56)						
GRO				0.07 (0.26)	0.80	0.05 (0.28)	0.84	0.01 (0.29)	0.97
Non-Statin User	1	1	1						
Statin User	1.17 (0.66, 2.06)	1.21 (0.65, 2.28)	1.09 (0.56, 2.13)						
HGF				0.31 (0.12)	0.01	0.24 (0.13)	0.08	0.22 (0.14)	0.13
Non-Statin User	1	1	1						
Statin User	1.07 (0.61, 1.90)	0.99 (0.53, 1.86)	1.01 (0.52, 1.96)						
TNF-α[b]				0.38 (0.36)	0.30	0.42 (0.39)	0.28	0.34 (0.42)	0.41
Non-Statin User	1	1	1						
Statin User	1.05 (0.58, 1.90)	1.04 (0.55, 1.98)	1.01 (0.51, 1.98)						
VEGF				0.07 (0.23)	0.77	0.21 (0.25)	0.40	0.23 (0.26)	0.38
Non-Statin User	1	1	1						
Statin User	1.51 (0.85, 2.67)	1.37 (0.73, 2.57)	1.31 (0.67, 2.55)						
TGF				-0.05 (0.07)	0.49	0.006 (0.08)	0.94	-0.001 (0.08)	0.99
Non-Statin User	1	1	1						
Statin User	0.76 (0.43, 1.35)	0.94 (0.50, 1.78)	0.95 (0.92, 0.99)						

* Adjusted for Age at diagnosis, Smoking status and ACE-27

a. Adjusted for Age at diagnosis, Smoking status, ACE-27, HPV status and disease site

b. Due to excess zeros a median split of the data results in a zero vs non-zero split

c. Low cytokines category ≤ median

Table 3.7: HPV-Stratified TILs Models (Dropping Missing HPV Status)

	# of Events	Non-Statin User	# of Events	Statin User
		OR (95% CI)*		OR (95% CI)*
TILws				
HPV status w/o *missing*				
HPV-Positive	64	1 (REF)	29	2.80 (1.03, 7.61)
HPV-Negative	72	1 (REF)	31	1.07 (0.57, 2.02)
P for interaction	0.1			
FoxP3				
HPV status w/o *missing*				
HPV-Positive	59	1 (REF)	32	4.15 (1.55, 11.14)
HPV-Negative	81	1 (REF)	27	0.81 (0.43, 1.52)
P for interaction	0.003			
CD4				
HPV status w/o *missing*				
HPV-Positive	62	1 (REF)	29	2.42 (1.00, 5.86)
HPV-Negative	73	1 (REF)	32	1.42 (0.75, 2.69)
P for interaction	0.32			
CD8				
HPV status w/o *missing*				
HPV-Positive	63	1 (REF)	29	1.84 (0.75, 4.55)
HPV-Negative	64	1 (REF)	30	1.10 (0.58, 2.07)
P for interaction	0.34			

* Adjusted for Age at diagnosis, Smoking status and ACE-27

Table 3.8: HPV-Stratified Cytokines Models (Dropping Missing HPV Status)

	Non-Statin User		Statin User	
	# of Events	**OR (95% CI)***	**# of Events**	**OR (95% CI)***
IFN-γ				
HPV status w/o *missing*				
HPV-Positive	24	1 (REF)	15	1.32 (0.50, 3.49)
HPV-Negative	34	1 (REF)	14	1.30 (0.50, 3.42)
P for interaction	1			
IL-6				
HPV status w/o *missing*				
HPV-Positive	22	1 (REF)	14	1.32 (0.50, 3.51)
HPV-Negative	38	1 (REF)	13	0.77 (0.29, 2.02)
P for interaction	0.42			
IL-8				
HPV status w/o *missing*				
HPV-Positive	21	1 (REF)	17	1.96 (0.73, 5.27)
HPV-Negative	33	1 (REF)	14	1.14 (0.44, 2.99)
P for interaction	0.43			
IL-10				
HPV status w/o *missing*				
HPV-Positive	26	1 (REF)	16	1.27 (0.48, 3.39)
HPV-Negative	35	1 (REF)	11	0.80 (0.30, 2.13)
P for interaction	0.50			
IL-17				
HPV status w/o *missing*				
HPV-Positive	9	1 (REF)	11	3.95 (1.26, 12.38)
HPV-Negative	11	1 (REF)	5	1.29 (0.36, 4.67)
P for interaction	0.17			
GRO				
HPV status w/o *missing*				
HPV-Positive	25	1 (REF)	16	1.39 (0.52, 3.72)
HPV-Negative	33	1 (REF)	12	0.95 (0.36, 2.53)
P for interaction	0.58			
HGF				
HPV status w/o *missing*				
HPV-Positive	20	1 (REF)	16	2.27 (0.82, 6.27)
HPV-Negative	38	1 (REF)	11	0.47 (0.17, 1.31)
P for interaction	0.03			
TNF–α				
HPV status w/o *missing*				
HPV-Positive	16	1 (REF)	12	1.69 (0.62, 4.64)
HPV-Negative	26	1 (REF)	8	0.73 (0.26, 2.05)
P for interaction	0.23			
VEGF				
HPV status w/o *missing*				
HPV-Positive	22	1 (REF)	19	2.46 (0.90, 6.78)
HPV-Negative	32	1 (REF)	11	0.76 (0.28, 2.02)
P for interaction	0.09			
TGF				
HPV status w/o *missing*				
HPV-Positive	28	1 (REF)	14	1.13 (0.41, 3.08)
HPV-Negative	34	1 (REF)	12	0.88 (0.32, 2.42)
P for interaction	0.73			

* Adjusted for Age at diagnosis, Smoking status and ACE-27

Table 3.9: HPV Stratified Analysis Using Various Strategies to Account for Missing HPV Status (TILs)

Outcomes	Non-Statin User		Statin User	
	No. of Events	OR (95% CI)[a]	No. of Events	OR (95% CI)[a,b]
TILws				
HPV status with missing				
HPV-Positive	64	1 (REF)	29	2.60 (0.96, 7.02)
HPV-Negative	72	1 (REF)	31	0.93 (0.50, 1.74)
HPV-Missing/Invalid	29	1 (REF)	13	0.87 (0.37, 2.03)
HPV status IPW				
HPV-Positive	57	1 (REF)	27	3.16 (1.06, 9.47)
HPV-Negative	70	1 (REF)	31	1.02 (0.51, 2.05)
FoxP3				
HPV status with missing				
HPV-Positive	59	1 (REF)	32	3.83 (1.44, 10.18)
HPV-Negative	81	1 (REF)	27	0.74 (0.40, 1.37)
HPV-Missing/Invalid	32	1 (REF)	12	0.81 (0.35, 1.88)
HPV status IPW				
HPV-Positive	52	1 (REF)	30	5.51 (1.91, 15.86)
HPV-Negative	79	1 (REF)	26	0.66 (0.34, 1.30)
CD4				
HPV status with missing				
HPV-Positive	62	1 (REF)	29	2.14 (0.89, 5.14)
HPV-Negative	73	1 (REF)	32	1.20 (0.65, 2.22)
HPV-Missing/Invalid	33	1 (REF)	12	0.74 (0.32, 1.72)
HPV status IPW				
HPV-Positive	57	1 (REF)	27	2.47 (0.98, 6.22)
HPV-Negative	69	1 (REF)	31	1.29 (0.64, 2.62)
CD8				
HPV status with missing				
HPV-Positive	63	1 (REF)	29	1.85 (0.75, 4.55)
HPV-Negative	64	1 (REF)	30	1.06 (0.57, 1.97)
HPV-Missing/Invalid	39	1 (REF)	16	0.69 (0.31, 1.55)
HPV status IPW				
HPV-Positive	57	1 (REF)	27	2.22 (0.82, 6.01)
HPV-Negative	62	1 (REF)	29	1.12 (0.56, 2.22)

a. Adjusted for Age at diagnosis, Smoking status and ACE-27
b. Inverse probability weighted model

Table 3.10: HPV Stratified Analysis Using Various Strategies to Account for Missing HPV Status (Cytokines)

Outcomes	Non-Statin User		Statin User	
	No. of Events	OR (95% CI)[a]	No. of Events	OR (95% CI)[a,b]
IFN-γ				
HPV status with missing				
HPV-Positive	24	1 (REF)	15	1.20 (0.46, 3.17)
HPV-Negative	34	1 (REF)	14	1.29 (0.50, 3.33)
HPV-Missing/Invalid	6	1 (REF)	10	0.997 (0.22, 4.55)
HPV status IPW				
HPV-Positive	24	1 (REF)	15	1.35 (0.49, 3.72)
HPV-Negative	34	1 (REF)	14	1.23 (0.46, 3.28)
IL-6				
HPV status with missing				
HPV-Positive	22	1 (REF)	14	1.32 (0.50, 3.49)
HPV-Negative	38	1 (REF)	13	0.83 (0.32, 2.17)
HPV-Missing/Invalid	8	1 (REF)	7	0.22 (0.04, 1.15)
HPV status IPW				
HPV-Positive	22	1 (REF)	14	1.48 (0.56, 3.94)
HPV-Negative	37	1 (REF)	13	1.03 (0.36, 2.90)
IL-8				
HPV status with missing				
HPV-Positive	21	1 (REF)	17	1.87 (0.70, 4.98)
HPV-Negative	33	1 (REF)	14	1.19 (0.46, 3.07)
HPV-Missing/Invalid	6	1 (REF)	13	1.68 (0.35, 7.95)
HPV status IPW				
HPV-Positive	21	1 (REF)	17	2.17 (0.80, 5.92)
HPV-Negative	32	1 (REF)	14	1.50 (0.54, 4.16)
IL-10				
HPV status with missing				
HPV-Positive	26	1 (REF)	16	1.18 (0.44, 3.12)
HPV-Negative	35	1 (REF)	11	0.78 (0.30, 2.05)
HPV-Missing/Invalid	5	1 (REF)	9	1.26 (0.27, 5.84)
HPV status IPW				
HPV-Positive	26	1 (REF)	16	1.31 (0.47, 3.63)
HPV-Negative	35	1 (REF)	11	0.81 (0.30, 2.17)
IL-17				
HPV status with missing				
HPV-Positive	9	1 (REF)	11	3.69 (1.20, 11.38)
HPV-Negative	11	1 (REF)	5	1.39 (0.40, 4.84)
HPV-Missing/Invalid	6	1 (REF)	6	0.41 (0.09, 1.99)
HPV status IPW				
HPV-Positive	9	1 (REF)	11	3.82 (1.17, 12.51)
HPV-Negative	11	1 (REF)	5	1.69 (0.42, 6.78)
GRO				
HPV status with missing				
HPV-Positive	25	1 (REF)	16	1.32 (0.49, 3.54)
HPV-Negative	33	1 (REF)	12	0.99 (0.38, 2.59)
HPV-Missing/Invalid	6	1 (REF)	11	1.21 (0.26, 5.71)
HPV status IPW				
HPV-Positive	25	1 (REF)	16	1.41 (0.51, 3.92)
HPV-Negative	33	1 (REF)	12	1.02 (0.37, 2.80)

HGF				
HPV status with missing				
HPV-Positive	20	1 (REF)	16	2.11 (0.78, 5.73)
HPV-Negative	38	1 (REF)	11	0.49 (0.18, 1.33)
HPV-Missing/Invalid	7	1 (REF)	11	0.65 (0.14, 3.15)
HPV status IPW				
HPV-Positive	20	1 (REF)	16	2.23 (0.83, 5.95)
HPV-Negative	37	1 (REF)	11	0.64 (0.20, 2.06)
TNF-α				
HPV status with missing				
HPV-Positive	16	1 (REF)	12	1.59 (0.58, 4.35)
HPV-Negative	26	1 (REF)	8	0.74 (0.27, 2.04)
HPV-Missing/Invalid	6	1 (REF)	8	0.64 (0.14, 2.95)
HPV status IPW				
HPV-Positive	16	1 (REF)	12	1.67 (0.58, 4.86)
HPV-Negative	25	1 (REF)	8	0.88 (0.29, 2.73)
VEGF				
HPV status with missing				
HPV-Positive	22	1 (REF)	19	2.32 (0.85, 6.32)
HPV-Negative	32	1 (REF)	11	0.74 (0.28, 1.95)
HPV-Missing/Invalid	7	1 (REF)	12	0.998 (0.21, 4.81)
HPV status IPW				
HPV-Positive	22	1 (REF)	19	2.82 (1.07, 7.44)
HPV-Negative	31	1 (REF)	11	0.64 (0.22, 1.84)
TGF				
HPV status with missing				
HPV-Positive	28	1 (REF)	14	1.14 (0.42, 3.05)
HPV-Negative	34	1 (REF)	12	0.99 (0.37, 2.66)
HPV-Missing/Invalid	7	1 (REF)	8	0.40 (0.08, 1.91)
HPV status IPW				
HPV-Positive	28	1 (REF)	14	1.25 (0.47, 3.35)
HPV-Negative	34	1 (REF)	12	0.80 (0.30, 2.16)

a. Adjusted for Age at diagnosis, Smoking status and ACE-27
b. Inverse probability weighted model

Table 3.11: PCA: Factor Patterns (3 Factors)

	Non-Rotated Factor Pattern			Orthogonally Rotated Factor Pattern		
	Factor 1	***Factor 2***	***Factor 3***	***Factor 1***	***Factor 2***	***Factor 3***
IFN-γ	0.76028	0.54601	-0.06271	0.25797	0.89390	0.12031
IL-6	0.90171	-0.16415	-0.09179	0.80381	0.41915	0.16325
IL-8	0.78838	-0.30819	0.03575	0.77488	0.22091	0.26185
IL-10	0.85849	0.39193	0.02470	0.40478	0.81898	0.23802
IL-17	0.85832	-0.25813	-0.16325	0.84544	0.32810	0.08711
GRO	0.75615	0.54284	-0.09241	0.26395	0.89278	0.09078
HGF	0.80145	-0.42410	-0.09475	0.88681	0.15378	0.14522
TNF-α	0.92307	-0.20291	-0.10222	0.84605	0.40252	0.16078
VEGF	0.56367	-0.03958	0.81847	0.25235	0.19829	0.94137

High factor loadings= **green**
Medium factor loadings=**yellow**
Low factor loadings=**red**

Table 3.12: PCA: Final Communality Factor Loadings

Cytokine	Final Communality Factor Loadings
IFN-γ	0.880
IL-6	0.848
IL-8	0.718
IL-10	0.891
IL-17	0.830
GRO	0.875
HGF	0.831
TNF-α	0.904
VEGF	0.989

Table 3.13: Logistic Regression: Association between Cytokine PCA Factor Patterns and Statin Use at Diagnosis

Factors (Median Split)	Logistic Regression, OR (95% CI)	
	Unadjusted	***Adjusted****
Factor 1	1.51 (0.85, 2.67)	1.36 (0.73, 2.53)
Factor 2	1.17 (0.66, 2.06)	1.16 (0.62, 2.17)
Factor 3	1.27 (0.72, 2.25)	1.38 (0.74, 2.58)

* Adjusted for age at diagnosis, smoking status and ACE-27

Table 3.14: Linear Regression: Association between Cytokine PCA Factor Patterns and Statin Use at Diagnosis

Factors (Continuous)	Linear Regression, Beta (SE)			
	Unadjusted	***p-value***	***Adjusted****	***p-value***
Factor 1	0.27 (0.14)	0.06	0.25 (0.16)	0.12
Factor 2	-0.06 (0.15)	0.70	-0.04 (0.16)	0.78
Factor 3	-0.02 (0.15)	0.87	0.07 (0.16)	0.64

* Adjusted for age at diagnosis, smoking status and ACE-27

Table 3.15: HPV-Stratified Cytokine PCA Factor Patterns Models (Dropping Missing HPV Status)

	Non-Statin User		Statin User	
	# of Events	**OR (95% CI)***	**# of Events**	**OR (95% CI)***
Factor 1				
HPV status w/o *missing*				
HPV-Positive	20	1 (REF)	18	2.13 (0.78, 5.81)
HPV-Negative	32	1 (REF)	13	1.04 (0.39, 2.75)
P for interaction	0.30			
Factor 2				
HPV status w/o *missing*				
HPV-Positive	27	1 (REF)	15	0.96 (0.36, 2.56)
HPV-Negative	32	1 (REF)	15	1.63 (0.61, 4.31)
P for interaction	0.44			
Factor 3				
HPV status w/o *missing*				
HPV-Positive	19	1 (REF)	20	4.55 (1.60, 12.96)
HPV-Negative	40	1 (REF)	10	0.51 (0.19, 1.38)
P for interaction	0.002			

* Adjusted for Age at diagnosis, Smoking status and ACE-27

Table 3.16: HPV Stratified Analysis Using Various Strategies to Account for Missing HPV Status (Cytokine PCA Factor Patterns)

	Non-Statin User		Statin User	
	# of Events	**OR (95% CI)***	**# of Events**	**OR (95% CI)***
Factor 1				
HPV status with *missing*				
HPV-Positive	20	1 (REF)	18	2.14 (0.79, 5.75)
HPV-Negative	32	1 (REF)	13	1.11 (0.43, 2.88)
HPV-Missing	9	1 (REF)	11	0.27 (0.04, 1.62)
HPV status IPW				
HPV-Positive	20	1 (REF)	18	2.28 (0.84, 6.18)
HPV-Negative	31	1 (REF)	13	1.25 (0.44, 3.54)
Factor 2				
HPV status with *missing*				
HPV-Positive	27	1 (REF)	15	0.88 (0.33, 2.35)
HPV-Negative	32	1 (REF)	15	1.60 (0.61, 4.19)
HPV-Missing	5	1 (REF)	9	1.09 (0.23, 5.07)
HPV status IPW				
HPV-Positive	27	1 (REF)	15	1.01 (0.36, 2.83)
HPV-Negative	32	1 (REF)	15	1.69 (0.62, 4.55)
Factor 3				
HPV status with *missing*				
HPV-Positive	19	1 (REF)	20	4.18 (1.49, 11.78)
HPV-Negative	40	1 (REF)	10	0.47 (0.18, 1.25)
HPV-Missing	4	1 (REF)	10	2.06 (0.43, 9.90)
HPV status IPW				
HPV-Positive	19	1 (REF)	20	4.97 (1.80, 13.78)
HPV-Negative	40	1 (REF)	10	0.43 (0.15, 1.18)

* Adjusted for Age at diagnosis, Smoking status and ACE-27

Table 3.17: Multivariable Models: TILs Using IPW to Account for Missing Values

	Crude Model Median TILs	Adjusted Model* Median TILs
TILws (N=427)		
Non-Statin User	1	1
Statin User	1.06 (0.67, 1.68)	1.08 (0.65, 1.80)
FoxP3 (N=437)		
Non-Statin User	1	1
Statin User	1.08 (0.69, 1.70)	1.23 (0.75, 2.01)
CD4 (N=433)		
Non-Statin User	1	1
Statin User	1.06 (0.67, 1.67)	1.21 (0.74, 1.98)
CD8[a] (N=433)		
Non-Statin User	1	1
Statin User	1.11 (0.70, 1.75)	1.05 (0.64, 1.74)

* Adjusted for Age at diagnosis, Smoking status and ACE-27

a. Low TILs category ≤ median

Table 3.18: Multivariable Models: Cytokines Using IPW to Account for Missing Values

	Crude Model Median Cytokines	Adjusted Model* Median Cytokines
IFN-γ (N=204)		
Non-Statin User	1	1
Statin User	0.78 (0.41, 1.51)	0.76 (0.37, 1.56)
IL-6 (N=204) [a]		
Non-Statin User	1	1
Statin User	0.76 (0.40, 1.45)	0.88 (0.45, 1.75)
IL-8 (N=204)		
Non-Statin User	1	1
Statin User	1.73 (0.89, 3.38)	1.87 (0.91, 3.82)
IL-10 (N=204)[a]		
Non-Statin User	1	1
Statin User	0.72 (0.38, 1.38)	0.80 (0.38, 1.68)
IL-17 (N=204)[b]		
Non-Statin User	1	1
Statin User	1.36 (0.64, 2.89)	1.63 (0.70, 3.77)
GRO (N=204)		
Non-Statin User	1	1
Statin User	0.86 (0.45, 1.66)	0.84 (0.40, 1.75)
HGF (N=204)		
Non-Statin User	1	1
Statin User	0.84 (0.44, 1.62)	0.81 (0.40, 1.66)
TNF-α (N=204) [b]		
Non-Statin User	1	1
Statin User	0.74 (0.38, 1.45)	0.74 (0.34, 1.60)
VEGF (N=204)		
Non-Statin User	1	1
Statin User	1.28 (0.66, 2.48)	1.29 (0.64, 2.61)
TGF (N=204)		
Non-Statin User	1	1
Statin User	0.91 (0.47, 1.76)	1.02 (0.49, 2.14)

* Adjusted for Age at diagnosis, Smoking status and ACE-27

a. Median split is ≤ Median, =0; >Median=1

b. Due to excess zeros a median split of the data results in a zero vs non-zero split

Chapter 4 : Association between Blood Lipid Levels and Head and Neck Squamous Cell Carcinoma Risk and Outcomes: A Mendelian Randomization Analysis

Abstract

Background: The protective association observed between statin use and head and neck squamous cell carcinoma (HNSCC) outcomes and risk of disease may be related to circulating cholesterol levels. Previous research has not clearly established how cholesterol levels influence cancer risk and outcomes, and it often appears to depend on the site of disease. The objective of this study is to explore whether cholesterol may be the mechanism by which statins exert any observed influence on HNSCC risk or outcomes through Mendelian randomization analysis; utilizing genetic variants to create instruments that predict hypercholesterolemia (total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides (TG)).

Methods: A case-control study was conducted among incident HNSCC patients diagnosed and/or treated at the University of Michigan Rogel Cancer Center and controls who did not have a HNSCC diagnosis, using a 1 to 3 match. We matched on age, gender, and race/ethnicity. Both cases and controls agreed to participate in the Michigan Genomics Initiative. The analytic cohort consisted of 864 participants (Cases=216 and Controls=648). Instruments were calculated for each cholesterol-related measure using single nucleotide polymorphisms (SNPs) that are statistically significantly associated with TC, LDL, HDL, and TG. Unconditional logistic regression analysis was conducted to estimate the association between cholesterol-related instruments and the risk of developing HNSCC. Cox proportional hazard models were utilized to

estimate the association between the cholesterol-related instruments, and HNSCC outcomes (overall death, disease-specific death, and recurrence) among cases.

Results: There does not appear to be an association between any of the cholesterol measures and risk of developing HNSCC, with the point estimates for all of the instruments relatively null; [(TC: OR=0.99, 95% CI=0.85, 1.15); (HDL: OR=1.01, 95% CI=0.87, 1.18); (LDL: OR=1.02, 95% CI=0.87, 1.19); (TG: OR=1.00, 95% CI=0.86, 1.17)]. There did appear to be a positive association between TC and overall death, and recurrence [(overall death: HR=1.36, 95% CI=1.01, 1.83); (recurrence: HR=1.30, 95% CI=0.98, 1.73)] and an inverse association was observed between TG and overall death and disease-specific death; [(overall death: HR=0.75, 95% CI=0.57, 0.99); (disease-specific death: HR=0.71, 95% CI=0.51, 0.98)]. HPV-status did not seem to modify any of the associations between the cholesterol-related instruments, and HNSCC risk or outcomes.

Conclusion: The findings from this study support a null association between circulating cholesterol levels and risk of HNSCC. Although there did appear to be an association between TC and TG, with HNSCC outcomes, given the small study sample, the findings are not precise. HPV status does not appear to modify the association between cholesterol levels and HNSCC risk, or outcomes. Future research investigating the association between circulating cholesterol levels and HNSCC outcomes in a larger, more diverse population of HNSCC patients is necessary in order to validate the presented study results.

Background

Statin drugs have various health effects including, anti-inflammation, immunomodulation but most notably cholesterol-lowering.[1] Cholesterol's effect on coronary heart disease is well established as detrimental, but the influence of cholesterol on cancer risk and outcomes associated with cancer is not as clear.[2] Statin use has been identified as having protective effects against cancer-related outcomes among patients suffering from disease in various sites.[3,4] More recently, research has identified a protective association between the use of statin drugs and HNSCC risk and outcomes [5–8], but the mechanism by which statins influence HNSCC risk and outcomes are not clearly established.

There are various biological explanations why cholesterol-lowering may be the mechanism by which statins inhibit cancer. These mechanisms are known to be related to cancer development at different disease sites (prostate, lung, breast, gastrointestinal, etc.), specifically the AKT pathway and hormones both of which are related to cholesterol through sterol-regulatory element-binding protein, an essential element in the regulation of cholesterol.[9–11] To our knowledge, the relationship between these pathways and cholesterol has not been investigated in patients with HNSCC. Epidemiologic studies have investigated the relationship between cholesterol and cancer risk across various cancer sites. Most studies have utilized observational designs but the results are not consistent and may be biased.[12] In particular, protective findings between high cholesterol and risk of cancer may be due to reverse causation.[13] Although there have been a plethora of studies investigating the association between circulating cholesterol levels and cancer at various sites, the literature is limited when exploring this association among patients with HNSCC.

The impact of HNSCC on patients with advanced disease can be quite devastating, with traditional treatment options including surgical excision of disease, chemotherapy, and radiation.[14] The behavioral or tertiary prevention recommendations are limited to smoking

cessation, which may not be applicable to all patients, specifically those with HPV-positive tumors who are less likely to smoke than patients with HPV-negative disease.[15] HPV- positive disease is becoming more prevalent in the United States in recent decades, whereas there has been a decrease in HNSCC sites that are associated with HPV-negative disease due to the drop in tobacco use.[16,17] Therefore, it is important to identify other behavioral or interventional approaches to improve HNSCC risk and prognosis due to this shift in disease etiology. If cholesterol levels are found to be associated with HNSCC risk and outcomes, it may be a health condition that can be prevented or mitigated through behavioral changes or medicinal intervention.

Identifying the impact of cholesterol as a mechanism to reduce HNSCC risk and outcomes may be beneficial for patients who may not tolerate the use of statin drugs. Statins, although relatively safe, have various adverse effects in some patients ranging from muscle pain and liver issues to increased risk of type 2 diabetes.[18,19] If the mechanism by which statins improve HNSCC risk and outcomes is through cholesterol-lowering, the use of other cholesterol-lowering medications may be an alternative treatment option for patients who may not be able to use statin drugs.

Given the presented limitations and mixed findings from research conducted previously investigating the relationship between cholesterol and cancer development in observational studies, conducting a Mendelian randomization analysis may assist with mitigating this potential bias. Through utilization of an instrumental variable approach, the issue of temporality may be eliminated due to the guarantee that the instrument (genetics) precedes the outcome. The objective of this study is to explore whether cholesterol may be the mechanism by which statins exert any observed influence on HNSCC risk or outcomes. We will use genetic data from the Michigan Genomics Initiative (MGI) to conduct a Mendelian randomization analysis examining the association between genetic variants through instruments that predict hypercholesterolemia

(high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG) and total cholesterol (TC)) and risk of developing HNSCC and outcomes among HNSCC patients. Given that HNSCC patients with HPV-positive disease have different patient characteristics and a dissimilar etiology of disease than HPV-negative patients, it is important to investigate these patients separately.

Methods

Study Population

A case-control study was conducted among a subset of incident HNSCC patients who agreed to participate in both the University of Michigan Head and Neck Cancer Specialized Program of Research Excellence (HNC SPORE), a longitudinal epidemiological study of HNSCC outcomes, and the MGI. HNC SPORE participants were recruited from 2003-2014, and HNSCC diagnosis was identified through medical record review of new patients visiting the University of Michigan Rogel Cancer Center for their HNSCC diagnosis with biopsy-confirmed disease. Outcomes information for cases were identified through yearly review of medical records for disease recurrence and progression. Overall death and disease-specific death were identified through medical record review; if this information was not available through the patient's medical record, the national social security death index and LexisNexis database were checked. All outcomes information was collected by trained study personnel and was reviewed by physicians for accuracy. Participants' outcome information was collected and updated through April 2016. Of the 216 patients with HNSCC in the final analytic sample, about 25.9% died of any cause (N=56), 18.1% died of HNSCC (N=39), and 25.9% (N=56) experienced recurrence, which is defined as never being free of disease after diagnosis or experiencing a local, regional or distant recurrence of disease. Additional information about the HNC SPORE cohort study recruitment and data collection was previously published.[20,21]

Of the 1,648 HNC SPORE participants, 228 also agreed to participate in the MGI. The MGI is a project that approached all patients who were undergoing procedures that require anesthesia at the University of Michigan starting in 2012.[22] Patients who agreed to participate in the MGI were asked to provide consent to collect blood to perform whole-genome sequencing using a customized Illumina Infinium CoreExome-24 bead arrays.[22] Our case-control study utilized a 3:1 match (3 controls per case). Controls were participants in the MGI who were not diagnosed with HNSCC. The controls were matched to the cases based on age (within 5 years), race/ethnicity, and gender. Additional covariate data on the controls was collected through the medical records system (MiChart) using DataDirect provided by the University of Michigan Data Office for Clinical and Translational Research. Genetic data from the MGI for cases who participated in the HNC SPORE were linked to the clinical, outcome, and behavioral data previously collected through prior studies conducted in the HNC SPORE. The final analytic sample excluded participants who identified as a race/ethnicity other than Caucasian in order to maintain a homogenous population. After excluding these participants, there were 216 cases and 648 controls.

Statistical Analysis

Specific gene variants that are associated with hypercholesterolemia were identified through the Global Lipids Genetics Consortium (GLGC) joint metabochip and genome-wide association study (GWAS) analysis.[23] Single nucleotide polymorphisms (SNP)s that were identified as being only statistically significantly associated ($P<5x10^{-8}$) with TC, HDL, LDL, and TG were isolated in the MGI data. We created instruments for HDL, LDL, TG, and TC through combining the individual SNPs that were only statistically significant for the corresponding cholesterol measure. Instruments were calculated using PLINK 2.0.[24,25] A table of the SNPs, identified through rs-id and chromosome location that were used to create each instrument can be found in Table 4.1. In addition to the TC instrument, we created another TC instrument (TCF)

that was composed of all of the SNPs that were statistically significantly associated with TC, even if there was overlap with the other cholesterol measures. We did this because TC encompasses all of the other cholesterol measures. Including all of the SNPs associated with TC may make the instrument stronger.

A previously published study utilizing a similar technique assessed the strength of the association between the instruments (containing almost all of the same SNPs) created for cholesterol measures (HDL, LDL, and TG) and the related cholesterol measure. After conducting linear regression, each instrument they created appeared to be statistically significantly associated with the corresponding cholesterol measure. HDL appeared to be the strongest instrument (beta coefficient=0.19 mmol/L HDL).[26] The LDL and TG instruments were associated with not only measured LDL (beta coefficient=0.56 mmol/L HDL) and TG (beta coefficient=0.35 mmol/L lnTG), respectively, but both were also associated with HDL.[26] Although the LDL and TG instruments were both associated with measured HDL, they were more strongly associated with their corresponding cholesterol measure.[26] Based on these previously published results, we can assume that the cholesterol-related instruments we created are strong indicators of the corresponding cholesterol measures.

To identify the association between instruments related to hypercholesterolemia and HNSCC risk, we conducted a Mendelian randomization analysis. Mendelian randomization analysis is an instrumental variable technique in which SNPs are selected that precede the exposure of interest. In order to properly conduct this analysis, certain assumptions must be met. The genetic variants (instrument) must only be associated with the outcome through the exposure and not independently. The genetic variants (instrument) must also not be associated with any confounders between the exposure and the outcome.[27] This relationship is displayed in the directed acyclic graph (DAG) found in Figure 4.1. A benefit of this analytic technique is that if all assumptions are met, unmeasured confounding between the exposure and outcome variable should not be an issue.

We operationalized the instruments continuously (1 unit increase=standard deviation increase(SD)), as well as created quartiles for analysis, given the approximately normal distribution of all of the instruments (histogram of the distribution of each instrument by standard deviation increase can be found in Figure 4.2). We also compared quartile 1 to quartiles 2-4 for the HDL instrument due to the distribution of the quartiles of the HDL instrument. In order to investigate the relationship between susceptibility to high cholesterol and odds of developing HNSCC, we conducted logistic regression models with the HNSCC cancer status (1= have HNSCC, case; 0=do not have HNSCC, control) as the outcome in the model and the instrument (HDL, LDL, TG and TC) as the exposure; with each instrument having a separate model.

We also investigated the association between the instruments (HDL, LDL, TG, and TC) and HNSCC outcomes (overall death, disease-specific death, and disease recurrence) among the cases. To explore this relationship, we conducted Cox proportional hazards models using the same continuous and quartile operationalized instruments as described above.

Since HNSCC is a diverse disease and patients who have HPV-positive associated tumors are etiologically different and also experience different prognosis than patients with HPV-negative tumors, we decided to investigate the potential effect modification of HPV-status through investigating the association between the risk of HNSCC and the listed instruments above for patients with HPV-positive and HPV-negative tumors separately. It is also important to investigate patients with and without HPV-associated disease separately because the impact of statin use on outcomes only appears to be protective for patients with HPV-positive tumors.[8] We will also assess the potential effect modification of disease site because it is so highly associated with HPV status, as well as stage of disease.

All analysis was conducted utilizing SAS version 9.4 (Cary, NC), and R; p-values were considered statistically significant at $p<0.05$.

Results

Descriptive statistics

Given the matched design of this case-control study, the distribution of age, gender and race are equal among cases and controls. Of the cases, the majority experience HNSCC in the oral cavity, with the second most frequent site of disease being the oropharynx. The majority of cases have stage 4 disease at diagnosis and are missing HPV status. For those without HPV status missing, there are slightly more patients with HPV-negative disease compared to those with HPV-positive disease. When comparing behavioral characteristics that were not matched between cases and controls, cases are more likely to be current smokers than controls. Although the frequency of former smokers appears to be similar between cases and controls, controls are much more likely to be never smokers. Drinking status is similar between cases and controls, and body mass index (BMI) is higher in controls than cases (Table 4.2). The distribution of the cholesterol-related instruments are very similar between cases and controls in this study (Figure 4.3).

HNSCC Risk

We observed no association between the instruments and HNSCC risk [per SD increase in score (TC: OR=0.99, 95% CI=0.85, 1.15); (HDL: OR=1.01, 95% CI=0.87, 1.18); (LDL: OR=1.02, 95% CI=0.87, 1.19); (TG: OR=1.00, 95% CI=0.86, 1.17)]; Table 4.3. The results remain null even when the instruments are operationalized as quartiles, Table 4.4.

When assessing how effect modification of HPV status may influence the association between instruments and risk of HNSCC, it appears as though there was a positive association between the TG instrument and risk of HNSCC when comparing cases with HPV-positive disease to their controls (TG: OR=1.29, 95% CI=0.97, 1.71); Table 4.5, but this association was

not statistically significant. A similar positive association was observed when assessing potential effect modification by disease site, particularly when comparing cases whose disease was located in the oropharynx to their controls (TG: OR=1.24, 95% CI=0.98, 1.57); Table 4.5, but again this association is not statistically significant.

The only other marginally significant finding is among disease stage where there is an inverse association between the TG instrument and risk of HNSCC when comparing cases with stage 0 or 1 disease to controls(TG: OR=0.71, 95% CI=0.48, 1.03); Table 4.5. Overall the association between all of the instruments and HNSCC risk were relatively null and remain null even after assessing the potential for effect modification of HPV status, disease site, and stage of disease at diagnosis among cases.

HNSCC Outcomes

The rate of overall death and disease recurrence is higher among cases with higher values of the TC instrument [(overall death: HR=1.36, 95% CI=1.01, 1.83); (recurrence: HR=1.30, 95% CI=0.98, 1.73)] but only the association between the TC instrument and overall death was statistically significant at a 0.05 alpha level. We observed an inverse association between the rate of overall death, disease-specific death and the TG instrument that was statistically significant [(overall death: HR=0.75, 95% CI=0.57, 0.99); (disease-specific death: HR=0.71, 95% CI=0.51, 0.98)]. A similar association was observed between disease-specific death and the LDL instrument, but this association was marginally statistically significant (disease-specific death: HR=0.74, 95% CI=0.52, 1.04); Table 4.6. Similar observations were observed when the instruments were operationalized as quartiles, which can be found in Table 4.8. There did not appear to be an association between the HDL instrument and HNSCC outcomes. The findings for the association between the TCF instrument and HNSCC outcomes were similar to those observed for the TC instrument and HNSCC outcomes.

In order to assess if statin use among cases is impacting the effect of the instruments as a proxy for cholesterol measures on HNSCC outcomes, we conducted the same models excluding cases who had ever used a statin. Additionally, we conducted models with statin use as a covariate in the model. The results from this analysis can be found in Table 4.10. The results largely remain the same as the previous models, not considering the impact of statin use, portraying mainly null associations. The only slightly different findings than what previously observed is that the statistically significant inverse association between the TG instrument and rate of disease-specific death was attenuated after excluded cases who used statin drugs (disease-specific death: HR=0.88, 95% CI=0.56, 1.37); Table 4.10.

When assessing the potential modification of HPV status on HNSCC outcomes among cases, the results again remain relatively null, except for a statistically significant association between the TG instrument and rate of disease-specific death when comparing cases with HPV-negative disease to controls (disease-specific death: HR=0.62, 95% CI=0.38, 0.99); Table 4.7. Although this point estimate is statistically significant, these are similar findings to what is observed among the patients with HPV-positive tumors as well. The only difference is the point estimate for HPV-negative patients is more precise, which is to be expected given the larger sample size. When assessing this effect modification after excluding cases who had ever used a statin, or when including statin use the model, the findings, although slightly different than what was observed previously, still remain relatively null; Table 4.11. The magnitude of the point estimates are stronger than what was observed when including all cases, but the point estimates are less precise. This is largely due to the very small sample size of the models conducted when limiting the sample to cases with certain HPV status (approximately 40% of cases are missing HPV status) and excluding cases on a statin (approximately 40% of cases had ever used statins).

Discussion

The findings from this study that explore the association between instruments that predict hypercholesterolemia and HNSCC risk and outcomes are mainly null. These findings are supported by the literature. A recently published paper by Gormley et al. investigating the association between SNPs identified as having the same effect as statins and other cholesterol-lowering medications as well as other SNPs that would be a proxy for LDL cholesterol levels also found mainly null associations between cholesterol-lowering and risk of HNSCC.[28] This paper conducted a two-sample Mendelian randomization technique to assess the association between the identified SNPs and HNSCC risk and also analyzed the risk of developing oral cavity and oropharynx HNSCC separately [28] They performed these analyses in the Genetic Associations and Mechanisms in Oncology (GAME-ON) and UK Biobank databases and the combined the findings of both studies through meta-analysis.[28] Although this study did not use the same approach to assess this association as our study their sample size was very large, and their methods were strong. The results from the meta-analysis combining the findings from the GAME-ON and UK Biobank databases identified an association between PCSK9 and risk of developing HNSCC of the oral cavity and oropharynx and an inverse association was observed between the LDL proxy measures and risk of HNSCC combined at both sites.[28] When the oral cavity and oropharynx disease sites were investigated separately the inverse association was not consistent across the databases and was only observed for the association between LDL proxy SNPs and risk of oral cavity HNSCC in the GAME-ON database. When assessing this association for SNPs as proxies for other cholesterol measures (HDL, TG and TC) no associations were observed.[28] The authors suggest that the associations observed between PCSK9 and HNSCC risk may not be through the cholesterol-lowering mechanism.[28]

Although the literature is limited when exploring the relationship between cholesterol and HNSCC, this association has been investigated across various different cancer sites with largely

null findings. There have been studies that have investigated the association between cholesterol and risk of cancer in general, prostate cancer, and colorectal cancer using Mendelian randomization, but to our knowledge, this will be the first study to examine the association using Mendelian randomization in HNSCC outcomes.[26,29,30] Previous studies that investigated the cholesterol-cancer relationship using this approach have found similar results. One study that investigated the relationship between low LDL and risk of cancer found that there was not a causal relationship between having genetically related low LDL and an increased risk of developing cancer.[30] Two papers looking at the association between cholesterol and colorectal cancer and prostate cancer, respectively, found increased triglycerides to be modestly associated with an increased risk of developing both prostate and colorectal cancer.[26,29] They both also determined that having a gene that influences Hydroxy-methylglutaryl-CoA (HMG-CoA) in a similar way to statins was protective against developing prostate cancer and statistically significantly protective against developing colorectal cancer.[26,29]

Other studies looking at the association between Apolipoprotein e (APOE) gene (a gene involved in the lipid-protein development that is associated with heart disease and Alzheimer's disease)[31] and risk of developing cancer and cancer-related mortality found mixed results. Different variants of APOE were utilized as proxy measures of LDL and HDL. One manuscript found no association between the APOE gene and risk of developing cancer and cancer-related mortality when using a Mendelian randomization approach.[32] Whereas another study found differences in cancer risk among the different genotypes of the APOE gene among an Asian population, where they observed an increased risk of cancer among those with a variant that was associated with lower levels of circulating HDL.[33] Additionally, a study of endometrial cancer patients identified an association between LDL and risk of developing endometrial cancer.[34]

Strengths and Limitations

Although this aim has quite a few strengths, including being the first to our knowledge to look at the relationship between genetic predisposition to high cholesterol levels and rate of overall death, disease-specific death and recurrence among HNSCC patients as well as exploring the potential effect modification of HPV-status on the association using this analytic technique, there are a few limitations.

The sample size of this study is quite small. It is possible that we do not have enough power to detect a statistically significant association between the instruments and HNSCC risk, death, or observe interaction. Also, larger sample size will increase power and precision but not necessarily change the magnitude of the point estimates observed. Since some of the point estimates observed borderline statistically significant associations between TC and HNSCC outcomes, a larger sample size may improve precision and provide statistical significance. But since the point estimates identified for the association between the instruments and HNSCC risk were quite precise, increasing sample size and power may not change the null study results.

It is possible that one of the assumptions necessary to complete Mendelian randomization analysis correctly may be violated, specifically the “exclusion restriction” assumption.[35] It is possible that having a predisposition to hypercholesterolemia would lead to the use of statin drugs. Statin drugs are known as being extremely effective at lowering cholesterol. They have also been identified as having an inverse association with outcomes related to HNSCC.[5,6,8] This violates the assumption that the instrument can only be associated with the outcome through the exposure. The DAG in Figure 4.4 displays this relationship. Since we are only trying to identify if an association between cholesterol and HNSCC risk/outcomes exists, it is possible this may not be a problem, but even keeping this in mind, the issue may not be completely addressed. It is reassuring the violation did not bias the associations given the findings are largely null.[35] Also, we investigated the association between the cholesterol-related

instruments and HNSCC outcomes, excluding cases who ever used a statin, and the results did not meaningfully change.

Given the case-control design of this study and the fact that we are using hospital-based controls, there may be an issue of selection bias (Berkson's bias) when assessing the association between cholesterol-related instruments and HNSCC risk. This bias may arise because our control selection may not be independent of the exposure variable. In this situation, our controls are not representative of the population from which the cases arose. Hospital-based controls may be more likely to have high cholesterol or have higher levels of the cholesterol-related instruments for TC, LDL, and TG. This is possible because, although we are not purposefully including participants who have diseases that are associated with high cholesterol (for example, cardiovascular disease), they are no excluded. Inclusion of too many controls with diseases that are associated with high cholesterol would create an over-representation of controls with higher levels of the exposure. If this occurs, we may observe bias toward the null or possibly a reverse association in which cholesterol is protective against HNSCC. This may explain the null association observed in our study if high cholesterol is truly a risk factor for developing HNSCC, but the distribution of the instruments from both cases and controls appears to be relatively normal (Figure 4.3).

Another possible issue with control selection is that we are not excluding controls who had a history of cancer other than HNSCC. It is possible that this will also bias our results toward the null if high cholesterol is associated with cancer at sites other than HNSCC, and there are a large proportion of controls who have a history of other cancers. This issue should be addressed in future research studies. It can be addressed through the collection of comorbidity information from controls. Controls with a medical history of cancer should be excluded since we have 3 controls for each case, we can make the ratio of controls to cases smaller. This will allow us to observe if including controls with a history of cancer biases our estimates.

We assume that the use of statin drugs may modify the association between the cholesterol-related instruments and developing HNSCC. Although we have collected data on statin use among the cases, we have not collected this data among the controls, which may lead to the analysis assessing HNSCC risk to be biased and failing to address all of the assumptions necessary for Mendelian randomization analysis. It is important for future research to collect medication information from controls to deal with this potential issue.

Conclusion

The findings from this study support a null association between circulating cholesterol levels and risk of HNSCC. Although there did appear to be an association between TC and TG with HNSCC outcomes, given the small study sample, the findings are not precise. HPV status does not appear to modify the association between cholesterol levels and HNSCC outcomes. Future research investigating the association between circulating cholesterol levels and HNSCC outcomes in a larger, more diverse population of HNSCC patients is necessary to validate the presented study results and provide more power to detect statistically significant associations between TC and TG with HNSCC outcomes.

Acknowledgments

This research was funded through the National Cancer Institute of the National Institutes of Health Award number R01CA129102. Research reported in this publication was also supported by the National Cancer Institute of the National Institutes of Health under Award Number P30CA046592 by the use of the following Cancer Center Shared Resource(s): Cancer Data Science.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Ethics Statement: Patients recruited through the HNC SPORE and the MGI provided written informed consent, and both studies were approved through the University of Michigan Medical School's Institutional Review Board.

References

1. Takemoto M, Liao JK. Pleiotropic effects of 3-hydroxy-2-methylglutaryl coenzyme a reductase inhibitors. *Arter Thromb Vas Biol*. 2001;21(11):1712-1719. doi:10.1161/hq1101.098486
2. Grundy SM. Cholesterol and coronary heart disease: a new era. *JAMA*. 1986;256(20):2849-2858. doi:10.1001/jama.1986.03380200087027
3. Mei Z, Liang M, Li L, Zhang Y, Wang Q, Yang W. Effects of statins on cancer mortality and progression: a systematic review and meta-analysis of 95 cohorts including 1,111,407 individuals. *Int J Cancer*. 2017;140(5):1068-1081. doi:10.1002/ijc.30526
4. Zhong S, Zhang X, Chen L, Ma T, Tang J, Zhao J. Statin use and mortality in cancer patients: systematic review and meta-analysis of observational studies. *Cancer Treat Rev*. 2015;41(6):554-567. doi:10.1016/j.ctrv.2015.04.005
5. Gupta A, Stokes W, Eguchi M, et al. Statin use associated with improved overall and cancer specific survival in patients with head and neck cancer. *Oral Oncol*. 2019;90:54-66. doi:10.1016/j.oraloncology.2019.01.019
6. Lebo NL, Griffiths R, Hall S, Dimitroulakos J, Johnson-Obaseki S. Effect of statin use on oncologic outcomes in head and neck squamous cell carcinoma. *Head Neck*. 2018;40(8):1697-1706. doi:10.1002/hed.25152
7. Kao L-T, Hung S-H, Kao P-F, Liu J-C, Lin H-C. Inverse association between statin use and head and neck cancer: population-based case-control study in Han population. *Head Neck*. 2019;41(5):1193-1198. doi:10.1002/hed.25501
8. Getz KR, Bellile E, Zarins KR, et al. Statin use and head and neck squamous cell carcinoma outcomes. *Int J Cancer*. 2021;148(10):2440-2448. doi:10.1002/ijc.33441
9. Kuzu OF, Noory MA, Robertson GP. The role of cholesterol in cancer. *Cancer Res*. 2016;76(8):2063-2070. doi:10.1158/0008-5472.CAN-15-2613
10. Brown AJ. Cholesterol, statins and cancer. *Clin Exp Pharmacol Physiol*. 2007;34(3):135-141. doi:10.1111/j.1440-1681.2007.04565.x
11. Altomare DA, Testa JR. Perturbations of the AKT signaling pathway in human cancer. *Oncogene*. 2005;24(50):7455-7464. doi:10.1038/sj.onc.1209085
12. Jacobs EJ, Gapstur SM. Cholesterol and cancer: answers and new questions. *Cancer Epidemiol Biomarkers Prev*. 2009;18(11):2805-2806. doi:10.1158/1055-9965.EPI-09-1027
13. Ahn J, Lim U, Weinstein SJ, et al. Prediagnostic total and high-density lipoprotein cholesterol and risk of cancer. *Cancer Epidemiol Biomarkers Prev*. 2009;18(11):2814-2821. doi:10.1158/1055-9965.EPI-08-1248
14. Chow LQM. Head and neck cancer. *N Engl J Med*. 2020;382(1):60-72. doi:10.1056/NEJMra1715715
15. Rettig EM, D'Souza G. Epidemiology of head and neck cancer. *Surg Oncol Clin N Am*. 2015;24(3):379-396. doi:10.1016/j.soc.2015.03.001
16. Sturgis EM, Cinciripini PM. Trends in head and neck cancer incidence in relation to smoking prevalence: an emerging epidemic of human papillomavirus-associated cancers? *Cancer*. 2007;110(7):1429-1435. doi:10.1002/cncr.22963

17. Marur S, D'Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. *Lancet Oncol*. 2010;11(8):781-789. doi:10.1016/S1470-2045(10)70017-6

18. Simic I, Reiner Z. Adverse effects of statins - myths and reality. *Curr Pharamceutical Des*. 2015;21(9):1220-1226. doi:10.2174/1381612820666141013134447

19. Kiortsis DN, Filippatos TD, Mikhailidis DP, Elisaf MS, Liberopoulos EN. Statin-associated adverse effects beyond muscle and liver toxicity. *Atherosclerosis*. 2007;195(1):7-16. doi:10.1016/j.atherosclerosis.2006.10.001

20. Peterson LA, Bellile EL, Wolf GT, et al. Cigarette use, comorbidities and prognosis in a prospective head and neck squamous cell carcinoma population. *Head Neck*. 2016;38(12):1810-1820. doi:10.1002/hed.24515

21. Duffy SA, Taylor JMG, Terrell JE, et al. Interleukin-6 predicts recurrence and survival among head and neck cancer patients. *Cancer*. 2008;113(4):750-757. doi:10.1002/cncr.23615

22. Fritsche LG, Gruber SB, Wu Z, et al. Association of polygenic risk scores for multiple cancers in a phenome-wide study: results from the michigan genomics initiative. *Am J Hum Genet*. 2018;102(6):1048-1061. doi:10.1016/j.ajhg.2018.04.001

23. Willer CJ, Schmidt EM, Sengupta S, et al. Discovery and refinement of loci associated with lipid levels. *Nat Genet*. 2013;45(11):1274-1283. doi:10.1038/ng.2797.

24. Shaun Purcell CC. PLINK 2.0. www.cog-genomics.org/plink/2.0/. Published 2021.

25. Chang CC, Chow CC, Tellier LCAM, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience*. 2015;4(1):s13742-015. doi:10.1186/s13742-015-0047-8

26. Bull CJ, Bonilla C, Holly JMP, et al. Blood lipids and prostate cancer: a mendelian randomization analysis. *Cancer Med*. 2016;5(6):1125-1136. doi:10.1002/cam4.695

27. Vanderweele TJ. *Explanation in Causal Inference Methods for Mediation and Interaction*. New York: Oxford University Press; 2015.

28. Gormley M, Yarmolinsky J, Dudding T, et al. Using genetic variants to evaluate the causal effect of cholesterol lowering on head and neck cancer risk: a mendelian randomization study. *PLOS Genet*. 2021;17(4):e1009525. doi:10.1101/2020.10.05.20206268

29. Rodriguez-Broadbent H, Law PJ, Sud A, et al. Mendelian randomisation implicates hyperlipidaemia as a risk factor for colorectal cancer. *Int J Cancer*. 2017;140(12):2701-2708. doi:10.1002/ijc.30709

30. Benn M, Tybjærg-Hansen A, Stender S, Frikke-Schmidt R, Nordestgaard BG. Low-density lipoprotein cholesterol and the risk of cancer: a mendelian randomization study. *J Natl Cancer Inst*. 2011;103(6):508-519. doi:10.1093/jnci/djr008

31. Rasmussen KL. Plasma levels of apolipoprotein e, apoe genotype and risk of dementia and ischemic heart disease: a review. *Atherosclerosis*. 2016;255:145-155. doi:10.1016/j.atherosclerosis.2016.10.037

32. Trompet S, Jukema JW, Katan MB, et al. Apolipoprotein e genotype, plasma cholesterol, and cancer: A mendelian randomization study. *Am J Epidemiol*. 2009;170(11):1415-1421. doi:10.1093/aje/kwp294

33. Yang C, Tian G, Mi J, et al. Causal relevance of circulating high-density lipoprotein cholesterol with cancer: A Mendelian randomization meta-analysis. *Sci Rep*. 2015;5:1-7. doi:10.1038/srep09495

34. Kho P, Amant F, Annibali D, et al. Mendelian randomization analyses suggest a role for cholesterol in the development of endometrial cancer. *Int J Cancer*. 2021;148(2):307-319. doi:10.1002/ijc.33206

35. Vanderweele TJ, Tchetgen Tchetgen EJ, Cornelis M, Kraft P. Methodological challenges in mendelian randomization. *Epidemiology*. 2014;25(3):427-435. doi:10.1097/EDE.0000000000000081.

Chapter 4: Figures/Tables

Figure 4.1: DAG Displaying Association between Lipid SNPs and HNSCC Risk/Outcomes

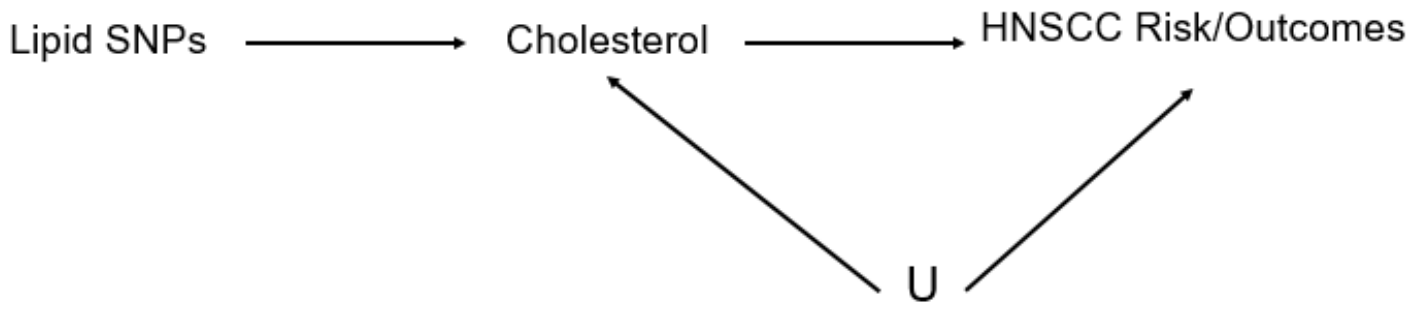


Figure 4.2: Distribution of Instruments (1-standard deviation increase)

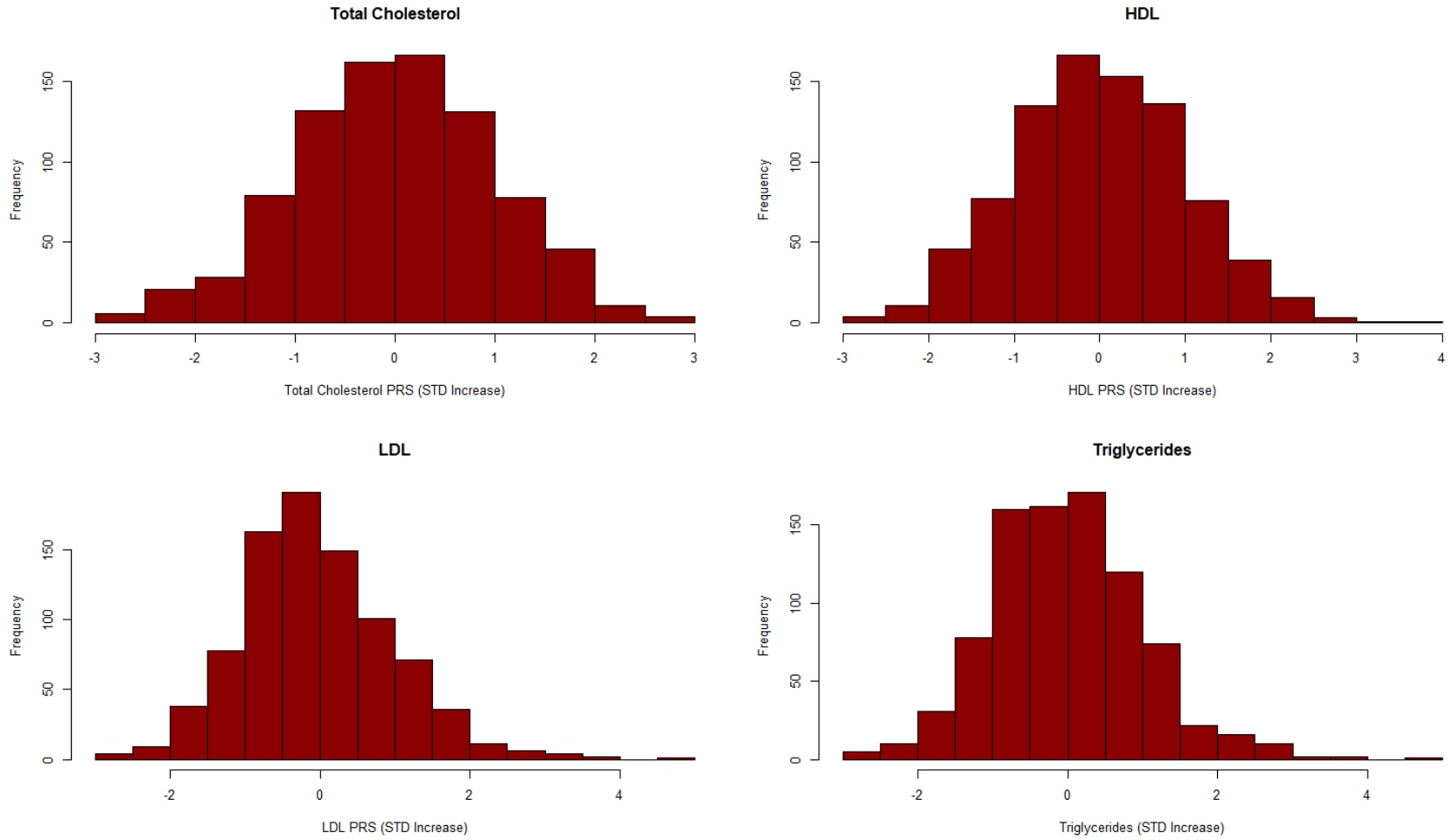


Figure 4.3: Overlaying Histograms of Instruments (1-standard deviation increase) by Case-Control Status

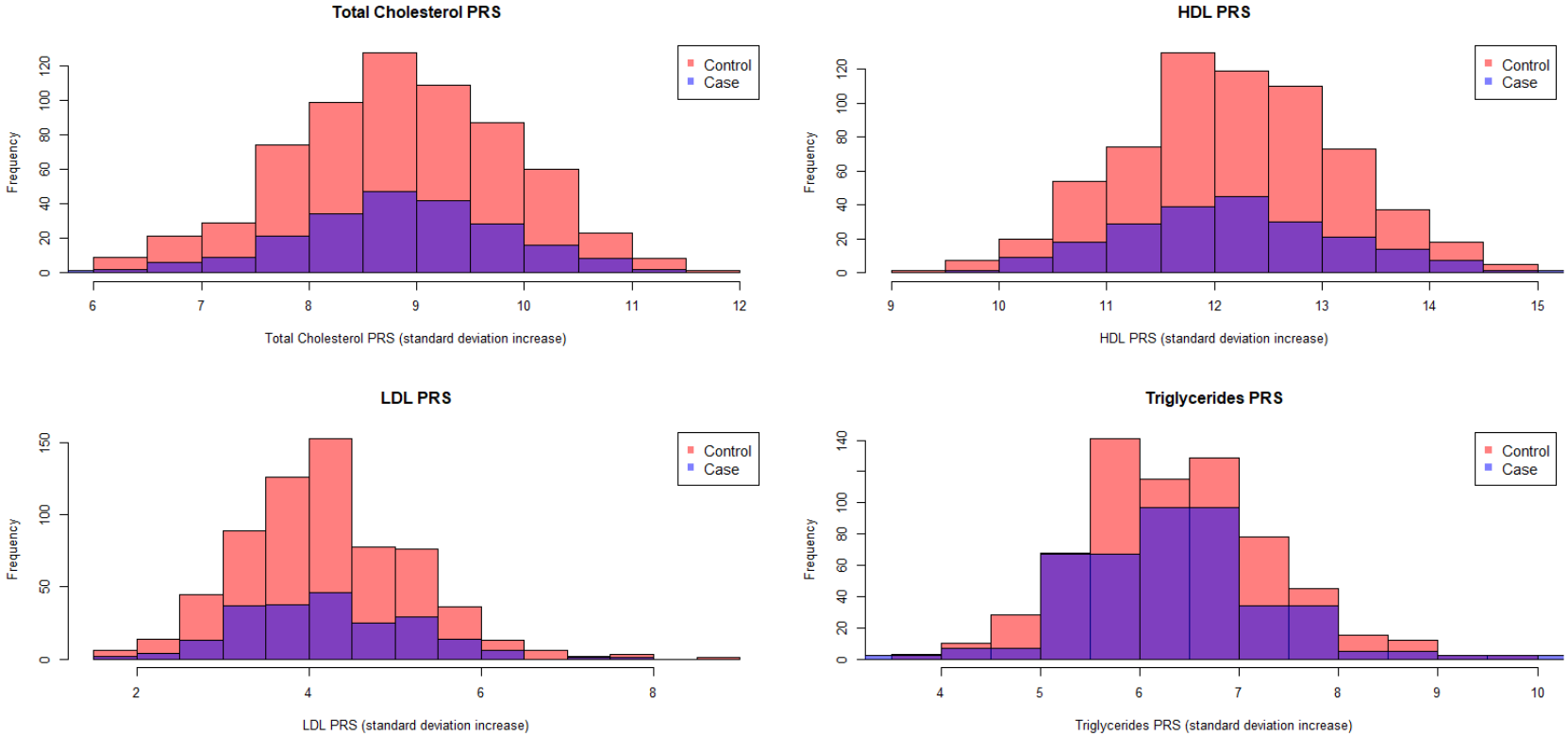


Figure 4.4: DAG Displaying Association between Lipid SNPs and HNSCC Risk/Outcomes

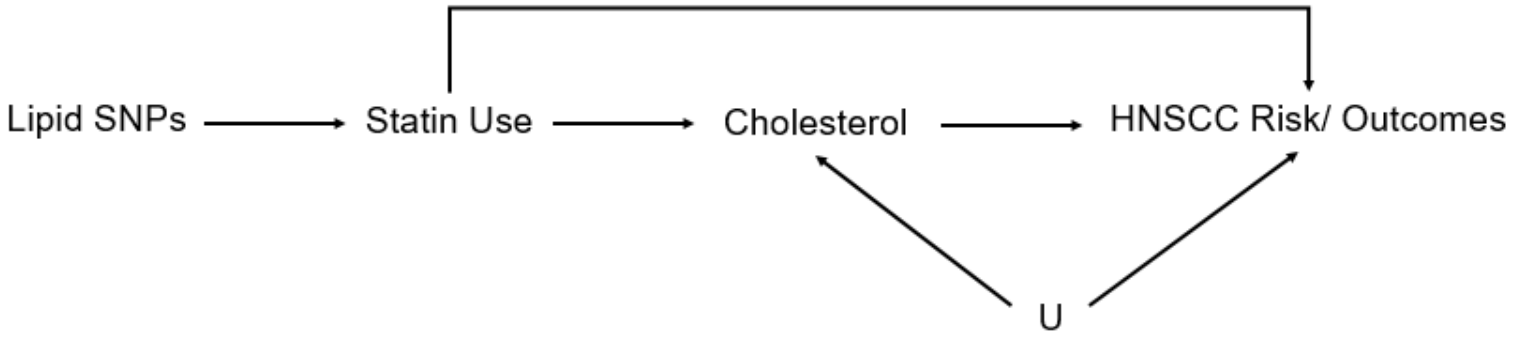


Table 4.1: SNPs Included in Cholesterol-Related Instruments

Total Cholesterol		HDL	
GRCh37 (hg19)	RSID	GRCh37 (hg19)	RSID
chr1:93009438	rs7515577	chr1:156700651	rs12145743
chr2:135837906	rs7570971	chr1:178515312	rs4650994
chr3:12628920	rs2290159	chr2:211540507	rs1047891
chr6:34546560	rs2814982	chr3:11400249	rs2606736
chr11:18632984	rs10128711	chr3:47061183	rs2290547
chr19:49206417	rs492602	chr3:50129399	rs2013208
chr20:34152782	rs2277862	chr3:52532118	rs13326165
chr1:23766233	rs1077514	chr3:119560606	rs6805251
chr2:169830155	rs2287623	chr4:26062990	rs10019888
chr2:203532304	rs11694172	chr4:89741269	rs3822072
chr3:58381287	rs13315871	chr4:100014805	rs2602836
chr6:39250837	rs2758886	chr7:6449272	rs702485
chr6:135411228	rs9376090	chr7:17919258	rs4142995
chr7:1083777	rs1997243	chr7:50305863	rs4917014
chr10:17260290	rs10904908	chr7:150529449	rs17173637
chr11:118486067	rs11603023	chr11:51512090	rs11246602
chr12:9082581	rs4883201	chr11:65391317	rs12801636
chr22:35711098	rs138777	chr11:75455021	rs499974
		chr14:105277209	rs4983559
		chr19:52324216	rs17695224
		chr1:40028180	rs4660293
		chr1:182168885	rs1689800
		chr2:165540800	rs12328675
		chr4:103188709	rs13107325
		chr5:53298025	rs6450176
		chr6:139829666	rs605066
		chr7:130433384	rs4731702
		chr8:116599199	rs2293889
		chr11:10388782	rs2923084
		chr11:46743247	rs3136441
		chr12:20473758	rs7134375
		chr12:110000193	rs7134594
		chr12:123796238	rs4759375
		chr12:125261593	rs838880
		chr15:63396867	rs2652834
		chr16:67928042	rs16942887
		chr16:81534790	rs2925979
		chr17:37813856	rs11869286
		chr17:66875294	rs4148008
		chr17:76403984	rs4129767
		chr18:57849023	rs12967135
		chr19:8433196	rs7255436
		chr19:11347493	rs737337
		chr19:54792761	rs386000
		chr22:21932068	rs181362
LDL		**Triglycerides**	
GRCh37 (hg19)	RSID	GRCh37 (hg19)	RSID
chr14:24883887	rs8017377	chr3:135926622	rs645040
chr1:150958836	rs267733	chr4:88030261	rs442177
chr2:63149557	rs2710642	chr5:55861786	rs9686661
chr2:216304384	rs1250229	chr7:72129667	rs13238203
chr13:32953388	rs4942486	chr8:10683929	rs11776767
chr17:64210580	rs1801689	chr10:65027610	rs10761731
chr20:12962718	rs364585	chr10:94839642	rs2068888
chr20:17845921	rs2328223	chr15:42683787	rs2412710
chr22:30378703	rs5763662	chr15:44245931	rs2929282
		chr16:30918487	rs11649653
		chr22:38546033	rs5756931
		chr7:116358044	rs38855
		chr10:5254847	rs1832007
		chr16:15129940	rs3198697
		chr17:41878166	rs8077889
		chr19:7224431	rs7248104

Table 4.2: Demographic and Clinical Characteristics by Case/Control Status

	Cases (N=216)	Controls (N=648)
Age at Diagnosis, years	60.81	NA
Sex (Male)	73.15%	73.15%
Death Status		
Alive	74.07%	95.22%
Deceased	25.93%	4.78%
Disease Site		
Larynx	16.20%	NA
Oral Cavity	45.37%	NA
Oropharynx	37.04%	NA
Hypopharynx	1.39%	NA
Stage at Diagnosis		
0	1.85%	NA
1	16.67%	NA
2	17.13%	NA
3	9.72%	NA
4	54.63%	NA
HPV status		
Negative	34.26%	NA
Positive	27.31%	NA
Invalid/Missing	38.43%	NA
BMI*	28.99	30.15[a]
Smoking Status		
Current	32.41%	13.43%
Former	37.50%	38.89%
Never	25.93%	47.22%
Missing	4.17%	0.46%
Drinking Status		
Current	59.72%	60.80%
Former	21.76%	NA
Never	14.35%	34.10%
Missing	4.17%	5.09%

*Cases, N=209; Controls, N=437

a. Mean of average BMI across multiple medical encounters

Table 4.3: Risk of HNSCC and Standard Deviation Increase of Cholesterol-Related Instruments

Instruments* (N=864)	OR (95% CI)
TC	0.99 (0.85. 1.15)
HDL	1.01 (0.87, 1.18)
LDL	1.02 (0.87, 1.19)
TG	1.00 (0.86, 1.17)
TCF[a]	0.996 (0.85, 1.16)

*****Standard deviation increase

a. Total cholesterol instrument containing all statistically significant SNPs

Table 4.4: Risk of HNSCC and Quartile Increase of Cholesterol-Related Instruments

Instruments	Case	Control	OR (95% CI)
TC			
Quartile 1	53	163	REF
Quartile 2	58	158	1.13 (0.73, 1.74)
Quartile 3	52	164	0.98 (0.63, 1.51)
Quartile 4	53	163	1.00 (0.65, 1.55)
HDL			
Quartile 1	59	157	REF
Quartile 2	50	166	0.80 (0.52, 1.24)
Quartile 3	53	163	0.87 (0.56, 1.33)
Quartile 4	54	162	0.89 (0.58, 1.36)
LDL			
Quartile 1	59	157	REF
Quartile 2	47	169	0.74 (0.48, 1.15)
Quartile 3	55	161	0.91 (0.59, 1.40)
Quartile 4	55	161	0.91 (0.59, 1.40)
TG			
Quartile 1	51	165	REF
Quartile 2	58	158	1.19 (0.77, 1.84)
Quartile 3	61	155	1.27 (0.83, 1.96)
Quartile 4	46	170	0.88 (0.56, 1.38)
TCF[a]			
Quartile 1	54	162	REF
Quartile 2	53	163	0.98 (0.63, 1.51)
Quartile 3	54	162	1.0 (0.65, 1.55)
Quartile 4	55	161	1.03 (0.66, 1.58)

	Case	Control	OR (95% CI)
HDL			
Quartile 1	59	157	REF
Quartile 2-4	157	491	0.85 (0.60, 1.21)

a. Total cholesterol instrument containing all statistically significant SNPs

Table 4.5: Risk of HNSCC by Standard Deviation Increase in Cholesterol-Related Instruments Stratified by HPV Status, Disease Site and Stage of Disease

Instruments*	**OR (95% CI)**	
HPV Status		
HPV-Positive (N=236)	*Case (N=59)*	*Control (N=177)*
TC	0.94 (0.70, 1.28)	
HDL	1.03 (0.75, 1.42)	
LDL	1.14 (0.88, 1.49)	
TG	1.29 (0.97, 1.71)	
TCF[a]	1.14 (0.86, 1.52)	
HPV-Negative (N=296)	*Case (N=74)*	*Control (N=222)*
TC	1.03 (0.79, 1.34)	
HDL	1.09 (0.83, 1.42)	
LDL	1.01 (0.76, 1.33)	
TG	1.00 (0.77, 1.30)	
TCF[a]	0.93 (0.70, 1.22)	
Disease Site		
Larynx (N=140)	*Case (N=35)*	*Control (N=140)*
TC	0.93 (0.64, 1.37)	
HDL	1.06 (0.73, 1.53)	
LDL	0.78 (0.51, 1.20)	
TG	0.91 (0.63, 1.31)	
TCF[a]	0.88 (0.60, 1.29)	
Oral Cavity (N=392)	*Case (N=98)*	*Control (N=294)*
TC	1.07 (0.85, 1.35)	
HDL	0.93 (0.74, 1.17)	
LDL	1.07 (0.84, 1.36)	
TG	0.86 (0.67, 1.10)	
TCF[a]	0.89 (0.70, 1.13)	
Oropharynx (N=320)	*Case (N=80)*	*Control (N=240)*
TC	0.93 (0.72, 1.20)	
HDL	1.10 (0.85, 1.44)	
LDL	1.07 (0.85, 1.36)	
TG	1.24 (0.98, 1.57)	
TCF[a]	1.10 (0.86, 1.40)	
Disease Stage		
0 and 1 (N=160)	*Case (N=40)*	*Control (N=120)*
TC	0.97 (0.67, 1.38)	
HDL	1.02 (0.70, 1.48)	
LDL	0.89 (0.63, 1.27)	
TG	0.71 (0.48, 1.03)	
TCF[a]	0.91 (0.64, 1.31)	
2 (N=148)	*Case (N=37)*	*Control (N=111)*
TC	0.98 (0.67, 1.42)	
HDL	1.04 (0.71, 1.52)	
LDL	0.99 (0.65, 1.51)	
TG	1.14 (0.78, 1.65)	
TCF[a]	0.95 (0.66, 1.36)	
3 (N=84)	*Case (N=21)*	*Control (N=63)*
TC	1.10 (0.64, 1.90)	
HDL	0.89 (0.53, 1.50)	
LDL	1.06 (0.66, 1.70)	
TG	1.03 (0.64, 1.66)	

TCF[a]	1.55 (0.94, 2.54)	
4 (N=472)	*Case (N=118)*	*Control (N=354)*
TC	0.98 (0.80, 1.21)	
HDL	1.02 (0.84, 1.26)	
LDL	1.06 (0.87, 1.31)	
TG	1.07 (0.87, 1.31)	
TCF[a]	0.96 (0.77, 1.19)	

*Standard deviation increase

a. Total cholesterol instrument containing all statistically significant SNPs

Table 4.6: Rate of HNSCC Outcomes by Standard Deviation Increase in Cholesterol-Related Instruments (Cases Only)

Instruments* (N=216)	HR (95% CI)
Overall Death (# Events=56)	
TC	1.36 (1.01, 1.83)
HDL	0.92 (0.71, 1.19)
LDL	0.93 (0.71, 1.22)
TG	0.75 (0.57, 0.99)
TCF[a]	1.13 (0.89, 1.44)
Disease-Specific Death (# Events=39)	
TC	1.22 (0.86, 1.73)
HDL	0.97 (0.72, 1.32)
LDL	0.74 (0.52, 1.04)
TG	0.71 (0.51, 0.98)
TCF[a]	1.23 (0.92, 1.64)
Recurrence (# Events=56)	
TC	1.30 (0.98, 1.73)
HDL	0.91 (0.81, 1.18)
LDL	0.83 (0.63, 1.10)
TG	0.83 (0.63, 1.09)
TCF[a]	1.09 (0.85, 1.39)

*Standard deviation increase

a. Total cholesterol instrument containing all statistically significant SNPs

Table 4.7: Rate of HNSCC Outcomes by Standard Deviation Increase in Cholesterol-Related Instruments by HPV Status (Cases Only)

Instruments*	HR (95% CI)
HPV-Positive (N=59)	
Overall Death (# Events=9)	
TC	0.98 (0.49, 1.97)
HDL	0.70 (0.31, 1.57)
LDL	0.80 (0.38, 1.67)
TG	0.77 (0.41, 1.45)
TCF[a]	1.03 (0.57, 1.85)
Disease-Specific Death (# Events=7)	
TC	1.06 (0.50, 2.27)
HDL	0.66 (0.28, 1.58)
LDL	0.58 (0.23, 1.44)
TG	0.82 (0.42, 1.60)
TCF[a]	1.07 (0.57, 2.00)
Recurrence (# Events=11)	
TC	1.26 (0.66, 2.37)
HDL	0.64 (0.33, 1.26)
LDL	0.64 (0.32, 1.29)
TG	0.82 (0.46, 1.46)
TCF[a]	1.20 (0.72, 2.02)
HPV-Negative (N=74)	
Overall Death (# Events=27)	
TC	1.61 (1.04, 2.52)
HDL	0.98 (0.72, 1.33)
LDL	0.83 (0.55, 1.26)
TG	0.76 (0.51, 1.13)
TCF[a]	1.49 (0.98, 2.26)
Disease-Specific Death (# Events=19)	
TC	1.40 (0.83, 2.36)
HDL	1.05 (0.72, 1.53)
LDL	0.65 (0.40, 1.07)
TG	0.62 (0.38, 0.99)
TCF[a]	1.56 (0.96, 2.54)
Recurrence (# Events=22)	
TC	1.01 (0.95, 2.40)
HDL	1.06 (0.75, 1.50)
LDL	0.65 (0.39, 1.07)
TG	0.70 (0.45, 1.09)
TCF[a]	1.29 (0.82, 2.02)

*****Standard deviation increase

a. Total cholesterol instrument containing all statistically significant SNPs

Table 4.8: Rate of HNSCC Outcomes by Cholesterol-Related Instrument Quartiles (Cases Only)

	# of Events	Person months	HR (95% CI)
Overall Death			
TC			
Quartile 1	11	2055.20	REF
Quartile 2	15	2038.97	1.38 (0.62, 3.07)
Quartile 3	12	2017.91	1.14 (0.49, 2.64)
Quartile 4	18	1911.82	1.89 (0.87, 4.09)
HDL			
Quartile 1	17	1955.06	REF
Quartile 2	10	2090.91	0.58 (0.26, 1.28)
Quartile 3	17	1895.33	1.03 (0.52, 2.02)
Quartile 4	12	1408.66	0.74 (0.35, 1.54)
LDL			
Quartile 1	15	2129.31	REF
Quartile 2	13	1923.68	1.06 (0.51, 2.24)
Quartile 3	14	2028.22	0.96 (0.46, 1.98)
Quartile 4	14	1942.67	1.09 (0.52, 2.26)
TG			
Quartile 1	17	1573.29	REF
Quartile 2	18	2359.92	0.82 (0.42, 1.60)
Quartile 3	15	2363.17	0.67 (0.33, 1.34)
Quartile 4	6	1175.46	0.36 (0.14, 0.91)
TCF[a]			
Quartile 1	9	2442.35	REF
Quartile 2	19	1723.66	2.98 (1.33, 6.66)
Quartile 3	13	2014.69	1.69 (0.72, 3.96)
Quartile 4	15	1843.19	2.14 (0.93, 4.94)
Disease-Specific Death			
TC			
Quartile 1	8	2055.20	REF
Quartile 2	10	2038.97	1.10 (0.44, 2.79)
Quartile 3	10	2017.91	1.17 (0.46, 2.97)
Quartile 4	11	1911.82	1.42 (0.57, 3.54)
HDL			
Quartile 1	12	1955.06	REF
Quartile 2	6	2090.91	0.55 (0.21, 1.47)
Quartile 3	12	1895.33	1.08 (0.48, 2.40)
Quartile 4	9	1408.66	0.81 (0.34, 1.93)
LDL			
Quartile 1	13	2129.31	REF
Quartile 2	10	1923.68	0.99 (0.43, 2.25)
Quartile 3	9	2028.22	0.73 (0.31, 1.70)
Quartile 4	7	1942.67	0.64 (0.25, 1.60)
TG			
Quartile 1	14	1573.29	REF
Quartile 2	10	2359.92	0.61 (0.27, 1.37)
Quartile 3	11	2363.17	0.64 (0.29, 1.41)
Quartile 4	4	1175.46	0.29 (0.10, 0.89)
TCF[a]			
Quartile 1	5	2442.35	REF
Quartile 2	14	1723.66	3.58 (1.29, 9.93)

Quartile 3	9	2014.69	1.97 (0.66, 5.89)
Quartile 4	11	1843.19	2.56 (0.89, 7.38)
Recurrence			
TC			
Quartile 1	10	1514.41	REF
Quartile 2	14	1593.82	1.23 (0.54, 2.76)
Quartile 3	17	1337.99	1.79 (0.82, 3.90)
Quartile 4	15	1275.30	1.73 (0.78, 3.86)
HDL			
Quartile 1	18	1472.72	REF
Quartile 2	12	1434.12	0.76 (0.37, 1.58)
Quartile 3	15	1406.03	0.92 (0.46, 1.82)
Quartile 4	11	1408.66	0.67 (0.32, 1.42)
LDL			
Quartile 1	13	1612.45	REF
Quartile 2	18	1284.50	1.75 (0.86, 3.57)
Quartile 3	14	1515.14	1.12 (0.53, 2.38)
Quartile 4	11	1309.44	0.97 (0.43, 2.16)
TG			
Quartile 1	16	1098.87	REF
Quartile 2	17	1667.19	0.81 (0.41, 1.60)
Quartile 3	15	1780.01	0.69 (0.34, 1.40)
Quartile 4	8	1175.46	0.51 (0.22, 1.20)
TCF[a]			
Quartile 1	12	1778.69	REF
Quartile 2	17	1131.76	1.89 (0.90, 3.98)
Quartile 3	12	1539.42	1.07 (0.48, 2.39)
Quartile 4	15	1271.66	1.53 (0.71, 3.27)

a. Total cholesterol instrument containing all statistically significant SNPs

Table 4.9: Demographic and Clinical Characteristics of Cases, Excluding Participants on a Statin

	Cases (N=129)
Age at Diagnosis, years	58.02
Sex (Male)	74.42%
Death Status	
Alive	75.97%
Deceased	24.03%
Disease Site	
Larynx	19.38%
Oral Cavity	41.09%
Oropharynx	38.76%
Hypopharynx	0.78%
Stage at Diagnosis	
0	1.55%
1	13.95%
2	20.16%
3	6.98%
4	57.36%
HPV status	
Negative	34.11%
Positive	26.36%
Invalid/Missing	39.53%
BMI (N=126)	28.49
Smoking Status	
Current	36.43%
Former	32.56%
Never	28.68%
Missing	2.33%
Drinking Status	
Current	65.89%
Former	18.60%
Never	12.40%
Missing	3.10%

Table 4.10: Rate of HNSCC Outcomes by Standard Deviation Increase in Cholesterol-Related Instruments (Cases only), Excluding Participants on a Statin

Instruments*	HR (95% CI)*,[a]	HR (95% CI)*,[b]
Overall Death	**(# Events=31)**	**(# Events=56)**
TC	1.42 (0.96, 2.09)	1.35 (1.00, 1.82)
HDL	0.92 (0.63, 1.34)	0.92 (0.71, 1.18)
LDL	1.08 (0.78, 1.49)	0.93 (0.71, 1.21)
TG	0.87 (0.60, 1.25)	0.75 (0.57, 0.99)
TCF[c]	1.15 (0.82, 1.60)	1.12 (0.88, 1.43)
Disease-Specific Death	**(# Events=20)**	**(# Events=39)**
TC	1.24 (0.77, 1.98)	1.20 (0.85, 1.71)
HDL	1.09 (0.69, 1.74)	0.97 (0.72, 1.30)
LDL	0.85 (0.55, 1.32)	0.73 (0.51, 1.03)
TG	0.88 (0.56, 1.37)	0.71 (0.52, 0.98)
TCF[c]	1.28 (0.85, 1.95)	1.20 (0.63, 2.29)
Recurrence	**(# Events=32)**	**(# Events=56)**
TC	1.37 (0.95, 1.99)	1.30 (0.97, 1.73)
HDL	0.90 (0.62, 1.29)	0.91 (0.71, 1.17)
LDL	0.95 (0.69, 1.32)	0.83 (0.63, 1.10)
TG	0.96 (0.67, 1.36)	0.83 (0.63, 1.09)
TCF[c]	1.11 (0.79, 1.56)	1.09 (0.85, 1.40)

*Standard deviation increase

a. Excluding participants on a statin, (N=129)

b. Adjusting for statin use, (N=215)

c. Total cholesterol instrument containing all statistically significant SNPs

Table 4.11: Rate of HNSCC Outcomes by Standard Deviation Increase in Cholesterol-Related Instruments by HPV Status (Cases Only), Excluding Participants on a Statin

Instruments*	HR (95% CI)*,[a]	HR (95% CI)*,[b]
HPV-Positive	**N=34**	**N=58**
Overall Death	*# Events=6*	*# Events=9*
TC	1.01 (0.47, 2.18)	1.03 (0.52, 2.05)
HDL	1.58 (0.61, 4.06)	0.69 (0.30, 1.59)
LDL	0.96 (0.44, 2.09)	0.78 (0.38, 1.60)
TG	0.30 (0.08, 1.15)	0.72 (0.36, 1.43)
TCF[c]	1.17 (0.50, 2.77)	1.10 (0.59, 2.04)
Disease-Specific Death	*# Events=4*	*# Events=7*
TC	1.07 (0.45, 2.57)	1.09 (0.51, 2.32)
HDL	1.72 (0.62, 4.79)	0.65 (0.27, 1.59)
LDL	0.72 (0.27, 1.95)	0.58 (0.24, 1.41)
TG	0.33 (0.07, 1.49)	0.79 (0.39, 1.59)
TCF[c]	1.24 (0.47, 3.28)	1.12 (0.58, 2.16)
Recurrence	*# Events=7*	*# Events=11*
TC	1.33 (0.65, 2.73)	1.35 (0.72, 2.54)
HDL	0.87 (0.34, 2.24)	0.61 (0.30, 1.24)
LDL	0.64 (0.29, 1.40)	0.62 (0.31, 1.22)
TG	0.29 (0.08, 1.11)	0.74 (0.39, 1.40)
TCF[c]	1.37 (0.61, 3.10)	1.35 (0.75, 2.42)
HPV-Negative	**N=44**	**N=74**
Overall Death	*# Events=14*	*# Events=27*
TC	1.88 (0.91, 3.89)	1.58 (1.02, 2.45)
HDL	0.73 (0.46, 1.16)	0.99 (0.73, 1.34)
LDL	1.17 (0.57, 2.36)	0.80 (0.53, 1.20)
TG	1.02 (0.61, 1.70)	0.76 (0.51, 1.12)
TCF[c]	1.39 (0.81, 2.41)	1.50 (0.99, 2.29)
Disease-Specific Death	*# Events=9*	*# Events=19*
TC	1.75 (0.71, 4.28)	1.36 (0.81, 2.27)
HDL	0.88 (0.48, 1.63)	1.06 (0.73, 1.53)
LDL	0.81 (0.35, 1.89)	0.64 (0.39, 1.03)
TG	0.85 (0.44, 1.64)	0.62 (0.39, 1.00)
TCF[c]	1.61 (0.82, 3.16)	1.56 (0.95, 2.58)
Recurrence	*# Events=12*	*# Events=22*
TC	2.16 (1.00, 4.66)	1.48 (0.93, 2.36)
HDL	0.92 (0.55, 1.52)	1.07 (0.76, 1.51)
LDL	0.88 (0.41, 1.91)	0.64 (0.39, 1.04)
TG	1.02 (0.58, 1.79)	0.71 (0.45, 1.09)
TCF[c]	1.13 (0.64, 1.99)	1.29 (0.82, 2.03)

*Standard deviation increase

a. Excluding participants on a statin

b. Adjusting for statin use

c. Total cholesterol instrument containing all statistically significant SNPs

Chapter 5 : Public Health Significance / Conclusion

The main public health implication from this project was to identify if statins improve health outcomes among head and neck squamous cell carcinoma (HNSCC) patients. In addition to identifying this association, this dissertation investigates the potential mechanisms by which statins may influence HNSCC risk as well as outcomes (overall death, disease-specific death, and recurrence). The biological processes that statin drugs may affect are various, and the main mechanisms investigated through this dissertation are their anti-inflammatory and immunomodulatory effects as well as their cholesterol-lowering properties.[1]

The results from Chapter 2 provide evidence that there is a protective association between the use of statins and overall death for all patients, and a similarly protective association was observed for disease-specific death and disease recurrence, particularly among patients whose tumors were human papillomavirus (HPV)-positive.[2] The association between statin use and overall death is not surprising given that the primary action of statin drugs is to lower cholesterol, which ultimately prevents coronary artery disease.[3,4] Since heart disease is the leading cause of death in the United States and the state of Michigan, the association between statin use and overall death even among HNSCC patients was expected.[5,6]

Although these findings are not ground-breaking, they may improve overall health outcomes for patients who have a poorer prognosis and are less likely to use statin drugs. African Americans and patients of low socioeconomic status (SES) with HNSCC often experience poorer outcomes than white patients and patients with higher SES, respectively.[7] African American patients and patients of lower SES are also less likely to be diagnosed with HPV-

positive associated HNSCC.[8,9] The literature has identified that African Americans are less likely to use and adhere to statin drugs, and those of lower SES have poorer adherence.[10,11] Identifying cholesterol levels and prescribing or promoting adherence to statin drugs among African American patients and patients of lower SES may ultimately improve overall survival regardless of the HPV status of their tumor, especially if they are identified as having high cholesterol levels and are not currently using statin drugs.

The innovative finding that statin drugs are additionally protective against HNSCC specific death and disease recurrence among patients with HPV-positive tumors is significant in that it may provide insight to not only the actions statins may have on cancer but the benefits these drugs may impose on patients who take them. Further research was conducted to help understand the potential mechanisms by which statin drugs may improve disease-specific outcomes among HNSCC patients. This is important to identify in order to clearly understand the disease processes and treatment options, particularly for patients with HPV-positive disease.

The results presented in Chapter 3 provide evidence supporting the hypothesis that the use of statin drugs have anti-inflammatory and immunomodulatory effects on head and neck tumors. Through this research, we observed that statin drugs were associated with higher levels of inflammatory biomarkers that have been identified as being associated with improved prognosis among HNSCC patients.[12,13] The biomarker that had the strongest association with statin use was the tumor-infiltrating lymphocyte (TIL), FoxP3, and this association was particularly observed among patients with HPV-positive tumors, consistent with our survival findings in Chapter 2. Although these inflammatory markers are not currently utilized in practice to identify prognosis, they may be implemented as a prognostic marker in clinical practice in the future. Further research on the association between statin drugs and TILs is necessary to better understand this relationship.

Although we did not identify a clear association between statin use and circulating cytokines, it is necessary to investigate this association further with a larger sample size through a more rigorous study design, particularly a randomized control trial. Higher levels of the pro-inflammatory cytokine IL-6 have been found to be associated with worse outcomes among HNSCC patients. The relationship between statin use and circulating pro-inflammatory cytokines has been explored previously with and demonstrated inverse results in other patient populations.[14–18]

Chapter 4 of this dissertation explored whether cholesterol may be the mechanism by which statins exert any observed influence on HNSCC risk or outcomes. To assess this association, I conducted a Mendelian randomization analysis through the utilization of instruments that were created using SNPs that were identified as being only statistically significantly associated ($P<5x10^{-8}$) with each cholesterol measure (total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides(TG)) based on a joint metabochip and genome-wide association study (GWAS) analysis conducted by the Global Lipids Genetics Consortium.[19]

The findings from this Chapter support a null association between cholesterol-related instruments and risk of developing HNSCC. These results are supported by a recently published paper investigating a similar relationship utilizing a different analytic technique in a larger study.[20] When exploring the influence of the instruments on HNSCC outcomes among the cases with HNSCC, however, there appeared to be protective associations between TG and overall death as well as disease-specific death. These results were mainly observed when statin users were included in the analytic sample. After excluding cases who used statin drugs from the analytic sample, the association became relatively null. This may be due to a violation of the "exclusion restriction" assumption that the instrument is only associated with the outcome through the exposure.[21] There was a positive association between TC and all of the outcomes

measured among cases (overall death, disease-specific death, and disease recurrence); although not consistently statistically significant, this association remained positive even after exclusion of cases using statin drugs. HPV status did not appear to modify the association between the cholesterol-related instruments and HNSCC risk or HNSCC outcomes. Findings for both outcomes were similar among those with HPV-positive and HPV-negative tumors.

Due to the limited analytic sample size, this study probably did not have adequate power to detect statistically significant associations, with a larger sample size it is possible that the positive association observed between TC and HNSCC outcomes would become more precise and prove to be statistically significant. Future research in a larger more diverse analytic sample is necessary.

Based on the findings described above, this dissertation identified a protective association between the use of statin drugs and HNSCC outcomes and two potential mechanisms that may explain this observed relationship (anti-inflammation/immunomodulation and cholesterol-lowering). These discoveries provide evidence to support the implementation of an adjuvant clinical trial to investigate the potential treatment properties of statins for HNSCC patients. Statins are a relatively safe medication with side effects occurring only rarely and generally with high doses.[22] Although it appears as though mortality and other cancer-related outcomes among HNSCC patients has improved over time, this may not be related to improvements in treatment but rather the increase in HPV-positive associated disease and a decrease in HPV-negative disease in the United States due to a decrease in smoking and tobacco use over time.[23]

Patients with HPV-positive disease have better prognosis than patients with HPV-negative disease. This may be because the etiology of HPV-positive disease is different, it has a stronger immune response, different patient characteristics, and HPV- positive patients have a more favorable response to treatment.[8,24] Currently there are not many tertiary prevention

options for HNSCC patients to improve survival and traditional treatment modalities often result in morbidities.[25] Depending on the site and the stage of the disease patients are recommended surgery at the primary site as well as possible neck dissection, radiation, chemotherapy or a combination of the three treatments.[26] Other more “targeted therapies” are emerging but these are still under investigation and are not a standard option.[27] Particularly immunotherapy may be a beneficial treatment option for HNSCC patients given the immune response some patients elicit.[28] Recently immunotherapy has appeared to improve outcomes among patients who have advanced disease in concurrence with other more conventional treatment.[29] It is possible that statin use may improve outcomes in conjunction with these other treatment modalities. Not only will the implementation of a statin potentially improve prognosis for disease-specific outcomes but also may improve death due to other causes such as heart disease. Cholesterol levels are relatively easy and routine to test. It may be beneficial to all patients to have this measured and statins prescribed during their oncologic visit or after treatment initiation if they are found to have hypercholesterolemia.

In addition to providing information about the potential beneficial effects of statins on HNSCC outcomes this dissertation supports the implementation of a risk stratification approach. Patients who develop HNSCC tumors’ are often tested for HPV. Especially if the site of the tumor is located within the oropharynx region. We have identified that patients whose disease was HPV-positive may benefit from statin use the most. HPV testing for all patients regardless of disease site may be valuable to identify the most optimal treatment plan and who may benefit the most from statins.

As it appears there is an association between cholesterol levels, particularly TC and HNSCC outcomes identified in Chapter 4. Although statins are a relatively safe drug, there are a few adverse effects that may inhibit a person from using them. The most common adverse effect is related to the muscles, particularly muscle pain and weakness.[30] In addition to muscle

pain, people who cannot tolerate statin drugs may also have issues related to the liver.[31] There are a plethora of other adverse effects that may occur but are not as common.[32] If cholesterol-lowering is the mechanism that is improving prognosis in patients with HNSCC, a patient who may not be able to tolerate statin drugs may still benefit from the use of other cholesterol-lowering medications such as bile acid sequestrants, fibrates or niacin.[33] These are also commonly used medications that can lower cholesterol, although they may not have the same anti-inflammatory or immunomodulatory impact as statin drugs.

Future research is necessary to better understand the relationship between statin use and HNSCC outcomes and the mechanisms that may influence this association in larger, more diverse study populations. It also important to have a better understand of the dose-response relationship as well as investigate how duration of statin use may influence HNSCC outcomes.

Unfortunately, the use of statins as primary prevention for HNSCC is not ethical or feasible. A large majority of the population uses statins; approximately 30% of American adults would be recommended statin use due to existing risk factors for cardiovascular disease.[34] Another issue is that HNSCC is quite rare, only occurring in 0.2-3.3 per 100,000 depending on the site of disease.[8] To develop a cohort and implement a randomized control trial in which statins are administered at random and follow subjects through time to identify which subjects develop HNSCC would be expensive, require a long period of time and an extremely large sample size. It is also not ethical given the wide use of statins among adults in the United States; it would not be possible to forbid patients who are not assigned the treatment from using statins. This would probably lead to a large amount of contamination in the non-treatment group and if an intent-to-treat analysis was performed, the association would probably be biased toward the null.

Although evaluating statins as a primary chemoprevention strategy is not feasible, assessing them for tertiary prevention is possible since they were found observationally to be

protective against mortality and recurrence, especially among patients whose tumors were HPV-positive. Prior to initiation of an adjuvant trial additional research is necessary. Identifying the type of statin (hydrophobic or hydrophilic), the dose and duration of use that is maximally beneficial is required. It would also be helpful to identify when after diagnosis or during treatment, the introduction of a statin would be the most helpful to improving prognosis. It is possible that because patients with HPV-positive disease have longer life-spans, they are benefiting the most from the use of statin drugs because they are possibly taking them for a longer period of time or are exposed to a larger dose over their lifetime. These are questions that are outstanding that need to be addressed before starting an adjuvant trial.

In order to have a better understanding about how the mechanisms of statin drugs may influence HNSCC risk and outcomes, additional research is necessary. It would be important to assess how TILs, particularly FoxP3 mediate the association between statin use and HNSCC outcomes and how this potential mediation is modified by HPV status. To further explore how the cholesterol-lowering mechanism impacts the HNSCC risk and outcomes, a much larger study must be conducted excluding all participants (cases and controls) who have used or are currently using statin drugs. Although the findings presented are promising, additional research is necessary to further elucidate the discoveries identified through this dissertation to provide evidence and inform treatment options for HNSCC patients.

References

1. Demierre M-F, Higgins PDR, Gruber SB, Hawk E, Lippman SM. Statins and cancer prevention. *Nat Rev Cancer*. 2005;5(12):930-942. doi:10.1038/nrc1751
2. Getz KR, Bellile E, Zarins KR, et al. Statin use and head and neck squamous cell carcinoma outcomes. *Int J Cancer*. 2021;148(10):2440-2448. doi:10.1002/ijc.33441
3. Maron DJ, Fazio S, Linton MF. Current perspectives on statins. *Circulation*. 2000;101(2):207-213. doi:10.1161/01.CIR.101.2.207
4. Brugts JJ, Yetgin T, Hoeks SE, et al. The benefits of statins in people without established cardiovascular disease but with cardiovascular risk factors: meta-analysis of randomised controlled trials. *BMJ*. 2009;338:b2376. doi:10.1136/bmj.b2376
5. National Center for Health Statstics. Michigan-Leading Causes of Death. https://www.cdc.gov/nchs/pressroom/states/michigan/mi.htm. Published 2019. Accessed May 26, 2021.
6. Kochanek KD, Xu J, Arias E. Mortality in the united states, 2019. *NCHS Data Brief*. 2020;(395):1-8.
7. Molina MA, Cheung MC, Perez EA, et al. African American and poor patients have a dramatically worse prognosis for head and neck cancer. *Cancer*. 2008;113(10):2797-2806. doi:10.1002/cncr.23889
8. Rettig EM, D'Souza G. Epidemiology of head and neck cancer. *Surg Oncol Clin N Am*. 2015;24(3):379-396. doi:10.1016/j.soc.2015.03.001
9. O'Rorke MA, Ellison MV, Murray LJ, Moran M, James J, Anderson LA. Human papillomavirus related head and neck cancer survival: a systematic review and meta-analysis. *Oral Oncol*. 2012;48(12):1191-1201. doi:10.1016/j.oraloncology.2012.06.019
10. Nanna MG, Navar AM, Zakroysky P, et al. Association of patient perceptions of cardiovascular risk and beliefs on statin drugs with racial differences in statin use insights from the patient and provider assessment of lipid management registry. *JAMA Cardiol*. 2018;3(8):739-748. doi:10.1001/jamacardio.2018.1511
11. Mauskop A, Borden WB. Predictors of statin adherence. *Curr Cardiol Rep*. 2011;13(6):553-558. doi:10.1007/s11886-011-0221-2
12. Spector ME, Bellile E, Amlani L, et al. Prognostic value of tumor-infiltrating lymphocytes in head and neck squamous cell carcinoma. *JAMA Otolaryngol Head Neck Surg*. 2019;145(11):1012-1019. doi:10.1001/jamaoto.2019.2427
13. Nguyen N, Bellile E, Thomas D, et al. Tumor infiltrating lymphocytes and survival in patients with head and neck squamous cell carcinoma (HNSCC). *Head Neck*. 2016;38(7):1074-1084. doi:10.1002/hed.24406
14. Duffy SA, Taylor JMG, Terrell JE, et al. Interleukin-6 predicts recurrence and survival among head and neck cancer patients. *Cancer*. 2008;113(4):750-757. doi:10.1002/cncr.23615
15. Malicki S, Winiarski M, Matlok M, Kostarczyk W, Guzdek A, Konturek P. Il-6 and il-8 responses of colorectal cancer in vivo and in vitro cancer cells subjected to simvastatin. *J Physiol pharamacology*. 2009;60(4):141-146.
16. Rezaie-Majd A, Maca T, Bucek RA, et al. Simvastatin reduces expression of cytokines

interleukin-6, interleukin-8, and monocyte chemoattractant protein-1 in circulating monocytes from hypercholesterolemic patients. *Arterioscler Thromb Vasc Biol*. 2002;22(7):1194-1199. doi:10.1161/01.ATV.0000022694.16328.CC

17. Ascer E, Bertolami MC, Venturinelli ML, et al. Atorvastatin reduces proinflammatory markers in hypercholesterolemic patients. *Atherosclerosis*. 2004;177(1):161-166. doi:10.1016/j.atherosclerosis.2004.07.003

18. Lyngdoh T, Vollenweider P, Waeber G, Marques-Vidal P. Association of statins with inflammatory cytokines: a population-based Colaus study. *Atherosclerosis*. 2011;219(1):253-258. doi:10.1016/j.atherosclerosis.2011.07.117

19. Willer CJ, Schmidt EM, Sengupta S, et al. Discovery and refinement of loci associated with lipid levels. *Nat Genet*. 2013;45(11):1274-1283. doi:10.1038/ng.2797.

20. Gormley M, Yarmolinsky J, Dudding T, et al. Using genetic variants to evaluate the causal effect of cholesterol lowering on head and neck cancer risk: a mendelian randomization study. *PLOS Genet*. 2021;17(4):e1009525. doi:10.1101/2020.10.05.20206268

21. Vanderweele TJ, Tchetgen Tchetgen EJ, Cornelis M, Kraft P. Methodological challenges in mendelian randomization. *Epidemiology*. 2014;25(3):427-435. doi:10.1097/EDE.0000000000000081.

22. Armitage J. The safety of statins in clinical practice. *Lancet*. 2007;370(9601):1781-1790. doi:10.1016/S0140-6736(07)60716-8

23. Sturgis EM, Cinciripini PM. Trends in head and neck cancer incidence in relation to smoking prevalence: an emerging epidemic of human papillomavirus-associated cancers? *Cancer*. 2007;110(7):1429-1435. doi:10.1002/cncr.22963

24. Marur S, D'Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. *Lancet Oncol*. 2010;11(8):781-789. doi:10.1016/S1470-2045(10)70017-6

25. Murphy BA, Deng J. Advances in supportive care for late effects of head and neck cancer. *J Clin Oncol*. 2015;33(29):3314-3321. doi:10.1200/JCO.2015.61.3836

26. Koyfman SA, Ismaila N, Crook D, et al. Management of the neck in squamous cell carcinoma of the oral cavity and oropharynx: ASCO clinical practice guideline. *J Clin Oncol*. 2019;37(20):1753-1774. doi:10.1200/JCO.18.01921

27. Marur S, Forastiere AA. Head and neck squamous cell carcinoma: update on epidemiology, diagnosis, and treatment. *Mayo Clin Proc*. 2016;91(3):386-396. doi:10.1016/j.mayocp.2015.12.017

28. Ferris RL. Immunology and immunotherapy of head and neck cancer. *J Clin Oncol*. 2015;33(29):3293-3304. doi:10.1200/JCO.2015.61.1509

29. Cramer JD, Burtness B, Ferris RL. Immunotherapy for head and neck cancer: recent advances and future directions. *Oral Oncol*. 2019;99:104460. doi:10.1016/j.oraloncology.2019.104460

30. Golomb BA, Evans MA. Statin adverse effects a review of the literature and evidence for a mitochondrial mechanism. *Am J Cardiovasc Drugs*. 2008;8(6):373-418.

31. Simic I, Reiner Z. Adverse effects of statins - myths and reality. *Curr Pharamceutical Des*. 2015;21(9):1220-1226. doi:10.2174/1381612820666141013134447

32. Kiortsis DN, Filippatos TD, Mikhailidis DP, Elisaf MS, Liberopoulos EN. Statin-associated adverse effects beyond muscle and liver toxicity. *Atherosclerosis*. 2007;195(1):7-16. doi:10.1016/j.atherosclerosis.2006.10.001

33. National Center for Chronic Disease Prevention and Health Promotion (NCCDPHP). Cholesterol-lowering Medicine. https://www.cdc.gov/cholesterol/treating_cholesterol.htm. Published 2017. Accessed June 9, 2021.

34. Ridker PM, Cook NR. Statins: new American guidelines for prevention of cardiovascular disease. *Lancet*. 2013;382(9907):1762-1765. doi:10.1016/S0140-6736(13)62388-0