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Lael Rayfield Date

Prognostic Relevance of HPV Infection in Cases of Anal Squamous Cell Carcinoma: A Weighted Propensity Score Analysis

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Prognostic Relevance of HPV Infection in Cases of Anal Squamous Cell Carcinoma: A Weighted Propensity Score Analysis

By

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B.S., University of Maryland Baltimore County, 2015

Thesis Advisor: Yuan Liu, PhD

An abstract of
A thesis submitted to the Faculty of the
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Abstract

Prognostic Relevance of HPV Infection in Cases of Anal Squamous Cell Carcinoma: A Weighted Propensity Score Analysis
By Lael Rayfield

Aim: Our goal was to evaluate the prognostic relevance of Human Papilloma Virus (HPV) infection for cases of non-metastatic anal squamous cell carcinoma treated with definitive concurrent chemo-radiation in the National Cancer Database (NCDB).

Methods: The 2014 Anal Cancer NCDB was queried for non-metastatic, histologically confirmed, anal squamous cell carcinoma patients diagnosed between the years 2008 and 2013. All eligible patients were required to have documented HPV status. Subjects were then stratified into two groups: HPV+ and HPV-. Univariate analysis (UVA) was performed using the χ^2 test for categorical covariates and ANOVA for numerical covariates. Multivariable analysis (MVA) was performed using Cox proportional hazard model for overall survival (OS). Hazard ratios (HR) and 95% confidence intervals (CI) were generated for each covariate. To minimize selection bias, propensity score (PS) weighting was implemented to balance OS related co-variates between the cohorts.

Results: A total of 1,063 patients fit the study inclusion criteria. Patients were stratified into HPV+ (n=498, 46.8%) and HPV- (n=565, 53.2%). After PS weighting, MVA for OS showed that for men, those with HPV infection had improved OS (HR 0.60, CI 0.38-0.96; p=0.034). However, for women, HPV infection demonstrated a statistical trend towards worse OS (HR 1.47, CI 0.96-2.25; p=0.074).

Conclusion: To our knowledge, this is the largest study evaluating the impact of HPV infection on OS in patients with anal cancer. We found that HPV infection confers a statistically significant survival advantage for men with ASCC. In contrast, for women, HPV infection portrayed a statistical trend towards survival detriment. HPV infection should be considered as a prognostic variable in future anal cancer clinical trials.

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Introduction

Background & Rationale

Although anal squamous cell carcinoma (ASCC) is quite rare, it is becoming a growing public health issue. In the United States, incidence rates of ASCC have been climbing significantly. Between the years 1973 and 2000, incidence increased by 160% in men and 78% in women (Johnson, 2004; SEER, 2003). ASCC is characterized by the formation of tumors in the squamous cells lining the anal canal and the anal margin. These tumors can be keratinizing or nonkeratinizing depending on where they are located, but both kinds have similar biology and prognosis (Ryan, 2000). ASCC is usually a loco-regional disease which metastasizes in only about 15% of patients (Ajani, 2008).

Initially, it was thought that ASCC was caused by benign chronic irritations such as hemorrhoids and fissures. Some also speculated that it was closely related to inflammatory bowel disease. In actuality, none of these are the true cause of ASCC, but several potential risk factors have been identified. For example, researchers found that sexual activity and genital viral infections are related to the pathogenesis of anal cancer (Daling 1982; Peters, 1983). Women with anal cancer were more likely to have a history of genital warts or chlamydia trachomatis infection than women with colon cancer. A similar comparison between men with anal cancer and men with colon cancer found that the former group of patients were more likely to have engaged in homosexual activity. Some other risk factors for anal cancer include history of cervical, vulvar or vaginal cancer, immunosuppression, cigarette smoking and having more than 10 sexual partners.

Studies have shown that combined chemo-radiation therapy is associated with improved loco-regional control of ASCC, eliminating much of the need for patients to undergo a colostomy (Bartelink, 1997; Flam, 1996; Ryan, 2000; UKCCR, 1996). Additionally, anal cancer has a much higher rate of response to chemotherapy and radiation than other gastrointestinal malignancies.

Patients with anal cancer have a significantly higher response rates than patients with rectal cancer, despite the fact that the anus and rectum are anatomically close to one another (Belluco, 2011; Gunderson, 2012). This difference in response rate could be attributed to histology, but some researchers speculate that human papilloma virus infection impacts the effectiveness of chemo-radiation therapy.

Anal cancer has been associated with sexual transmission of human papilloma virus (HPV), making it an important prognostic factor (Zur Hausen, 2002). According to the CDC, HPV is the most common sexually transmitted disease and about one in four people in the United States is infected at some point in their lives (CDC, 2016). HPV can lead to anal intraepithelial neoplasia, often found in areas adjacent to those typically affected by ASCC (Fenger, 1991). The virus infects the squamous epithelial cells, which cover the skin and mucous membranes. If a person is infected, the virus can enter these cells and create proteins. These proteins, particularly E6 and E7, interfere with normal cell functioning and cause excessive cell growth. They also inhibit p53 and Rb, two proteins that suppress tumors. If a person's immune system does not act on the malignant cells, they continue to divide until lesions develop. Eventually, these lesions can become cancerous tumors.

There are 200 known genotypes of HPV virus, and not all of them cause cancer. In this study, the investigators were particularly interested in type 16, a high-risk subtype of HPV infection identified as an etiological factor for ASCC (Daling 2004; Frisch 1997; Frisch, 1999; Yhim, 2011). Surprisingly, patients with HPV16 have better progression-free survival and superior time to loco-regional failure (Yhim, 2011). Some other high risk types are 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 70. The prevalence of HPV DNA in cases of ASCC is between 75 and 100% and an estimated 92% of anal cancers are caused by types 16 and 18 alone (HPV and Anal Cancer Foundation, 2016).

The link between carcinoma and HPV infection has become a growing topic of research. Previous studies on patients with head and neck squamous cell carcinoma (HNSCC) demonstrated that HPV infected tumor cells have an intrinsic sensitivity to both chemotherapy and radiation therapy (Kimple, 2013; Rieckmann, 2013). Several single-institution retrospective studies have investigated the effect of HPV on ASCC, but most had limited sample sizes ranging from 47-153 patients (Gilbert, 2013; Mai, 2015; Yhim, 2011). This study seeks to answer to same question, but with superior statistical power and data from multiple institutions.

Study Objective

The primary aim of this study is to evaluate the survival rates of HPV positive and HPV negative patients with non-metastatic anal squamous cell carcinoma treated with concurrent chemo-radiation. Based on previous scientific findings, the investigators hypothesized that HPV infection would confer a better prognosis.

A secondary statistical aim of this thesis was to compare the performance of a traditional propensity score analysis to that of a generalized propensity score approach. We hypothesized that the generalized method would control for differences among treatment groups more effectively. These topics are expanded upon in the Propensity Score Analysis portion of the Methods section.

Methods

Data Source

Data used were from the National Cancer Database (NCDB), a nationally recognized clinical oncology database containing more than 70% of cancer cases diagnosed annually in the United States (Winchester, 2010). It is jointly sponsored by the American Cancer Society and the American College of Surgeons. Data is collected from a variety of reporting centers including community hospitals, academic medical centers and National Cancer Institute (NCI)-designated Comprehensive Cancer Centers. Data specifically came from the NCDB 2014 Participant User File (PUF) for anal cancer, a Health Insurance Portability and Accountability Act (HIPAA) compliant data file. The PUF contains patient level data, but no identifying information pertaining to hospitals, healthcare providers or individual patients. Included variables were patient demographics, disease characteristics, treatment details and survival outcomes.

Study Population

The original NCDB Anal PUF included 54,069 anal squamous cell carcinoma cases diagnosed between 2004 and 2013. The database was queried to retrieve patients who fit the investigators' inclusion/exclusion criteria. In order to be eligible for the study, cases needed a histologic confirmation of malignancy and a tumor located in the anus or anal canal. All included cases had confirmed test results indicating they were HPV positive or HPV negative. Patients with in-situ tumor behavior, missing date of death or last contact, any form of metastasis at diagnosis, adenocarcinoma, disease involving the cloacogenic zone or perianal skin, non-squamous histology or carcinoma in situ were excluded. Patients with AJCC Clinical Stage 0, Stage 1 or Stage 4 carcinoma were excluded, leaving only those with Stage 2, Stage 3, Stage 3A or Stage 3B. T stage 0-4 and N Stage 0-3 were included.

All included cases received concurrent chemo-radiation within 2 weeks apart and had radiation to the pelvis, abdomen or other/unknown region. Cases who received less than 4400 cGy or more than 7000 cGy of radiation were excluded. Cases who had an incomplete course of radiation therapy were also excluded. The following radiation treatment modalities were excluded: protons, stereotactic radiosurgery, linac radiosurgery, gamma knife, all forms of brachytherapy, radioisotopes and strontium-90. Once all inclusion and exclusion criteria were applied, the final analytic dataset contained 1,063 ASCC cases. A detailed record of how many patients were removed by each exclusion criterion can be found in **Table 15** of the Appendix.

Study Cohorts

The cohorts of interest were HPV positive and HPV negative cases. HPV status was defined by Collaborative Stage Site Specific Factor 1. This variable in the PUF contains recorded results of any HPV testing performed on pathologic specimens from the primary tumor or a metastatic site, including lymph nodes. Patients coded as 0 were considered HPV negative and patients coded as 010, 020, 030, 040, 050, 060 or 070 were considered HPV positive (the latter group included both high and low-risk HPV types). Additionally, we created a variable which classified HPV positive patients into two groups: High Risk (Site Specific Factor 1 of 030 or 040) and Low Risk/Unknown HPV Type (Site Specific Factor 1 of 010, 020, 050, 060, 070). Patients with no infection were categorized as HPV Negative. A detailed description of each Collaborative Stage Site Specific Factor 1 code can be found in **Table 9** of the Appendix.

Covariates of Interest

Study variables were defined by the NCDB Participant User File dictionary (link to full 2014 dictionary: http://ncdbpuf.facs.org/?q=print-pdf-all). The outcome of interest was overall survival (OS) defined as months from the date of diagnosis to death or last follow-up.

There were 9 demographic covariates of interest: gender, race, facility type, insurance status, median income, education level, urban/rural status, distance from treatment facility (also called great circle distance) and age at diagnosis. Due to the small number of patients in certain categories, some variable groups were collapsed. Gender was a binary variable and patients were coded as either male or female. Race was collapsed into two groups, white and non-white/other. Age at diagnosis was measured in years. Facility type was categorized as Community/Integrated Network Cancer Program, Comprehensive Community Cancer Program or Academic/Research Program. Insurance status (labeled in tables as primary payor) was designated as Not Insured/Unknown, Private Medicaid or Medicare/Other Government Insurance. Data on median income and education level (percent with no high school degree) were categorized by quartiles and taken from the 2000 US Census. Urban/rural status was based on data from the 2003 US Census and categorized as metro, urban or rural. Great circle distance is defined by the distance in miles between the patient's residence and the hospital that reported the case of cancer. Zip code centroids were used to estimate the patient's area of residence. This variable was categorized by quartiles.

Additionally, there were 10 patient-specific disease variables of interest: grade of tumor, sequence number, primary site, size/extension of primary tumor (or AJCC Clinical T Stage), absence/presence of lymph metastasis (or AJCC Clinical N Stage), agent of chemotherapy, Charlson-Deyo score, radiation treatment modality, year of diagnosis, and total radiation dose measured in centigray (cGy).

Tumor grade was categorized as well/moderately differentiated, poorly differentiated/undifferentiated and undetermined cell type. A tumor that is well differentiated (low

grade) bears resemblance to normal tissue, while an undifferentiated (high grade) tumor is least like normal tissue. Sequence number was defined as either single malignant primary tumor or subsequent malignant primary tumor. This variable indicates the sequence of malignant and nonmalignant neoplasms over the patient's lifetime. Primary site is the site of origin where the cancer was found. In this study, included primary sites were the anus and the anal canal. Year of diagnosis was coded as quartiles and years ranged from 2008 to 2013.

Size or extension of tumor is also defined as the AJCC Clinical T Stage. Categories were Stage 0, Stage 1, Stage 2, Stage 3, and Stage 4 and X. Absence/presence of lymph metastasis is also defined as the AJCC Clinical N Stage. Categories were Stage 0, Stage 1, Stage 2, and Stage 3 and X. The NCDB PUF Data Dictionary states that some cases are coded as X as a result of the Commission on Caner's restriction on the allowable range of registry coding. It is likely that data on their T and N Stage was beyond the information documented by the managing physician. The principal investigators requested that these patients be kept in the dataset. For the purposes of this analysis, Stage X can be treated as Other or Unknown.

The Charlson-Deyo Score was derived from scores for comorbid conditions found in the Charlson Comorbidity Score Mapping Table. Conditions such as myocardial infarction, AIDS and diabetes are included in the Comorbidity Mapping Table, and each condition has an assigned score ranging from 0 to 25. Higher numbers indicate more severe morbidity. The final Charlson-Deyo Score is a weighted sum of the scores for each individual comorbid condition. This covariate was categorized as 0 or 1+, the latter indicating a Charlson score of 1 or higher.

Agent of chemotherapy was categorized as single-agent, multi-agent or agent not documented. Radiation treatment modality was defined as the method by which the most clinically

significant regional dose of radiation therapy was administered during a patient' first course of treatment. In the event that more than one modality is used, the dominant one is recorded. In this analysis, the investigators were particularly interested to see which patients received Intensity Modulated Radiation Therapy (IMRT) and Conformal/3D Therapy. Both are external beam techniques, but the latter uses beams that match the shape of the tumor for added precision. Thus, the categories for this covariate were IMRT, Conformal/3D, or Other. The total radiation dose was kept as continuous variable and included both the regional radiation dose and any boost doses administered.

Statistical Analysis

Statistical procedures were conducted using SAS version 9.4 (Cary, NC) and SAS Macros developed by the Winship Cancer Institute Biostatistics and Bioinformatics Shared Resource (Nickleach, 2013). All hypothesis tests were two sided and the significance level was 0.05 unless otherwise specified.

Descriptive & Univariate Statistics

First, descriptive statistics were calculated for each variable in the analytic dataset. Reported values included mean, median and standard deviation for continuous variables and frequency counts and percentages for categorical variables. Next, univariate statistics and p-values were generated. These statistics examine the associations between the covariates of interest and HPV infection. For categorical variables, a chi-square test of independence was used to calculate the unadjusted associations. If expected contingency table cell-counts were less than 5, the Fisher's Exact test was used in the place of the chi-square test. For continuous variables, independent t-tests and analysis of variance (ANOVA) were used to determine whether or not a significant difference in mean existed between the HPV positive and HPV negative cohorts.

Univariate & Multivariable Survival Analysis

The univariate association between each covariate of interest and the outcome (overall survival in months since date of diagnosis) was assessed using a Cox proportional hazard model and the log-rank test. An initial Kaplan-Meier plot was generated to examine the difference in survival between the two study cohorts. Additional Kaplan-Meier plots were generated to examine how survival probability differed by gender and HPV risk level.

To determine which covariates were associated with HPV infection, a multivariable Cox proportional hazard (PH) model with response variable of overall survival was fit using the backward selection method and a removal criterion of 0.20. In backward selection, you begin by fitting a model with all covariates of interest or potential confounders. Then, the least significant variable is dropped, taking into account the chosen removal criterion. The model is then re-fitted with the remaining variables, and the least significant variable is dropped. This process continues until all remaining variables are statistically significant.

First, selected covariates were added to a multivariable Cox proportional hazard model. These variables were chosen because they were significantly associated with HPV status, identified as potential confounders or had high prognostic relevance. Using an option in the SAS Macro, HPV status was kept in the model and was not subject to removal. The hazard ratio in each level of the chosen covariates was generated along with the corresponding 95% confidence interval and log-rank test p-value.

Next, stratified analyses was conducted by including an interaction term between the HPV study cohorts and the stratification variable in a multivariable Cox proportional hazard model. Stratification variables used were those deemed particularly relevant to prognosis. Backward

selection was used to remove insignificant covariates. Using an option in the SAS Macro, HPV status, the stratification variable and the interaction term were kept in the model and not subject to removal. The hazard ratio was estimated for study cohorts in each level of the stratification variable.

Median follow-up times for all patients and patients in each study cohort were evaluated. Follow-up time was measured in months from diagnosis. The median, 25^{th} and 75^{th} percentiles were recorded along with corresponding 95% confidence intervals. The results of the log-rank, Wilcoxon and likelihood ratio tests were also reported to assess any difference in follow-up times between HPV+ and HPV- cases.

Propensity Score Analysis

Background on the Propensity Score

Usually, a randomized control trial (RCT) is the standard for examining the effect of a treatment, exposure or intervention on a given outcome. The RCT is ideal because, as the name implies, treatment is assigned by randomization. Thus, the unbiased average treatment effect (ATE) can be computed directly from the study data. For a continuous variable, the ATE is defined as a difference in means and for dichotomous variables it is often a difference in proportions. In mathematical notation, the ATE can be written as $E[Y_i(1) - Y_i(0)] = E[Y_i(1)] - E[Y_i(0)]$. Typically, $Y_i(1)$ represents the outcome for those assigned treatment 1 (intervention) and $Y_i(0)$ represents the outcome for those assigned treatment 0 (control).

Since, data collected in the NCDB are observational, patients in the HPV positive cohort may have starkly different baseline characteristics than those in the HPV negative cohort. This is because randomization is not used to assign patients to a treatment group. Additionally, there is

high likelihood of confounding due to selection bias. This means that certain patient characteristics can be associated with both the outcome of interest and the likelihood of receiving treatment. Selection bias often results in type I errors in which treatment effects are attributed to the treatment itself rather than the confounding variables (Starks, 2009). To control for confounding and account for systematic differences in baseline characteristics, we implemented a propensity score analysis.

The propensity score was first introduced by Rosenbaum and Rubin in 1983 and is defined as the conditional probability of receiving treatment given the pre-treatment covariates (Imbens, 2000; Rosenbaum & Rubin, 1983a). It is used to balance the distribution of baseline characteristics among treatment and control groups in an observational study. As a result, making comparisons between the two groups is much easier and effects of confounding are reduced.

In mathematical notation, the propensity score can be written as $e_i = P(Z_i = 1|X_i)$. (Austin 2011; Rosenbaum & Rubin, 1983). In this expression, e_i is the propensity score, Z is the treatment group and X are the observed covariates. Z can take the value of 0 (control group) or 1 (treatment group). In an observational study, the propensity score is not known, but can be estimated using the study data. Scores are generated using a propensity score model (usually a logistic regression model), which uses the treatment group as its dependent variable and a set of chosen covariates as independent variables.

In order for the propensity score to provide an unbiased estimate of ATE, strongly ignorable treatment assignment must hold true (Rosenbaum & Rubin, 1983). There are two components of strongly ignorable treatment: treatment assignment must be independent of outcome conditional

on the baseline covariates and every subject must have a non-zero probability of receiving either treatment.

Generalized Propensity Score

Until recently, the propensity score was applied exclusively to studies with two treatment arms. However, in many observational studies, treatment is non-binary. To address this issue, some researchers have begun to explore generalized propensity score analysis, which is used in studies with multiple treatment arms. One of the first to investigate this approach was Imbens, who proposed the use of a multinomial logistic regression model to predict each subject's treatment assignment (Imbens, 2000). For a study with $K > 2$ treatment levels, the model would be defined as $logit(p_{ij}) = a_{0j} + a_{1j} * X_i$ with $j = 1,2, \dots, K$. This model would create a vector of propensity scores, $(P_{i1}, P_{i2}, \dots, P_{ik})$ indicating the subjects' probability to being assigned to each treatment given their baseline characteristics (Liu, 2013).

During our analysis, we discovered a significant interaction between HPV and patient gender. Consequently, we decided to use the following treatment arms in our propensity score model: HPV+ Male, HPV+ Female, HPV- Male, and HPV- Female. We hoped to mimic the effects of the generalized propensity score approach and reduce imbalance among all four of these groups. To test the effectiveness of this method, we compared it to a more standard 2-group approach. In the standard analysis, the dataset was split into two, one dataset for males and another for females, and propensity scores were generated for subjects in each using two treatment arms: HPV positive and HPV negative. We were interested in comparing the effectiveness of the two and four group analysis, since literature on the generalized propensity score is quite sparse. This could also be an interesting topic to explore further in methodology research or simulation studies.

Variable Selection for Propensity Score Model

Once we decided on the treatment arms, the next step was to select variables for the propensity score model. At this time, there is not a clear consensus as to which covariates should or should not be included (Austin, 2011). Most literature suggests using variables that are hypothesized to be associated with the outcome of interest and the treatment, because these are potential confounders. It is also acceptable to include variables associated with outcome but not the treatment. However, variables that are associated with treatment but not the outcome, affected by the treatment or predict treatment status perfectly should be avoided (Austin 2011; Garrido, 2014a; Garrido, 2014b). Included covariates that are highly correlated with one another can also be problematic. After selecting our variables, we placed them in a multinomial logistic regression model with assigned treatment group as the dependent variable.

Once propensity scores were generated, histograms of score distributions were generated in SAS for each group. These histograms were visually examined to assess the amount of overlap among each score distribution. Similar distributions are indicative of balance among the treatment groups, while dissimilar distributions indicate imbalance.

Adjusted Inverse Probability Weighting

Inverse probability of treatment weights (IPTW) estimates were calculated from the propensity scores. Using these weights creates a "synthetic sample" in which the distribution of measured covariates is independent of treatment assignment (Austin, 2011). Each subject's weight is equal to the inverse of the probability of receiving the treatment that the subject is ultimately assigned. Mathematically, the weight equation can be written like so: $w_i = \frac{Z_i}{e_i} + \frac{(1-Z_i)}{1-e_i}$ (Austin, 2011). Incorporating weights makes the sample more representative of the general population and usually retains more patients than propensity score matching (Halpern, 2014). For all conducted

analyses, weights were stabilized and normalized to add up to the original sample size. The stabilized weight formula is written as $sw_i = \frac{P(Z_i=j)}{e_i}$. Stabilized weighting is useful in cases where the propensity score, e_i, is very small and the number of patients is very unbalanced among treatment groups (Cole, 2004; Liu, 2013).

Balance Diagnostics

Finally, to assess the balance of propensity scores in the weighted sample, we used standardized differences. This is a simple way to check whether or not the propensity model has been misspecified. The standardized difference was calculated to compare the mean of continuous and binary variables between pairs of groups. Multilevel categorical variables, were represented using a set of multiple binary indicator variables. The standardized difference for a continuous variable was calculated using the following formula:

$$d = \frac{(\bar{x}_{treatment} - \bar{x}_{control})}{\sqrt{\frac{s^2_{treatment} + s^2_{control}}{2}}}$$

Here, the $\bar{x}_{treatment}$ and $\bar{x}_{control}$ represent the sample mean of the covariate in subjects who are treated and untreated while $s^2_{treatment}$ and $s^2_{control}$ represent the sample variance in the treated and untreated subjects.

For binary variables, the following formula is used to calculate the standardized difference:

$$d = \frac{(\hat{p}_{treatment} - \hat{p}_{control})}{\sqrt{\frac{\hat{p}_{treatment}(1-\hat{p}_{treatment}) + \hat{p}_{control}(1-\hat{p}_{control})}{2}}}$$

In this equation, $\hat{p}_{control}$ and $\hat{p}_{treatment}$ represent the prevalence or mean of the dichotomous variable in each cohort.

Since there were four treatment groups in total, the maximum possible standardized difference based on the pairwise comparisons was reported in the output table. A parametric p-value was also generated using either the chi-square test of independence or the independent t test to see if there was a significant difference in patient characteristics among the four groups. According to the literature, a difference of 0.1 or less is usually considered negligible, but some studies use 0.2 or even 0.3 (Austin, 2009). For our analysis, we used a slightly less conservative cutoff of 0.15. We assessed the standardized differences both before and after applying IPTW to test the effectiveness of the weights. The effects of HPV status were recalculated using the IPTW with a Cox proportional hazard model. Finally, weighted survival curves were generated comparing treatment groups. The Breslow method was used for these aforementioned comparisons (Cole, 2004).

Alternative Two-Group Propensity Score Analysis

A secondary statistical aim of the study was to compare the generalized four-group PS analysis to that of a more traditional two-group analysis. Due to the strong interaction between gender and overall survival, we created two separate datasets: one containing all the female patients in the study, and another containing all the males. Next, propensity scores were generated for subjects in each dataset using a logistic regression model. Covariates included were the same ones used in the four-group analysis. IPTW weights were generated for each dataset using the propensity scores and were multiplied by the marginal probability of or receiving the observed treatment. Finally, we evaluated the balance of baseline covariates in each weighted sample using

standardized differences. To examine the effectiveness of this method, we compared standardized differences from the weighted male and female cohorts to the standardized differences generated by the weighted four-group analysis. We hypothesized that the four group analysis would have better balance overall.

Results

Note: in all results, HPV negative was coded as the reference group. Female gender was also used as the reference group for any gender comparisons.

Descriptive Statistics

Full tabulated descriptive results can be found in **Table 1** of the Appendix. The final analytic cohort consisted of 1,063 patients extracted from the NCDB Anal PUF. A total of 480 (45.2%) cases had a tumor in the anus and 583 (54.8%) had a tumor in the anal canal. Roughly half of tumors were well or moderately differentiated (47.6%), 28.9% were poorly differentiated or undifferentiated and the remaining 23.5% were of an undetermined cell type. AJCC Clinical stage groups were almost evenly split with 51.5% in Stage 2 and 48.5% in Stage 3, 3A or 3B. Patients with AJCC Clinical T Stage 0 (0.1%), 1 (4.3%), 2 (58.1%), 3 (26.2%) 4 (9.5%) and Other/X (1.9%) were included in the sample. About half (54.1%) the cases classified as AJCC Clinical N Stage 0, 12.5% were Stage 1, 19.2% were Stage 2, 13.2% were Stage 3 and the remaining 1.0% were Other/X. Nearly all of the cases consisted of single malignant primary diagnoses (94.6%). 498 patients (46.8%) were HPV + and 565 were HPV – (53.2%). Of the HPV positive cases, the majority (64.3%) had a high risk type of HPV infection. 187 patients (17.6%) had Type 16 HPV and only 2 (0.2%) had Type 18.

718 patients were female (67.5%) and 345 were male (32.5%). The majority were White (86.7%) and lived in a metropolitan area (83.4%). 15.0% came from urban areas and only 1.6% from rural areas. Surprisingly, more than half (60.6%) of the sample lived in census tracts where <20% of the population had a high school diploma. About 39.8% lived in census tracts where residents made upward of $46,000 a year, 29.2% made $36,000 - $45,999 a year, 17.5% made $30,000 - $35,999 a year and 13.6% made less than $30,000 a year. The median age was 57 years and year of diagnosis ranged from 2008 to 2013.

Most patients were treated at a Comprehensive Community Cancer program (43%), about a quarter came from Community/Integrated Network Cancer programs (21.8%) and the remaining cases (35.2%) received treatment at Academic/Research Centers. Treatment facilities were located all over the country: 33.6% were treated in the South, 25.4% in the Midwest, 23.5% in the Northeast and 17.5% in the West. Median distance from residence to treatment facility was 8 miles. Only a small fraction of cases (7.1%) had no insurance of any kind. The majority had private insurance (52%), 30.4% had Medicare or Other Government insurance and the remaining cases had Medicaid (10.4%). 79.7% of cases had a Charlson-Deyo morbidity score of 0 and the remaining 20.3% had a score of 1 or higher.

As previously stated, all patients received both chemotherapy and radiation. The majority received multiagent chemotherapy (88.2%) while 7.7% received single-agent and 4.0% received an undocumented agent. We examined both regional radiation dose and boost radiation dose for all patients. More than half of cases (60.1%) received intensity-modulated radiotherapy (IMRT) and very small fraction (4.1%) received Conformal/3D Therapy. The remaining cases received Other, which consisted of the following modalities: external beam, photons, electrons, photons and electrons mixed and Other (NOS). About half of the patients (54.1%) did not receive any boost

radiation. 21.9% received a boost dose of IMRT, 4% received a boost dose of Conformal/3D Therapy, and 20% received Other. The median radiation dose was 5,400 cGy, or 54 Gy.

A Kaplan-Meier plot comparing the survival times of each study cohort showed no significant difference in HPV + and HPV - patients (p = 0.662). This graph can be found in **Figure 1** of the Appendix. Median follow-up for all patients was 32.4 (31.5 – 33.4) months. We did not observe a significant difference in median follow-up times of the HPV + and HPV - cohorts (31.9 vs 32.7, log-rank p = 0.158). See **Tables 6-8** of the Appendix for follow-up time results.

Univariate Association with HPV Status

Univariate associations between covariates of interest and HPV status are listed in **Table 2** of the Appendix. Gender, AJCC Clinical Stage Group, agent of chemotherapy, Charlson-Deyo score and age at diagnosis were significantly associated with HPV status. Thus, HPV + patients were more likely to be male (37.4% vs. 26.9%), younger aged (median 55 vs. 58 years, p <.001), have a more advanced clinical stage at diagnosis (Stage 3/3A/3B 51.9% vs. Stage 2 44.8%, p = 0.021), treated with single-agent chemotherapy (9.2% vs. 6.02%) and have a Charlson-Deyo morbidity score of 1 or greater (22.7% vs. 17.7%, p = 0.044). A significant difference was not found in race, grade of tumor, treatment facility type, insurance status, median income, year of diagnosis, radiation therapy dosage or radiation modality between the two study cohorts.

Univariate Association with Overall Survival

Results of this portion of the analysis are presented in **Table 3** of the Appendix. Male patients are associated with significantly lower survival probability than females (HR = 2.08 [1.54 – 2.80], p = <.001). When examining AJCC Clinical Stage Group, we found that higher stages of cancer had worse survival probabilities (HR = 1.92 [1.41 – 2.62], p <.001). Medicare and Medicaid patients

had lower survival than uninsured patients, but those with private insurance had higher survival probability. Those with a single malignant primary tumor lived longer than those who'd developed a subsequent malignant tumor (HR = 2.06 [1.26 – 3.29], p = 0.002). Unsurprisingly, higher T and N Stage patients had worse survival than those in Stage 0 or 1. Finally, those with a Charlson-Deyo score of 1 or more had significantly worse probability of survival (HR = 1.89 [1.37 – 2.61], p < .001).

Multivariable Survival Analysis

Results of this portion of the analysis can be found in **Tables 4-5** of the Appendix. The unadjusted multivariable analysis (MVA) showed that HPV infection was not statistically associated with time to death (HR = 1.97 [0.71 – 1.37], p = 0.936). However, male gender (HR = 1.71 [1.22 – 2.40], p = 0.002), clinical Stage 3 (HR = 1.97 [1.42 – 2.75], p < .001) and Charlson-Deyo greater than 1 (HR = 1.82 [1.228 – 2.58], p <.001) were found to be significantly associated with overall survival.

In addition to the initial MVA, potential interactions between certain covariates and HPV status were explored. Covariates chosen were age, race, gender, tumor grade and Charlson-Deyo score. The only covariate to yield a significant interaction with HPV status was patient gender. When stratifying by gender, we observed that the presence of HPV infection was statistically significant for men (HR = 0.60 [0.39 – 0.94], p = 0.025). This result shows that HPV had a protective effect on male patients in the dataset. However, HPV tended to have a harmful effect on women (HR = 1.43 [0.93 – 2.18], p = 0.101). While this result was not statistically significant, it is still quite intriguing. We did not expect to observe opposite survival trends when stratifying by patient gender. Additionally, there was a significant difference in overall survival between men and women in the stratified MVA (type III p = 0.006).

Propensity Score Analysis

Generalized Propensity Score Approach

Due to the significant interaction between gender and HPV status, patients were assigned to four groups: HPV- Male (n = 134; 12.6%), HPV + Male (n = 211; 19.9%), HPV – Female (n = 364; 50.7%) and HPV + Female (n = 354; 49.3%). Covariates included in the propensity score (PS) model were AJCC clinical stage group, facility type, insurance status, urban/rural location, distance from treatment facility, year of diagnosis, Charlson-Deyo score, agent of chemotherapy, age at diagnosis and education level. Chosen covariates were either associated with overall survival or important demographic/prognostic factors. Propensity scores were generated and score distributions compared using histograms **(Figure 6).** Distributions were quite similar across all four gender/HPV groups.

Balance between the four groups was assessed using standardized differences. These results can be found in **Tables 10-11** of the Appendix. As aforementioned in the methods section, the investigators considered a difference of 0.15 or greater to be an indication of imbalance in the sample. Nearly all covariates included in the PS model yielded standardized differences greater than 0.15, and balance was deemed poor.

Next, stabilized inverse probability of treatment weights (IPTW) were applied to the sample. The final weighted sample contained 958 patients: 118 HPV – Males (12.3%), 179 HPV + Males (18.7%), 338 HPV – Females (35.3%) and 323 HPV + Females (33.7%). Standardized differences were again evaluated. This time, all differences were below 0.15 indicating good balance among the four PS groups. The highest observed difference was 0.115 for year of diagnosis between 2011 and 2012. Thus, the weighted PS analysis was successful.

The IPTW was incorporated into a multivariable Cox PH model containing gender, HPV status and the interaction between HPV status and gender. The results show that HPV + men had significantly higher survival probability than HPV – men (HR = 0.60 [0.38 – 0.96], p = 0.034), but HPV had a harmful effect on women's chances of survival (HR = 1.47 [0.96 – 2.25], p = 0.074). Results can be found in **Table 14** of the Appendix.

Kaplan-Meir plots were generated for the weighted PS samples. Long-rank p-values were used to compare survival probabilities of study cohorts. The weighted plot stratified by gender **(Figure 3)** confirms that HPV+ men tend to have improved overall survival when compared to HPV- men with 5-year overall survival of 56.4% (29.4% - 76.5%) and 50% (31.0% - 66.4%), p=0.034. PS weighted KM plot for women is shown in **Figure 4** which demonstrates a statistical trend towards worse 5-yr overall survival for HPV+ women when compared to HPV- women (78.9% [70% - 85.4%] vs 85.6% [79.4% - 90.1%], p=0.074). When all four weighted PS groups are compared, we observe a statistically significant difference in 5-year overall survival ($p < .0001$). This plot is found in **Figure 5** of the Appendix.

Alternative Propensity Score Approach

In the alternative PS analysis, we began by splitting the dataset into two: one containing the males and another containing the females. Covariates included in the propensity score (PS) model were AJCC clinical stage group, facility type, insurance status, urban/rural location, distance from treatment facility, year of diagnosis, Charlson-Deyo score, agent of chemotherapy, age at diagnosis and education level. Chosen covariates were either associated with overall survival or important demographic/prognostic factors. Propensity scores were generated and score distributions compared using histograms **(Figures 7-8).** The distribution of scores appeared similar for both datasets.

Stabilized inverse probability of treatment weights (IPTW) were applied to the male and female datasets. The weighted male sample contained 68 HPV – patients (22.7%) and 232 HPV + patients (77.3%). Balance in the sample was checked using standardized differences. The investigators considered a difference of 0.15 or greater to be an indication of imbalance in the sample. Many standardized differences were quite large in the male dataset. The largest was 0.827 for age at diagnosis. This may be because the number of HPV – males in the weighted sample was small. The weighted female sample contained 274 HPV – patients (41.6%) and 384 HPV + patients (58.4%). Compared to the males, standardized differences were not as large, but several were above 0.15. The largest was 0.380 for single-agent chemotherapy. Balance checks can be found in **Tables 12-13** of the Appendix.

After finishing the balance assessment, we concluded that the balance was poor among both groups of patients. Balance in the four group analysis was clearly superior, indicating that the generalized propensity score model was the better choice.

Discussion

This is the first large, multi-institutional study to examine the impact of HPV infection on ASCC prognosis. Our results show that HPV infection has a protective effect in men but tends to worsen probability of survival in women. Currently, the explanation for these contrasting effects is a bit of a mystery. We know from previous studies that HPV infected patients have an increased sensitivity to chemo-radiation therapy. This sensitivity could be attributed to increased levels of excision repair gene expansion, modulation of protein kinase B activation or restoration of

apoptotic cell death and upregulation of tumor suppressor p53 (Hampson, 2001; Gupta, 2009; Kimple, 2013; Rieckmann, 2013). However, HPV induced sensitivity to chemo-radiation has not been significantly associated with gender. From a statistical standpoint, we found that the weighted generalized propensity score analysis performed best and generated well-balanced sample. The alternative two-group analysis may have performed poorly because it required splitting the data, resulting in loss of statistical power.

Despite our exciting results, this analysis has several limitations. The NCDB PUF file contained observational data, which is prone to selection bias. Going forward, researchers should consider investigating the impact of HPV on ASCC in a prospective, randomized clinical trial (RCT). Ideally, patients in this trial would be stratified by gender. An RCT design could also create study cohorts with similar baseline characteristics, eliminating the need for such a rigorous PS analysis.

Data on specific clinical markers were not available in the NCDB PUF. For example, we only had information on whether a patient tested HPV positive or negative. We had no data describing the method of testing (in-situ hybridization or polymerase chain reaction) that was utilized. Also, we did not know which patients had p16 protein overexpression, a clinical marker for HPV infection. Finally, data on human immunodeficiency virus (HIV) status and specific chemotherapy agent (mitomycin or cisplatin) were unavailable, but would have aided our analysis.

Our propensity score analysis also had some limitations. While the IPTW approach was quite successful in balancing the study cohorts, the model itself can be unstable around the tails if the probability of treatment assignment is very small. To protect against instability, we used stabilizing weights in our analysis. Model misspecification can also be a problem in a PS analysis.

Although we evaluated our model using standardized differences, some additional analyses to check model misspecification could have aided our study.

In conclusion, more research is needed to determine the impact of HPV on ASCC prognosis. Our analysis successfully applied generalized propensity score weighting, an innovative technique that is rarely used in clinical studies. We hope our findings have shed some light on a burgeoning subject and look forward to seeing where they lead.

References

Ajani, J. A., Winter, K. A., Gunderson, L. L., Pedersen, J., Benson, A. B., Thomas, C. R., ... & Willett, C. (2008). *Jama*, *299*(16), 1914-1921.

Bartelink, H., Roelofsen, F., Eschwege, F., Rougier, P., Bosset, J. F., Gonzalez, D. G., ... & Pierart, M. (1997). Concomitant radiotherapy and chemotherapy is superior to radiotherapy alone in the treatment of locally advanced anal cancer: results of a phase III randomized trial of the European Organization for Research and Treatment of Cancer Radiotherapy and Gastrointestinal Cooperative Groups. *Journal of clinical oncology*, *15*(5), 2040-2049.

Belluco, C., De Paoli, A., Canzonieri, V., Sigon, R., Fornasarig, M., Buonadonna, A., … De Marchi, F. (2011). Long-Term Outcome of Patients with Complete Pathologic Response after Neoadjuvant Chemoradiation for cT3 Rectal Cancer: Implications for Local Excision Surgical Strategies. *Annals of Surgical Oncology, 18*(13), 3686–3693.

Centers for Disease Control and Prevention. (2016). *What is HPV?* Retrieved February 20, 2017 from: https://www.cdc.gov/hpv/index.html

Cole, S. R., & Hernán, M. A. (2004). Adjusted survival curves with inverse probability weights. *Computer Methods and Programs in Biomedicine*, *75*(1), 45-49.

Daling, J. R., Weiss, N. S., Klopfenstein, L. L., Cochran, L. E., Chow, W. H., & Daifuku, R. (1982). Correlates of homosexual behavior and the incidence of anal cancer. *Jama*, *247*(14), 1988-1990.

Daling, J. R., Madeleine, M. M., Johnson, L. G., Schwartz, S. M., Shera, K. A., Wurscher, M. A., ... & McDougall, J. K. (2004). Human papillomavirus, smoking, and sexual practices in the etiology of anal cancer. *Cancer*, *101*(2), 270-280.

Dawid, A. P. (1979). Conditional independence in statistical theory. *Journal of the Royal Statistical Society*. Series B (Methodological), 1-31.

Fenger, C. (1991). Anal neoplasia and its precursors: facts and controversies. *Semin Diagn Pathol*, *8*(3), 190-201.

Flam, M., John, M., Pajak, T. F., Petrelli, N., Myerson, R., Doggett, S., ... & Murray, K. (1996). Role of mitomycin in combination with fluorouracil and radiotherapy, and of salvage chemoradiation in the definitive nonsurgical treatment of epidermoid carcinoma of the anal canal: results of a phase III randomized intergroup study. *Journal of Clinical Oncology*, *14*(9), 2527-2539.

Frisch, M., Glimelius, B., van den Brule, A. J., Wohlfahrt, J., Meijer, C. J., Walboomers, J. M., ... & Melbye, M. (1997). Sexually transmitted infection as a cause of anal cancer. *New England Journal of Medicine*, *337*(19), 1350-1358.

Frisch, M., Fenger, C., van den Brule, A. J., Sørensen, P., Meijer, C. J., Walboomers, J. M., ... & Glimelius, B. (1999). Variants of squamous cell carcinoma of the anal canal and perianal skin and their relation to human papillomaviruses. *Cancer Research*, *59*(3), 753-757.

Garrido, M. M. (2014a). Propensity scores: a practical method for assessing treatment effects in pain and symptom management research. *Journal of pain and symptom management, 48*(4), 711-718.

Garrido, M. M., Kelley, A. S., Paris, J., Roza, K., Meier, D. E., Morrison, R. S., & Aldridge, M. D. (2014b). Methods for constructing and assessing propensity scores. *Health services research, 49*(5), 1701-1720.

Gilbert, D. C., Williams, A., Allan, K., Stokoe, J., Jackson, T., Linsdall, S., ... & Summers, J. (2013). p16 INK4A, p53, EGFR expression and KRAS mutation status in squamous cell cancers of the anus: correlation with outcomes following chemo-radiotherapy. *Radiotherapy and Oncology*, *109*(1), 146-151.

Gunderson, L. L., Winter, K. A., Ajani, J. A., Pedersen, J. E., Moughan, J., Benson, A. B., … Willett, C. G. (2012). Long-Term Update of US GI Intergroup RTOG 98-11 Phase III Trial for Anal Carcinoma: Survival, Relapse, and Colostomy Failure With Concurrent Chemoradiation Involving Fluorouracil/Mitomycin Versus Fluorouracil/Cisplatin. *Journal of Clinical Oncology, 30*(35), 4344–4351.

Gupta, A. K., Lee, J. H., Wilke, W. W., Quon, H., Smith, G., Maity, A., ... & Spitz, D. R. (2009). Radiation Response in Two HPV-Infected Head-and-Neck Cancer Cell Lines in Comparison to a Non–HPV-Infected Cell Line and Relationship to Signaling Through AKT. *International Journal of Radiation Oncology* Biology* Physics*, *74*(3), 928-933.

Hampson, L., EL SAID, A. E. H., Moore, J. V., Kitchener, H., & Hampson, I. N. (2001). The HPV16 E6 and E7 proteins and the radiation resistance of cervical carcinoma. *The FASEB Journal*, *15*(8), 1445-1447.

HPV and Anal Cancer Foundation. *HPV & Cancer.* Retrieved February 20, 2017 from: http://www.analcancerfoundation.org/about-hpv/hpv-cancer/

Imai, K., & Van Dyk, D. A. (2004). Causal inference with general treatment regimes: Generalizing the propensity score. Journal of the American Statistical Association, *99*(467), 854-866.

Imbens, G. (2000). The Role of the Propensity Score in Estimating Dose-Response Functions. *Biometrika*, *87*(3), 706-710.

Johnson, L. G., Madeleine, M. M., Newcomer, L. M., Schwartz, S. M., & Daling, J. R. (2004). Anal cancer incidence and survival: the surveillance, epidemiology, and end results experience, 1973–2000. *Cancer*, 101(2), 281-288.

Kimple, R. J., Smith, M. A., Blitzer, G. C., Torres, A. D., Martin, J. A., Yang, R. Z., ... & Lambert, P. F. (2013). Enhanced radiation sensitivity in HPV-positive head and neck cancer. *Cancer research*, *73*(15), 4791-4800.

Liu, Y., Nickleach, D., & Lipscomb, J. (2013). Propensity score matching for multiple treatment comparisons in observational studies. In *The 59th World Statistics Congress Proceeding.*

Mai, S., Welzel, G., Ottstadt, M., Lohr, F., Severa, S., Prigge, E. S., ... & Reuschenbach, M. (2015).

Prognostic relevance of HPV infection and p16 overexpression in squamous cell anal cancer. *International Journal of Radiation Oncology* Biology* Physics*, *93*(4), 819-827.

Nickleach D, Liu Y, Shrewsberry A, Ogan K, Kim S, Wang Z. (2013). SAS® Macros to Conduct Common Biostatistical Analyses and Generate Reports. *SESUG 2013: The Proceeding of the SouthEast SAS User Group.* URL: http://analytics.ncsu.edu/sesug/2013/PO-05.pdf.

Peters, R. K., & Mack, T. M. (1983). Patterns of anal carcinoma by gender and marital status in Los Angeles County. *British Journal of cancer*, *48*(5), 629.

Rieckmann, T., Tribius, S., Grob, T. J., Meyer, F., Busch, C. J., Petersen, C., ... & Kriegs, M. (2013). HNSCC cell lines positive for HPV and p16 possess higher cellular radiosensitivity due to an impaired DSB repair capacity. *Radiotherapy and oncology*, *107*(2), 242-246.

Rosenbaum, P. R., & Rubin, D. B. (1983a). The central role of the propensity score in observational studies for causal effects. *Biometrika*, *70*(1), 41-55.

Rosenbaum, P. R., & Rubin, D. B. (1983b). Assessing sensitivity to an unobserved binary covariate in an observational study with binary outcome. *Journal of the Royal Statistical Society*. Series B (Methodological), 212-218.

Ryan, D. P., Compton, C. C., & Mayer, R. J. (2000). Carcinoma of the anal canal. *New England Journal of Medicine*, *342*(11), 792-800.

Surveillance, Epidemiology, and End Results (SEER) Program. SEER*Stat database: incidence—SEER 9 regs public-use, based on the November, 2002 submission (1973–2000), released April, 2003. Bethesda: National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, 2003. URL: http://www.seer.cancer.gov

UKCCCR Anal Cancer Trial Working Party. (1996). Epidermoid anal cancer: results from the UKCCCR randomised trial of radiotherapy alone versus radiotherapy, 5-fluorouracil, and mitomycin. *The Lancet*, *348*(9034), 1049-1054.

Winchester, D. P., Stewart, A. K., Phillips, J. L., & Ward, E. E. (2010). "The National Cancer Data Base: Past, Present, and Future." *Annals of Surgical Oncology*, *17*(1), 4–7.

Yhim, H. Y., Lee, N. R., Song, E. K., Kwak, J. Y., Lee, S. T., Kim, J. H., ... & Hwang, J. E. (2011). The prognostic significance of tumor human papillomavirus status for patients with anal squamous cell carcinoma treated with combined chemoradiotherapy. *International journal of cancer*, *129*(7), 1752-1760.

Zur Hausen, H. (2002). Papillomaviruses and cancer: from basic studies to clinical application. *Nature Reviews Cancer, 2*(5), 342-350.

Appendix

Table 1: Characteristics of Full Study Population

Variable	Level	N (%) = 1063
HPV	Negative	498 (46.8)
	Positive	565 (53.2)
HPV Risk Level	HPV Negative	498 (46.8)
	Low Risk/Unknown HPV Type	245 (23.0)
	High Risk HPV	320 (30.1)
Sex	Male	345 (32.5)
	Female	718 (67.5)
Race	White	922 (86.7)
	Non-White, Other or Unknown	141 (13.3)
Grade	Well/Moderately Differentiated	506 (47.6)
	Poorly Differentiated/Undifferentiated	307 (28.9)
	Cell Type Not Determined	250 (23.5)
AJCC Clinical Stage Group	Stage 2	547 (51.5)
	Stage 3/3A/3B	516 (48.5)
Facility Type	Community/Integrated Network Cancer Program	221 (21.8)
	Comprehensive Community Cancer Program	436 (43.0)
	Academic/Research Program	357 (35.2)
	Missing	49

Variable	Level	N (%) = 1063
Primary Payor	Not Insured/Unknown	76 (7.1)
	Private	553 (52.0)
	Medicaid	111 (10.4)
	Medicare/Other Government	323 (30.4)
Median Income Quartiles 2000	Not Available	32
	< $30,000	140 (13.6)
	$30,000 - $35,999	180 (17.5)
	$36,000 - $45,999	301 (29.2)
	$46,000 +	410 (39.8)
Percent No High School Degree Quartiles 2000	Not Available	32
	>=29%	183 (17.7)
	20-28.9%	223 (21.6)
	14-19.9%	237 (23.0)
	< 14%	388 (37.6)
Urban/Rural 2003	Metro	862 (83.4)
	Urban	155 (15.0)
	Rural	17 (1.6)
	Missing	29
Sequence Number	Single Malignant Primary	1006 (94.6)
	Subsequent Malignant Tumor	57 (5.4)
Primary Site	Anus	480 (45.2)
	Anal Canal	583 (54.8)
Great Circle Distance in Miles (quartile)	Less than 4 miles	266 (25.0)
	Between 4 and 8 miles	266 (25.0)
	Between 8 to 18 miles	268 (25.2)
	Greater than 18 miles	263 (24.7)

Variable	Level	N (%) = 1063
AJCC Clinical T	Stage 0	1 (0.1)
	Stage 1	45 (4.3)
	Stage 2	614 (58.1)
	Stage 3	277 (26.2)
	Stage 4	100 (9.5)
	X	20 (1.9)
	Missing	6
AJCC Clinical N	Stage 0	572 (54.1)
	Stage 1	132 (12.5)
	Stage 2	203 (19.2)
	Stage 3	139 (13.2)
	X	11 (1.0)
	Missing	6
Agent of Chemotherapy	Agent Not Documented	43 (4.0)
	Single-Agent	82 (7.7)
	Multiagent	938 (88.2)
Diagnosis Year (quartile)	>=2008, <=2011	386 (36.3)
	>2011, <=2012	307 (28.9)
	>2012, <=2013	370 (34.8)
Charlson-Deyo Score	0	847 (79.7)
	1+	216 (20.3)
Radiation Treatment Modality	Other	380 (35.7)
	IMRT	639 (60.1)
	Conformal/3D Therapy	44 (4.1)
Boost Treatment Modality	None	575 (54.1)
	Other	213 (20.0)
	IMRT	233 (21.9)
	Conformal or 3D Therapy	42 (4.0)

Variable	Level	N (%) = 1063
Age at Diagnosis	Mean	57.33
	Median	57.00
	Minimum	23.00
	Maximum	90.00
	Std Dev	11.12
	Missing	0.00
Regional+Boost Radiation Dose (cGY)	Mean	5403.43
	Median	5400.00
	Minimum	4400.00
	Maximum	6840.00
	Std Dev	442.49
	Missing	0.00

Table 2: Univariate Associations with HPV Status

Covariate	Statistics	Level	HPV		Parametric P-value*
			Negative N=498	Positive N=565	
Sex	N (Row %)	Male	134 (38.84)	211 (61.16)	**<.001**
	N (Row %)	Female	364 (50.7)	354 (49.3)	
Race	N (Row %)	White	441 (47.83)	481 (52.17)	0.101
	N (Row %)	Non-White, Other or Unknown	57 (40.43)	84 (59.57)	
Grade	N (Row %)	Well/Moderately Differentiated	237 (46.84)	269 (53.16)	1.000
	N (Row %)	Poorly Differentiated/Undifferentiated	144 (46.91)	163 (53.09)	
	N (Row %)	Cell Type Not Determined	117 (46.8)	133 (53.2)	
AJCC Clinical Stage Group	N (Row %)	Stage 2	275 (50.27)	272 (49.73)	**0.021**
	N (Row %)	Stage 3/3A/3B	223 (43.22)	293 (56.78)	
Facility Type	N (Row %)	Community/Integrated Network Cancer Program	103 (46.61)	118 (53.39)	0.835
	N (Row %)	Comprehensive Community Cancer Program	214 (49.08)	222 (50.92)	
	N (Row %)	Academic/Research Program	172 (48.18)	185 (51.82)	
Primary Payor	N (Row %)	Not Insured/Unknown	30 (39.47)	46 (60.53)	0.353
	N (Row %)	Private	254 (45.93)	299 (54.07)	
	N (Row %)	Medicaid	52 (46.85)	59 (53.15)	
	N (Row %)	Medicare/Other Government	162 (50.15)	161 (49.85)	
Median Income Quartiles 2000	N (Row %)	< $30,000	61 (43.57)	79 (56.43)	0.881
	N (Row %)	$30,000 - $35,999	86 (47.78)	94 (52.22)	
	N (Row %)	$36,000 - $45,999	139 (46.18)	162 (53.82)	
	N (Row %)	$46,000 +	193 (47.07)	217 (52.93)	

Covariate	Statistics	Level	HPV		Parametric P-value*
			Negative N=498	Positive N=565	
Percent No High School Degree Quartiles 2000	N (Row %)	>=29%	88 (48.09)	95 (51.91)	0.591
	N (Row %)	20-28.9%	111 (49.78)	112 (50.22)	
	N (Row %)	14-19.9%	105 (44.3)	132 (55.7)	
	N (Row %)	< 14%	175 (45.1)	213 (54.9)	
Urban/Rural 2003	N (Row %)	Metro	402 (46.64)	460 (53.36)	0.826
	N (Row %)	Urban	75 (48.39)	80 (51.61)	
	N (Row %)	Rural	7 (41.18)	10 (58.82)	
Sequence Number	N (Row %)	Single Malignant Primary	476 (47.32)	530 (52.68)	0.199
	N (Row %)	Subsequent Malignant Tumor	22 (38.6)	35 (61.4)	
Primary Site	N (Row %)	Anus	219 (45.63)	261 (54.38)	0.468
	N (Row %)	Anal Canal	279 (47.86)	304 (52.14)	
Great Circle Distance in Miles (quartile)	N (Row %)	Less than 4 miles	116 (43.61)	150 (56.39)	0.253
	N (Row %)	Between 4 and 8 miles	117 (43.98)	149 (56.02)	
	N (Row %)	Between 8 to 18 miles	132 (49.25)	136 (50.75)	
	N (Row %)	Greater than 18 miles	133 (50.57)	130 (49.43)	
AJCC Clinical T	N (Row %)	Stage 0	0 (0)	1 (100)	0.655
	N (Row %)	Stage 1	19 (42.22)	26 (57.78)	
	N (Row %)	Stage 2	300 (48.86)	314 (51.14)	
	N (Row %)	Stage 3	125 (45.13)	152 (54.87)	
	N (Row %)	Stage 4	44 (44)	56 (56)	
	N (Row %)	X	8 (40)	12 (60)	
AJCC Clinical N	N (Row %)	Stage 0	286 (50)	286 (50)	0.193
	N (Row %)	Stage 1	55 (41.67)	77 (58.33)	
	N (Row %)	Stage 2	95 (46.8)	108 (53.2)	
	N (Row %)	Stage 3	57 (41.01)	82 (58.99)	
	N (Row %)	X	4 (36.36)	7 (63.64)	

Covariate	Statistics	Level	HPV Negative N=498	HPV Positive N=565	Parametric P-value*
Agent of Chemotherapy	N (Row %)	Agent Not Documented	28 (65.12)	15 (34.88)	**0.010**
	N (Row %)	Single-Agent	30 (36.59)	52 (63.41)	
	N (Row %)	Multiagent	440 (46.91)	498 (53.09)	
Diagnosis Year (quartile)	N (Row %)	>=2008, <=2011	186 (48.19)	200 (51.81)	0.409
	N (Row %)	>2011, <=2012	149 (48.53)	158 (51.47)	
	N (Row %)	>2012, <=2013	163 (44.05)	207 (55.95)	
Charlson-Deyo Score	N (Row %)	0	410 (48.41)	437 (51.59)	**0.044**
	N (Row %)	1+	88 (40.74)	128 (59.26)	
Radiation Treatment Modality	N (Row %)	Other	174 (45.79)	206 (54.21)	0.823
	N (Row %)	IMRT	302 (47.26)	337 (52.74)	
	N (Row %)	Conformal/3D Therapy	22 (50)	22 (50)	
Boost Treatment Modality	N (Row %)	None	271 (47.13)	304 (52.87)	0.443
	N (Row %)	Other	93 (43.66)	120 (56.34)	
	N (Row %)	IMRT	110 (47.21)	123 (52.79)	
	N (Row %)	Conformal or 3D Therapy	24 (57.14)	18 (42.86)	
Age at Diagnosis	N		498	565	**<.001**
	Mean		59.42	55.49	
	Median		58	55	
	Min		24	23	
	Max		89	90	
	Std Dev		10.74	11.13	
Regional+Boost Radiation Dose (cGY)	N		498	565	0.901
	Mean		5401.63	5405.01	
	Median		5400	5400	
	Min		4400	4500	
	Max		6840	6840	
	Std Dev		464.19	422.84	

Covariate	Statistics	Level	HPV		Parametric P-value*
			Negative N=498	Positive N=565	
Last Contact or Death, Months from Dx	N		498	565	0.130
	Mean		31.53	30.18	
	Median		30.55	29.14	
	Min		2.17	2.23	
	Max		70.37	87.43	
	Std Dev		14.66	14.34	

* The parametric p-value is calculated by ANOVA for numerical covariates and chi-square test for categorical covariates.

Table 3: Univariate Association with Overall Survival

			Survived Months from Diagnosis		
Covariate	**Level**	**N**	**Hazard Ratio (95% CI)**	**HR P-value**	**Log-rank P-value**
Sex	Male	345	2.08 (1.54-2.80)	**<.001**	**<.001**
	Female	718	-	-	
Race	Non-White, Other or Unknown	141	1.16 (0.75-1.79)	0.496	0.496
	White	922	-	-	
Grade	Cell Type Not Determined	250	1.32 (0.92-1.91)	0.130	0.309
	Poorly Differentiated/Undifferentiated	307	1.16 (0.81-1.65)	0.426	
	Well/Moderately Differentiated	506	-	-	
AJCC Clinical Stage Group	Stage 3/3A/3B	516	1.92 (1.41-2.62)	**<.001**	**<.001**
	Stage 2	547	-	-	
Facility Type	Academic/Research Program	357	0.93 (0.62-1.38)	0.703	0.132
	Comprehensive Community Cancer Program	436	0.69 (0.46-1.03)	0.072	
	Community/Integrated Network Cancer Program	221	-	-	
Primary Payor	Medicare/Other Government	323	1.54 (0.86-2.77)	0.148	**<.001**
	Medicaid	111	1.32 (0.67-2.57)	0.421	
	Private	553	0.58 (0.32-1.05)	0.074	
	Not Insured/Unknown	76	-	-	
Median Income Quartiles 2000	$46,000 +	410	0.71 (0.44-1.14)	0.155	0.093
	$36,000 - $45,999	301	1.06 (0.67-1.69)	0.802	
	$30,000 - $35,999	180	1.14 (0.68-1.89)	0.618	
	< $30,000	140	-	-	

			Survived Months from Diagnosis		
Covariate	**Level**	**N**	**Hazard Ratio (95% CI)**	**HR P-value**	**Log-rank P-value**
Percent No High School Degree Quartiles 2000	< 14%	388	0.67 (0.44-1.02)	0.064	0.159
	14-19.9%	237	0.89 (0.56-1.40)	0.603	
	20-28.9%	223	1.00 (0.64-1.56)	0.990	
	>=29%	183	-	-	
Urban/Rural 2003	Rural	17	1.89 (0.77-4.61)	0.164	0.367
	Urban	155	1.02 (0.67-1.56)	0.928	
	Metro	862	-	-	
Sequence Number	Subsequent Malignant Tumor	57	2.06 (1.29-3.29)	**0.002**	**0.002**
	Single Malignant Primary	1006	-	-	
Primary Site	Anal Canal	583	1.10 (0.81-1.48)	0.551	0.549
	Anus	480	-	-	
Great Circle Distance in Miles (quartile)	Greater than 18 miles	263	1.09 (0.72-1.65)	0.672	0.234
	Between 8 to 18 miles	268	0.73 (0.47-1.14)	0.162	
	Between 4 and 8 miles	266	1.10 (0.73-1.65)	0.644	
	Less than 4 miles	266	-	-	
AJCC Clinical T	X	20	0.69 (0.08-6.16)	0.740	**<.001**
	Stage 4	100	3.74 (1.30-10.71)	**0.014**	
	Stage 3	277	3.23 (1.17-8.89)	**0.023**	
	Stage 2	614	1.72 (0.63-4.70)	0.289	
	Stage 0-1	46	-	-	
AJCC Clinical N	X	11	0.65 (0.09-4.69)	0.671	**<.001**
	Stage 3	139	2.18 (1.45-3.26)	**<.001**	
	Stage 2	203	1.90 (1.30-2.78)	**<.001**	
	Stage 1	132	1.39 (0.86-2.24)	0.175	
	Stage 0	572	-	-	

			Survived Months from Diagnosis		
Covariate	**Level**	**N**	**Hazard Ratio (95% CI)**	**HR P-value**	**Log-rank P-value**
Agent of Chemotherapy	Multiagent	938	0.77 (0.39-1.51)	0.451	0.199
	Single-Agent	82	1.17 (0.53-2.61)	0.696	
	Agent Not Documented	43	-	-	
Age at Diagnosis	Age >= 50 years	812	0.92 (0.65-1.29)	0.621	0.621
	Age < 50 years	251	-	-	
Diagnosis Year (quartile)	>2012, <=2013	370	0.97 (0.66-1.42)	0.878	0.108
	>2011, <=2012	307	0.67 (0.45-0.99)	**0.044**	
	>=2008, <=2011	386	-	-	
Charlson-Deyo Score	1+	216	1.89 (1.37-2.61)	**<.001**	**<.001**
	0	847	-	-	
HPV Risk Level	High Risk HPV	320	1.14 (0.81-1.60)	0.460	0.707
	Low Risk/Unknown HPV Type	245	0.98 (0.67-1.45)	0.929	
	HPV Negative	498	-	-	
Radiation Treatment Modality	Conformal/3D Therapy	44	0.86 (0.39-1.87)	0.696	0.820
	IMRT	639	0.91 (0.67-1.25)	0.567	
	Other	380	-	-	
Boost Treatment Modality	Other	213	1.01 (0.68-1.51)	0.948	0.639
	IMRT	233	1.26 (0.87-1.81)	0.218	
	Conformal or 3D Therapy	42	1.17 (0.57-2.40)	0.678	
	None	575	-	-	
HPV	Positive	565	1.07 (0.79-1.44)	0.663	0.662
	Negative	498	-	-	
Regional+Boost Radiation Dose (cGY)		1063	1.00 (1.00-1.00)	0.190	-

Table 4: Multivariable Survival Analysis

			Survived Months from Diagnosis		
Covariate	**Level**	**N**	**Hazard Ratio (95% CI)**	**HR P-value**	**Type3 P-value**
HPV	Positive	501	0.99 (0.71-1.37)	0.936	0.936
	Negative	457	-	-	
Sex	Male	300	1.71 (1.22-2.40)	**0.002**	**0.002**
	Female	658	-	-	
AJCC Clinical Stage Group	Stage 3/3A/3B	459	1.97 (1.42-2.75)	**<.001**	**<.001**
	Stage 2	499	-	-	
Facility Type	Academic/Research Program	337	0.88 (0.58-1.34)	0.551	0.196
	Comprehensive Community Cancer Program	410	0.69 (0.46-1.05)	0.083	
	Community/Integrated Network Cancer Program	211	-	-	
Primary Payor	Medicare/Other Government	305	1.63 (0.84-3.15)	0.146	**<.001**
	Medicaid	89	1.59 (0.74-3.42)	0.239	
	Private	497	0.76 (0.39-1.49)	0.420	
	Not Insured/Unknown	67	-	-	
Median Income Quartiles 2000	$46,000 +	386	1.36 (0.78-2.37)	0.279	0.149
	$36,000 - $45,999	276	1.82 (1.05-3.17)	**0.033**	
	$30,000 - $35,999	170	1.58 (0.89-2.80)	0.117	
	< $30,000	126	-	-	
Urban/Rural 2003	Rural	16	2.57 (0.99-6.65)	0.052	0.146
	Urban	138	1.14 (0.71-1.83)	0.580	
	Metro	804	-	-	
Diagnosis Year (quartile)	>2012, <=2013	339	1.23 (0.81-1.87)	0.322	0.098
	>2011, <=2012	284	0.75 (0.49-1.14)	0.175	
	>=2008, <=2011	335	-	-	
Charlson-Deyo Score	1+	200	1.82 (1.28-2.58)	**<.001**	**<.001**
	0	758	-	-	

			Survived Months from Diagnosis		

Covariate	**Level**	**N**	**Hazard Ratio (95% CI)**	**HR P-value**	**Type3 P-value**

* Number of observations in the original data set = 1063. Number of observations used = 958.
** Backward selection with an alpha level of removal of .20 was used. The following variables were removed from the model: Great Circle Distance in Miles (quartile), Grade, Percent No High School Degree Quartiles 2000, Primary Site, Race, Regional+Boost Radiation Dose (cGY), Age at Diagnosis, Agent of Chemotherapy, and HPV Risk Level.

Table 5: Multivariable Survival Analysis Stratified by Patient Gender

			Survived Months from Diagnosis		
Covariate	**Level**	**N**	**Hazard Ratio (95% CI)**	**HR P-value**	**Type3 P-value**
AJCC Clinical Stage Group	Stage 3/3A/3B	503	2.06 (1.50-2.82)	**<.001**	**<.001**
	Stage 2	528	-	-	
Primary Payor	Medicare/Other Government	319	1.48 (0.82-2.70)	0.196	**<.001**
	Medicaid	106	1.35 (0.68-2.68)	0.392	
	Private	533	0.70 (0.38-1.28)	0.243	
	Not Insured/Unknown	73	-	-	
Median Income Quartiles 2000	$46,000 +	410	1.05 (0.65-1.71)	0.841	0.162
	$36,000 - $45,999	301	1.51 (0.94-2.45)	0.089	
	$30,000 - $35,999	180	1.37 (0.82-2.31)	0.232	
	< $30,000	140	-	-	
Diagnosis Year (quartile)	>2012, <=2013	361	1.04 (0.70-1.54)	0.845	0.167
	>2011, <=2012	297	0.71 (0.48-1.06)	0.092	
	>=2008, <=2011	373	-	-	
Charlson-Deyo Score	1+	214	1.63 (1.16-2.29)	**0.005**	**0.005**
	0	817	-	-	
Comparisons Stratified by Sex :	**HPV :**		-	-	**0.006**
Male	Positive vs. Negative	204 vs. 130	0.60 (0.39-0.94)	**0.025**	-

			Survived Months from Diagnosis		
Covariate	**Level**	**N**	**Hazard Ratio (95% CI)**	**HR P-value**	**Type3 P-value**
Female	Positive vs. Negative	348 vs. 349	1.43 (0.93-2.18)	0.101	-

* Number of observations in the original data set = 1063. Number of observations used = 1031.
** Backward selection with an alpha level of removal of .20 was used. The following variables were removed from the model: Great Circle Distance in Miles (quartile), Facility Type, Grade, Percent No High School Degree Quartiles 2000, Primary Site, Race, Regional+Boost Radiation Dose (cGY), Urban/Rural 2003, Age at Diagnosis, and Agent of Chemotherapy.

Table 6: Median Follow-Up for All Patients

Quartile Estimates				
Percent	**Point Estimate**	**95% Confidence Interval**		
		Transform	**[Lower**	**Upper)**
75	43.7300	LOGLOG	41.9200	45.6300
50	32.4300	LOGLOG	31.4700	33.3800
25	24.3100	LOGLOG	23.4600	25.2300

Table 7: Median Follow-Up for HPV Positive Cohort

Quartile Estimates				
Percent	**Point Estimate**	**95% Confidence Interval**		
		Transform	**[Lower**	**Upper)**
75	42.6400	LOGLOG	39.9500	45.1700
50	31.9300	LOGLOG	30.7200	33.6800
25	23.8500	LOGLOG	22.1800	25.1300

Table 8: Median Follow-Up for HPV Negative Cohort

Quartile Estimates				
Percent	Point Estimate	95% Confidence Interval		
		Transform	[Lower	Upper)
75	45.6300	LOGLOG	42.3200	47.9700
50	32.6600	LOGLOG	31.6700	34.8900
25	24.8400	LOGLOG	23.5600	26.1800

Table 9: Codes Used for HPV Status in NCDB Dataset

Code	Description
000	HPV negative for high-risk and low-risk types HPV negative for high-risk types with no mention of low-risk types Negative, NOS
010	HPV positive for low-risk types only
020	HPV positive for specified high risk type(s) other than types 16 or 18
030	HPV positive for high-risk type 16 WITHOUT positive results for high-risk type18 or positivity of high-risk type 18 unknown
040	HPV positive for high-risk type18 WITHOUT positive results for high-risk type 16 or positivity of high-risk type 16 unknown
050	HPV positive for high-risk types16 AND 18
060	HPV positive for high-risk type(s), NOS, high-risk type(s) not stated
070	HPV positive, NOS, risk and type(s) not stated
888	OBSOLETE DATA CONVERTED V0200 See code 988 Not applicable for this site
988	Not applicable: Information not collected for this case (May include cases converted from code 888 used in CSv1 for "Not applicable" or when the item was not collected. If this item is required to drive T, N, M, or any stage, use of code 988 may result in an error.)
997	Test ordered, results not in chart
998	Test not done (test not ordered and not performed) No pathologic specimen available for HPV testing
999	Unknown or no information Not documented in patient record

Table 10: Patient Characteristics in Unweighted Sample

Covariate	Level	Statistics	Gender/HPV Group: Male HPV Negative N=120	Male HPV Positive N=180	Female HPV Negative N=337	Female HPV Positive N=321	Parametric P-value*	Standardized Difference
AJCC Clinical Stage Group	Stage 2	N (Col%)	66 (55)	93 (51.67)	190 (56.38)	150 (46.73)	0.086	**0.194**
	Stage 3/3A/3B	N (Col%)	54 (45)	87 (48.33)	147 (43.62)	171 (53.27)		**0.194**
Facility Type	Community/Integrated Network Cancer Program	N (Col%)	22 (18.33)	39 (21.67)	76 (22.55)	74 (23.05)	0.367	**0.117**
	Comprehensive Community Cancer Program	N (Col%)	49 (40.83)	68 (37.78)	148 (43.92)	145 (45.17)		**0.150**
	Academic/Research Program	N (Col%)	49 (40.83)	73 (40.56)	113 (33.53)	102 (31.78)		**0.189**
Primary Payor	Not Insured/Unknown	N (Col%)	7 (5.83)	17 (9.44)	18 (5.34)	25 (7.79)	**<.001**	**0.157**
	Private	N (Col%)	43 (35.83)	72 (40)	189 (56.08)	193 (60.12)		**0.501**
	Medicaid	N (Col%)	16 (13.33)	16 (8.89)	30 (8.9)	27 (8.41)		**0.159**
	Medicare/Other Government	N (Col%)	54 (45)	75 (41.67)	100 (29.67)	76 (23.68)		**0.461**
Urban/Rural 2003	Metro	N (Col%)	99 (82.5)	153 (85)	284 (84.27)	268 (83.49)	0.925	0.068
	Urban	N (Col%)	18 (15)	25 (13.89)	49 (14.54)	46 (14.33)		0.032
	Rural	N (Col%)	3 (2.5)	2 (1.11)	4 (1.19)	7 (2.18)		**0.104**
Great Circle Distance in Miles (quartile)	Less than 4 miles	N (Col%)	34 (28.33)	56 (31.11)	71 (21.07)	80 (24.92)	**0.042**	**0.230**
	Between 4 and 8 miles	N (Col%)	21 (17.5)	50 (27.78)	90 (26.71)	81 (25.23)		**0.247**
	Between 8 to 18 miles	N (Col%)	29 (24.17)	29 (16.11)	92 (27.3)	86 (26.79)		**0.274**
	Greater than 18 miles	N (Col%)	36 (30)	45 (25)	84 (24.93)	74 (23.05)		**0.158**

Covariate	Level	Statistics	Gender/HPV Group: Male HPV Negative N=120	Male HPV Positive N=180	Female HPV Negative N=337	Female HPV Positive N=321	Parametric P-value*	Standardized Difference
Diagnosis Year (quartile)	>=2008, <=2011	N (Col%)	45 (37.5)	67 (37.22)	120 (35.61)	103 (32.09)	0.472	**0.114**
	>2011, <=2012	N (Col%)	37 (30.83)	57 (31.67)	101 (29.97)	89 (27.73)		0.086
	>2012, <=2013	N (Col%)	38 (31.67)	56 (31.11)	116 (34.42)	129 (40.19)		**0.190**
Charlson-Deyo Score	0	N (Col%)	98 (81.67)	120 (66.67)	276 (81.9)	264 (82.24)	**<.001**	**0.363**
	1+	N (Col%)	22 (18.33)	60 (33.33)	61 (18.1)	57 (17.76)		**0.363**
Agent of Chemotherapy	Agent Not Documented	N (Col%)	11 (9.17)	7 (3.89)	17 (5.04)	6 (1.87)	**<.001**	**0.324**
	Single-Agent	N (Col%)	14 (11.67)	15 (8.33)	14 (4.15)	31 (9.66)		**0.281**
	Multiagent	N (Col%)	95 (79.17)	158 (87.78)	306 (90.8)	284 (88.47)		**0.330**
Age at Diagnosis	Age < 50 years	N (Col%)	20 (16.67)	67 (37.22)	50 (14.84)	55 (17.13)	**<.001**	**0.528**
	Age >= 50 years	N (Col%)	100 (83.33)	113 (62.78)	287 (85.16)	266 (82.87)		**0.528**
Percent No High School Degree Quartiles 2000	>=29%	N (Col%)	26 (21.67)	40 (22.22)	60 (17.8)	43 (13.4)	0.226	**0.232**
	20-28.9%	N (Col%)	29 (24.17)	35 (19.44)	76 (22.55)	66 (20.56)		**0.115**
	14-19.9%	N (Col%)	26 (21.67)	44 (24.44)	71 (21.07)	76 (23.68)		0.081
	< 14%	N (Col%)	39 (32.5)	61 (33.89)	130 (38.58)	136 (42.37)		**0.205**

* The parametric p value is calculated by ANOVA for numerical covariates and Chi-Square test for categorical covariates.

Table 11: Patient Characteristics in Weighted Sample

Covariate	Level	Statistics	Gender/HPV Group				Parametric P-value*	Standardized Difference
			Male HPV Negative N=118	Male HPV Positive N=179	Female HPV Negative N=338	Female HPV Positive N=323		
AJCC Clinical Stage Group	Stage 2	N (Col%)	64 (54.18)	93 (52.51)	172 (50.88)	172 (53.32)	0.903	0.066
	Stage 3/3A/3B	N (Col%)	54 (45.82)	84 (47.49)	166 (49.12)	150 (46.68)		0.066
Facility Type	Community/Integrated Network Cancer Program	N (Col%)	26 (22.51)	40 (22.62)	71 (21.21)	69 (21.49)	0.999	0.034
	Comprehensive Community Cancer Program	N (Col%)	49 (41.48)	77 (43.13)	147 (43.6)	139 (43.03)		0.043
	Academic/Research Program	N (Col%)	42 (36.01)	61 (34.25)	118 (35.2)	114 (35.48)		0.037
Primary Payor	Not Insured/Unknown	N (Col%)	9 (7.77)	13 (7.45)	24 (7.23)	20 (6.48)	0.999	0.050
	Private	N (Col%)	58 (49.49)	93 (52.54)	177 (52.49)	164 (51.07)		0.061
	Medicaid	N (Col%)	11 (9.34)	14 (7.9)	28 (8.57)	31 (9.91)		0.071
	Medicare/Other Government	N (Col%)	39 (33.41)	57 (32.11)	107 (31.71)	105 (32.55)		0.036
Urban/Rural 2003	Metro	N (Col%)	103 (87.22)	151 (85.04)	286 (84.74)	270 (83.67)	0.985	**0.101**
	Urban	N (Col%)	13 (11.38)	24 (13.87)	46 (13.86)	47 (14.85)		**0.103**
	Rural	N (Col%)	1 (1.4)	1 (1.1)	4 (1.4)	4 (1.48)		0.034
Great Circle Distance in Miles (quartile)	Less than 4 miles	N (Col%)	30 (26.03)	47 (26.3)	81 (24.15)	84 (26.06)	0.999	0.050
	Between 4 and 8 miles	N (Col%)	28 (24.46)	47 (26.5)	87 (25.97)	84 (26.05)		0.047
	Between 8 to 18 miles	N (Col%)	29 (24.58)	43 (24.53)	83 (24.77)	73 (22.83)		0.045
	Greater than 18 miles	N (Col%)	29 (24.93)	40 (22.66)	84 (25.11)	80 (25.05)		0.057
Diagnosis Year (quartile)	>=2008, <=2011	N (Col%)	40 (34.29)	67 (37.92)	119 (35.25)	113 (35.19)	0.978	0.076
	>2011, <=2012	N (Col%)	38 (32.43)	48 (27.17)	98 (29.17)	96 (29.78)		**0.115**
	>2012, <=2013	N (Col%)	39 (33.29)	62 (34.91)	120 (35.58)	113 (35.02)		0.048
Charlson-Deyo Score	0	N (Col%)	95 (80.73)	138 (77.48)	264 (78.39)	251 (77.89)	0.916	0.080
	1+	N (Col%)	22 (19.27)	40 (22.52)	73 (21.61)	71 (22.11)		0.080

Covariate	Level	Statistics	Gender/HPV Group				Parametric P-value*	Standardized Difference
			Male HPV Negative N=118	Male HPV Positive N=179	Female HPV Negative N=338	Female HPV Positive N=323		
Agent of Chemotherapy	Agent Not Documented	N (Col%)	5 (4.71)	9 (5.05)	15 (4.54)	11 (3.53)	0.984	0.075
	Single-Agent	N (Col%)	9 (8.08)	12 (6.86)	25 (7.52)	26 (8.09)		0.047
	Multiagent	N (Col%)	103 (87.21)	157 (88.09)	297 (87.94)	285 (88.38)		0.036
Age at Diagnosis	Age < 50 years	N (Col%)	25 (21.24)	34 (19.55)	70 (20.98)	64 (19.97)	0.971	0.042
	Age >= 50 years	N (Col%)	93 (78.76)	143 (80.45)	267 (79.02)	258 (80.03)		0.042
Percent No High School Degree Quartiles 2000	>=29%	N (Col%)	20 (17.64)	32 (18.03)	61 (18.18)	60 (18.6)	0.996	0.025
	20-28.9%	N (Col%)	26 (22.38)	32 (18.44)	73 (21.63)	71 (22.2)		0.098
	14-19.9%	N (Col%)	24 (21.11)	43 (24.35)	77 (23)	70 (21.83)		0.077
	< 14%	N (Col%)	45 (38.87)	70 (39.18)	125 (37.19)	120 (37.37)		0.041

* The parametric p value is calculated by ANOVA for numerical covariates and Chi-Square test for categorical covariates.

Table 12: Patient Characteristics in Unweighted Male Sample (Alternative PS Analysis)

Covariate	Level	Statistics	Gender/HPV Group: Male HPV Negative N=68	Gender/HPV Group: Male HPV Positive N=232	Parametric P-value*	Standardized Difference
AJCC Clinical Stage Group	Stage 2	N (Col%)	36 (53.38)	115 (50.01)	0.624	0.068
	Stage 3/3A/3B	N (Col%)	31 (46.62)	115 (49.99)		0.068
Facility Type	Community/Integrated Network Cancer Program	N (Col%)	13 (19.07)	54 (23.63)	0.719	**0.112**
	Comprehensive Community Cancer Program	N (Col%)	26 (39.28)	83 (36.04)		0.067
	Academic/Research Program	N (Col%)	28 (41.65)	93 (40.33)		0.027
Primary Payor	Not Insured/Unknown	N (Col%)	3 (5.38)	28 (12.12)	0.155	**0.240**
	Private	N (Col%)	25 (37.23)	98 (42.65)		**0.111**
	Medicaid	N (Col%)	8 (11.85)	14 (6.45)		**0.188**
	Medicare/Other Government	N (Col%)	31 (45.54)	89 (38.78)		**0.137**
Urban/Rural 2003	Metro	N (Col%)	56 (82.74)	195 (84.52)	0.718	0.048
	Urban	N (Col%)	10 (15.19)	33 (14.58)		0.017
	Rural	N (Col%)	1 (2.07)	2 (0.89)		0.098
Great Circle Distance in Miles (quartile)	Less than 4 miles	N (Col%)	20 (30.28)	66 (28.74)	0.111	0.034
	Between 4 and 8 miles	N (Col%)	13 (19.38)	77 (33.51)		**0.325**
	Between 8 to 18 miles	N (Col%)	14 (21.49)	32 (13.82)		**0.202**
	Greater than 18 miles	N (Col%)	19 (28.85)	55 (23.93)		**0.112**
Diagnosis Year (quartile)	>=2008, <=2011	N (Col%)	25 (37.4)	87 (37.87)	0.914	0.010
	>2011, <=2012	N (Col%)	21 (31.65)	67 (29.16)		0.054
	>2012, <=2013	N (Col%)	21 (30.95)	76 (32.97)		0.043
Charlson-Deyo Score	0	N (Col%)	55 (80.74)	134 (58.1)	**<.001**	**0.507**
	1+	N (Col%)	13 (19.26)	97 (41.9)		**0.507**

Covariate	Level	Statistics	Gender/HPV Group: Male HPV Negative N=68	Gender/HPV Group: Male HPV Positive N=232	Parametric P-value*	Standardized Difference
Agent of Chemotherapy	Agent Not Documented	N (Col%)	5 (7.46)	7 (3.35)	0.239	**0.183**
	Single-Agent	N (Col%)	7 (10.96)	18 (8.11)		0.097
	Multiagent	N (Col%)	55 (81.58)	205 (88.54)		**0.196**
Age at Diagnosis	Age < 50 years	N (Col%)	11 (16.56)	122 (52.97)	**<.001**	**0.827**
	Age >= 50 years	N (Col%)	56 (83.44)	108 (47.03)		**0.827**
Percent No High School Degree Quartiles 2000	>=29%	N (Col%)	14 (21.93)	48 (21.03)	0.739	0.022
	20-28.9%	N (Col%)	15 (22.13)	43 (18.64)		0.087
	14-19.9%	N (Col%)	15 (22.56)	67 (29.15)		**0.151**
	< 14%	N (Col%)	22 (33.38)	72 (31.18)		0.047

* The parametric p value is calculated by ANOVA for numerical covariates and Chi-Square test for categorical covariates.

Table 13: Patient Characteristics in Unweighted Female Sample (Alternative PS Analysis)

			Gender/HPV Group			
Covariate	**Level**	**Statistics**	**Female HPV Negative N=274**	**Female HPV Positive N=384**	**Parametric P-value***	**Standardized Difference**
AJCC Clinical Stage Group	Stage 2	N (Col%)	153 (56)	174 (45.43)	**0.008**	**0.213**
	Stage 3/3A/3B	N (Col%)	120 (44)	209 (54.57)		**0.213**
Facility Type	Community/Integrated Network Cancer Program	N (Col%)	61 (22.55)	85 (22.27)	0.875	0.007
	Comprehensive Community Cancer Program	N (Col%)	119 (43.82)	175 (45.73)		0.038
	Academic/Research Program	N (Col%)	91 (33.64)	123 (32)		0.035
Primary Payor	Not Insured/Unknown	N (Col%)	14 (5.21)	35 (9.25)	0.137	**0.157**
	Private	N (Col%)	155 (56.75)	225 (58.65)		0.038
	Medicaid	N (Col%)	24 (8.91)	32 (8.57)		0.012
	Medicare/Other Government	N (Col%)	79 (29.13)	90 (23.53)		**0.128**
Urban/Rural 2003	Metro	N (Col%)	229 (84.02)	322 (83.95)	0.304	0.002
	Urban	N (Col%)	40 (14.81)	50 (13.21)		0.046
	Rural	N (Col%)	3 (1.17)	10 (2.84)		**0.120**
Great Circle Distance in Miles (quartile)	Less than 4 miles	N (Col%)	56 (20.84)	100 (26.26)	0.460	**0.128**
	Between 4 and 8 miles	N (Col%)	73 (26.98)	96 (25.22)		0.040
	Between 8 to 18 miles	N (Col%)	74 (27.34)	98 (25.62)		0.039
	Greater than 18 miles	N (Col%)	67 (24.84)	88 (22.9)		0.046
Diagnosis Year (quartile)	>=2008, <=2011	N (Col%)	97 (35.6)	119 (30.96)	0.182	0.099
	>2011, <=2012	N (Col%)	81 (29.82)	105 (27.44)		0.053
	>2012, <=2013	N (Col%)	94 (34.57)	159 (41.6)		**0.145**
Charlson-Deyo Score	0	N (Col%)	223 (81.73)	318 (82.96)	0.683	0.032
	1+	N (Col%)	49 (18.27)	65 (17.04)		0.032
Agent of Chemotherapy	Agent Not Documented	N (Col%)	10 (3.98)	9 (2.45)	**<.001**	0.087
	Single-Agent	N (Col%)	10 (3.67)	55 (14.38)		**0.380**
	Multiagent	N (Col%)	252 (92.35)	319 (83.18)		**0.283**

Covariate	Level	Statistics	Gender/HPV Group: Female HPV Negative N=274	Gender/HPV Group: Female HPV Positive N=384	Parametric P-value*	Standardized Difference
Age at Diagnosis	Age < 50 years	N (Col%)	39 (14.51)	64 (16.9)	0.410	0.066
	Age >= 50 years	N (Col%)	233 (85.49)	319 (83.1)		0.066
Percent No High School Degree Quartiles 2000	>=29%	N (Col%)	46 (16.97)	53 (13.89)	0.532	0.085
	20-28.9%	N (Col%)	60 (22.3)	77 (20.03)		0.056
	14-19.9%	N (Col%)	58 (21.44)	93 (24.28)		0.068
	< 14%	N (Col%)	107 (39.28)	160 (41.8)		0.051

* The parametric p value is calculated by ANOVA for numerical covariates and Chi-Square test for categorical covariates.

Table 14: PS Weighted Multivariable Analysis of Overall Survival – Interaction with Gender

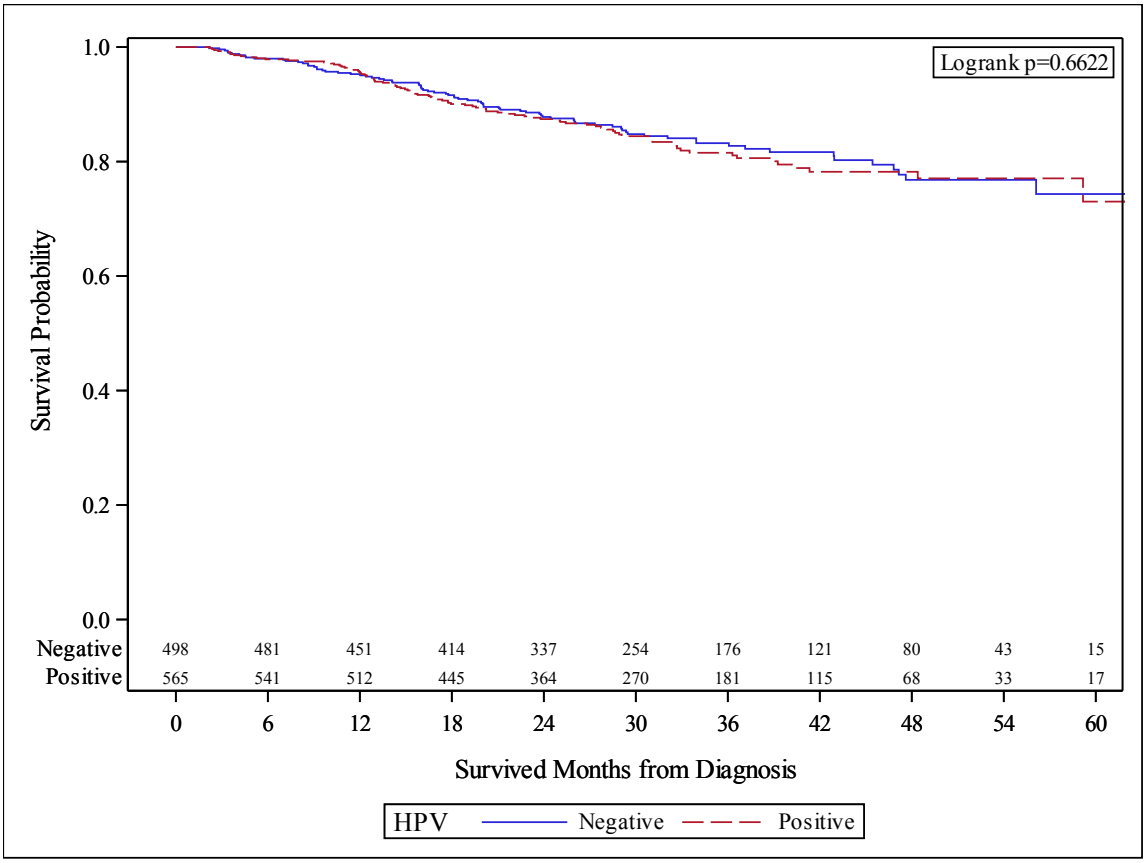
Covariate	Level	Survived Months from Diagnosis Hazard Ratio (95% CI)	HR P-value	Type3 P-value
Comparisons Stratified by Sex :	**HPV :**	-	-	**0.006**
Male	Positive vs. Negative	0.60 (0.38-0.96)	**0.034**	-
Female	Positive vs. Negative	1.47 (0.96-2.25)	0.074	-

* Number of observations in the original data set = 958. Number of observations used = 958.
** Backward selection with an alpha level of removal of .20 was used. No variables were removed from the model.
*** The estimated stratified treatement effect was controlled by: None

Table 15: Diagram of Selection/Exclusion Criteria

Selection and Exclusion Criteria	Sample Size	Excluded
NCDB Anus PUF Cancer Cases	54069	-
Include Behavior=invasive, Sequence Number in 0&1, Class of Case >0	37300	16769
Include Primary Sites Anus C210 and Anal Canal Only C211	31145	6155
Include Clinical Stage II-IIIB, Exclude Metastasis Cases	17815	13330
Include Histology as 807, Squamous Cell	14939	2876
Include DIAGNOSTIC_CONFIRMATION = 1	14831	108
Include Cases with Concurrent Chemo-radiation (within 2 weeks of start date)	12461	2370
Include Cases with desired parameter for radiation: Radiation Dose between 4400 and 7000, Radiation Volume in Anus, Radiation Modality not in 40 42 50 53 54	9870	2591
Include HPV Status Positive or Negative	1425	8445
Exclude Missing Outcome	1063	362

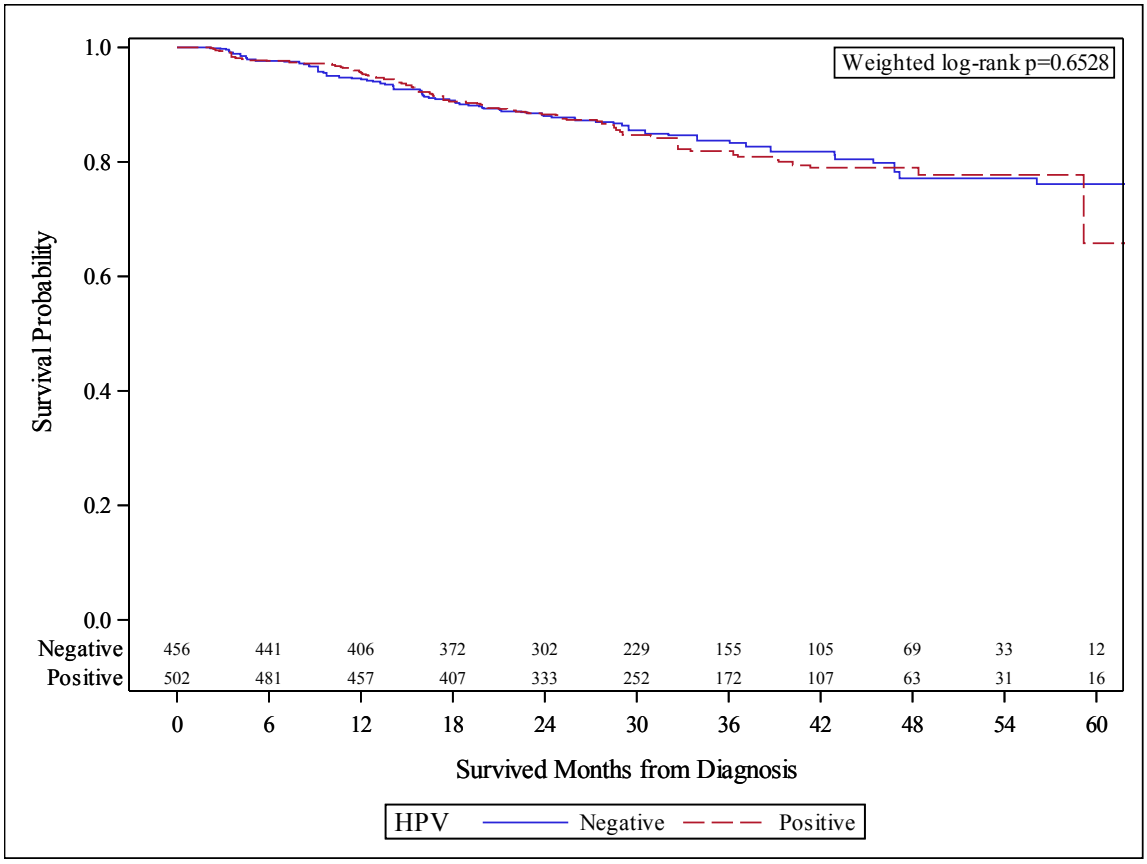
Figure 1: Preliminary Kaplan-Meier Plot of Study Cohorts



HPV	No. of Subject	Event	Censored	Median Survival (95% CI)	6 Mo Survival	12 Mo Survival	18 Mo Survival	24 Mo Survival	30 Mo Survival	36 Mo Survival
Negative	498	80 (16%)	418 (84%)	NA (NA, NA)	98.0% (96.3%, 98.9%)	95.3% (93.0%, 96.8%)	91.6% (88.7%, 93.8%)	87.8% (84.4%, 90.5%)	84.8% (81.0%, 87.9%)	83.2% (79.1%, 86.6%)
Positive	565	93 (16%)	472 (84%)	NA (64.7, NA)	97.9% (96.3%, 98.8%)	95.6% (93.6%, 97.1%)	90.1% (87.2%, 92.3%)	87.4% (84.2%, 90.0%)	84.4% (80.8%, 87.4%)	81.5% (77.5%, 85.0%)

42 Mo Survival	48 Mo Survival	54 Mo Survival	60 Mo Survival
81.7% (77.2%, 85.3%)	76.8% (70.9%, 81.7%)	76.8% (70.9%, 81.7%)	74.3% (66.5%, 80.6%)
78.2% (73.4%, 82.3%)	78.2% (73.4%, 82.3%)	77.1% (71.7%, 81.6%)	73.0% (62.7%, 80.9%)

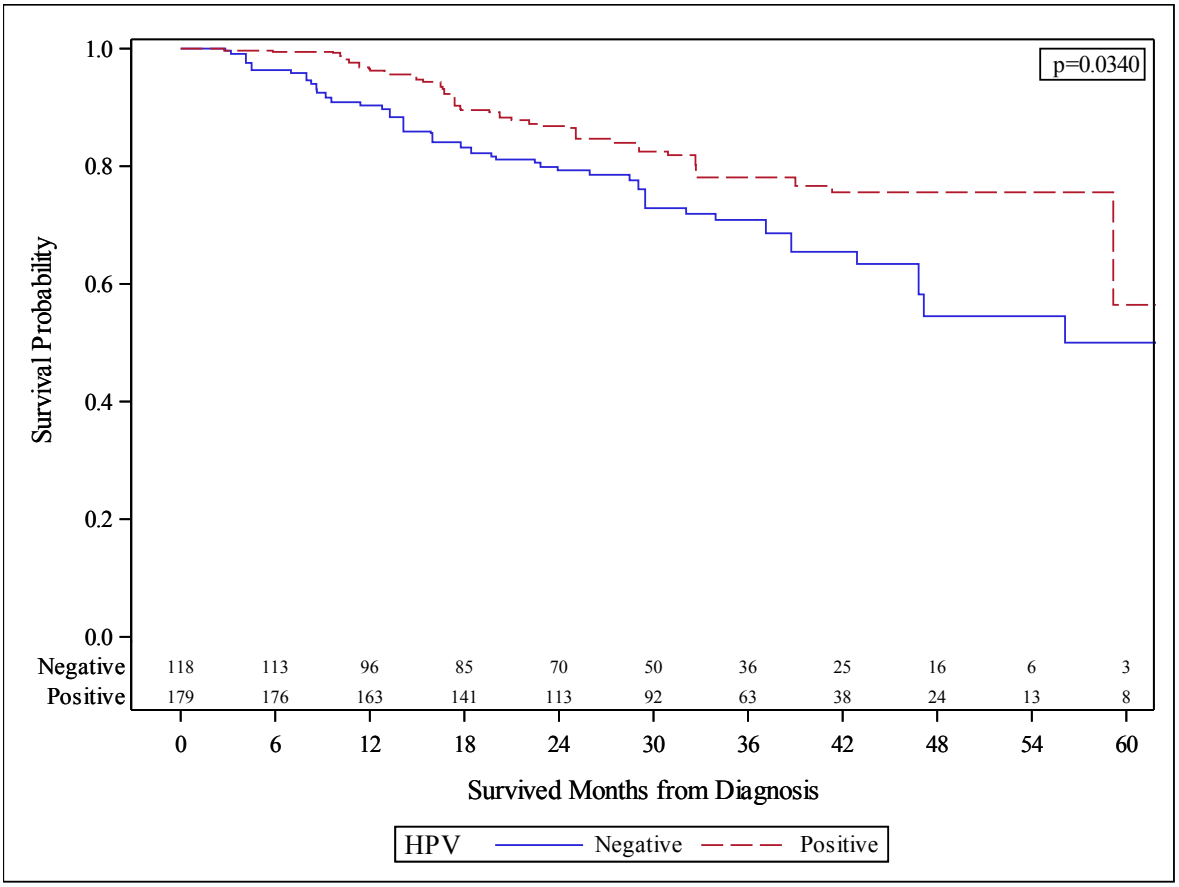
Figure 2: Weighted Kaplan-Meier of All Patients



HPV	No. of Subject	Event	Censored	Median Survival (95% CI)	6 Mo Survival	12 Mo Survival	18 Mo Survival	24 Mo Survival	30 Mo Survival	36 Mo Survival
Negative	457	70 (15%)	387 (85%)	NA (NA, NA)	97.6% (95.5%, 98.8%)	94.6% (91.8%, 96.5%)	90.7% (87.3%, 93.3%)	88.0% (84.2%, 90.9%)	85.5% (81.3%, 88.9%)	83.7% (79.1%, 87.4%)
Positive	501	81 (16%)	420 (84%)	64.7 (59.2, NA)	97.7% (95.6%, 98.8%)	95.7% (93.1%, 97.3%)	90.5% (87.1%, 93.1%)	88.3% (84.5%, 91.2%)	84.7% (80.3%, 88.2%)	81.9% (77.0%, 85.9%)

42 Mo Survival	48 Mo Survival	54 Mo Survival	60 Mo Survival
81.8% (76.7%, 85.9%)	77.1% (70.3%, 82.6%)	77.1% (70.3%, 82.6%)	76.1% (68.4%, 82.2%)

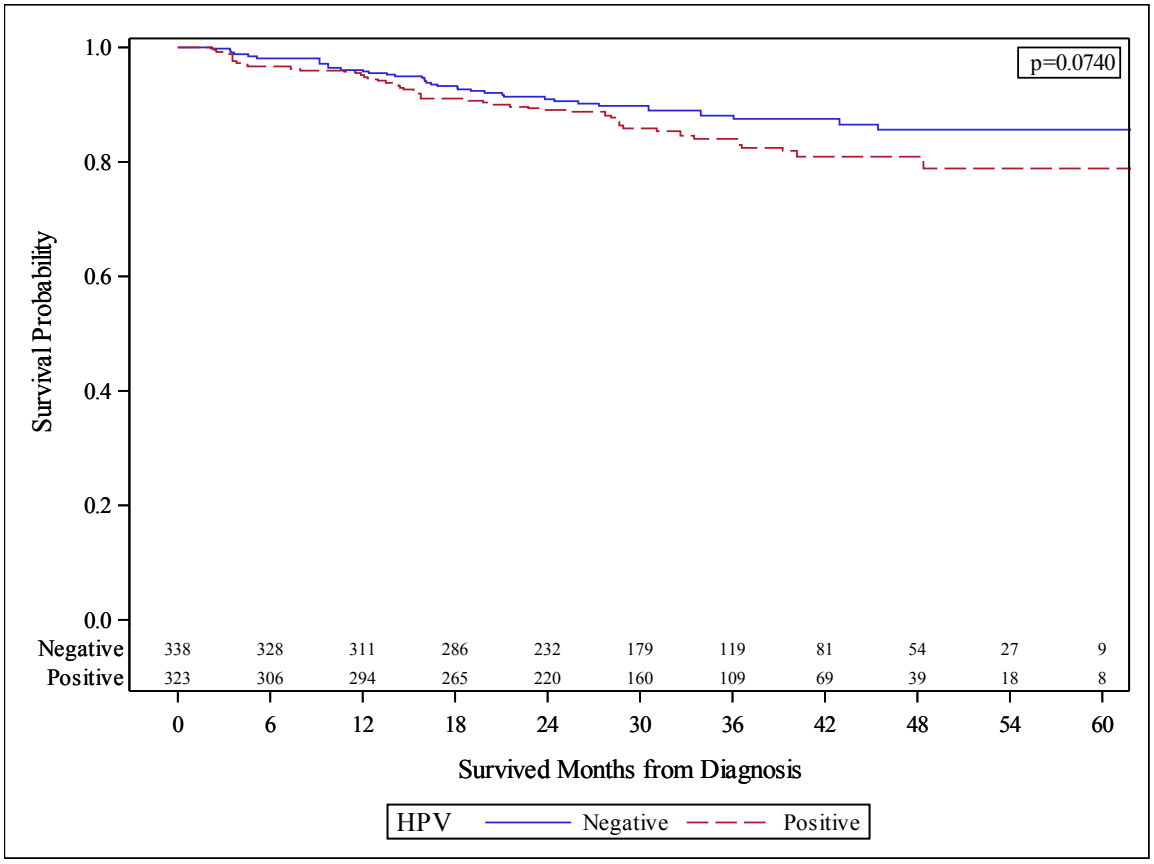
Figure 3: Weighted Kaplan-Meier (Males)



HPV	No. of Subject	Event	Censored	Median Survival (95% CI)	6 Mo Survival	12 Mo Survival	18 Mo Survival	24 Mo Survival	30 Mo Survival	36 Mo Survival
Negative	120	36 (30%)	84 (70%)	NA (42.9, NA)	96.3% (89.6%, 98.7%)	90.3% (81.9%, 94.9%)	83.2% (73.6%, 89.5%)	79.3% (69.1%, 86.5%)	72.9% (61.5%, 81.4%)	70.9% (59.2%, 79.8%)
Positive	180	34 (19%)	146 (81%)	NA (59.2, NA)	99.4% (94.5%, 99.9%)	96.6% (91.3%, 98.7%)	89.5% (82.3%, 93.9%)	86.8% (78.9%, 91.9%)	82.5% (73.5%, 88.6%)	78.1% (68.1%, 85.3%)

42 Mo Survival	48 Mo Survival	54 Mo Survival	60 Mo Survival
65.5% (52.5%, 75.7%)	54.5% (39.2%, 67.5%)	54.5% (39.2%, 67.5%)	50.0% (31.0%, 66.4%)
75.6% (64.4%, 83.7%)	75.6% (64.4%, 83.7%)	75.6% (64.4%, 83.7%)	56.4% (29.4%, 76.5%)

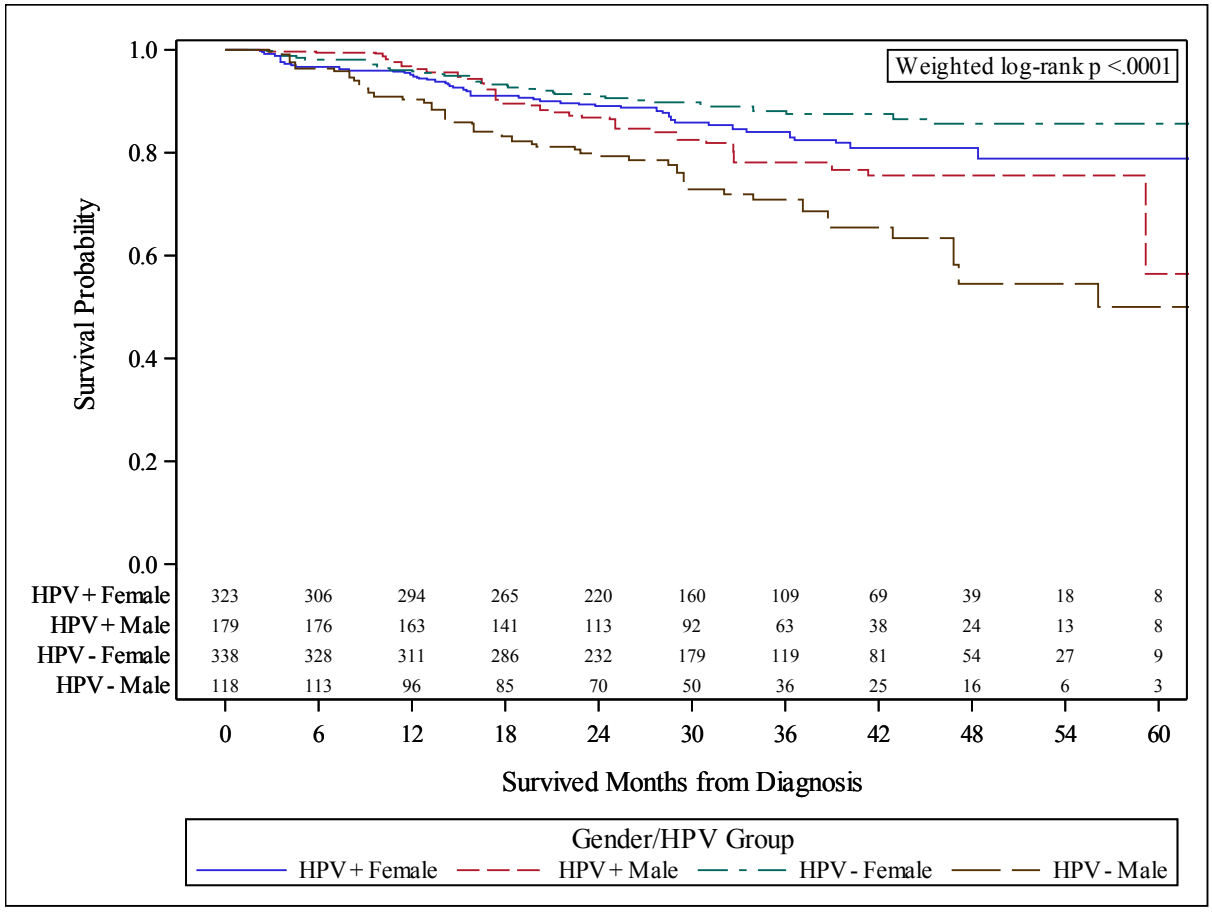
Figure 4: Weighted Kaplan-Meier (Females)



HPV	No. of Subject	Event	Censored	Median Survival (95% CI)	6 Mo Survival	12 Mo Survival	18 Mo Survival	24 Mo Survival	30 Mo Survival	36 Mo Survival
Negative	337	34 (10%)	303 (90%)	NA (NA, NA)	98.1% (95.7%, 99.2%)	96.0% (93.1%, 97.7%)	93.3% (89.7%, 95.6%)	90.9% (86.9%, 93.8%)	89.8% (85.5%, 92.9%)	88.1% (83.2%, 91.6%)
Positive	321	47 (15%)	274 (85%)	64.7 (64.7, NA)	96.7% (93.7%, 98.3%)	95.2% (91.8%, 97.2%)	91.1% (86.9%, 93.9%)	89.1% (84.6%, 92.3%)	85.9% (80.6%, 89.8%)	84.0% (78.3%, 88.4%)

42 Mo Survival	48 Mo Survival	54 Mo Survival	60 Mo Survival
87.5% (82.5%, 91.2%)	85.6% (79.4%, 90.1%)	85.6% (79.4%, 90.1%)	85.6% (79.4%, 90.1%)
80.9% (74.0%, 86.2%)	80.9% (74.0%, 86.2%)	78.9% (70.0%, 85.4%)	78.9% (70.0%, 85.4%)

Figure 5: Weighted Kaplan-Meier of All Propensity Score Groups



Gender HPV Group	No. of Subject	Event	Censored	Median Survival (95% CI)	6 Mo Survival	12 Mo Survival	18 Mo Survival	24 Mo Survival	30 Mo Survival	36 Mo Survival
HPV + Female	321	47 (15%)	274 (85%)	64.7 (64.7, NA)	96.7% (93.7%, 98.3%)	95.2% (91.8%, 97.2%)	91.1% (86.9%, 93.9%)	89.1% (84.6%, 92.3%)	85.9% (80.6%, 89.8%)	84.0% (78.3%, 88.4%)
HPV + Male	180	34 (19%)	146 (81%)	NA (59.2, NA)	99.4% (94.5%, 99.9%)	96.6% (91.3%, 98.7%)	89.5% (82.3%, 93.9%)	86.8% (78.9%, 91.9%)	82.5% (73.5%, 88.6%)	78.1% (68.1%, 85.3%)
HPV - Female	337	34 (10%)	303 (90%)	NA (NA, NA)	98.1% (95.7%, 99.2%)	96.0% (93.1%, 97.7%)	93.3% (89.7%, 95.6%)	90.9% (86.9%, 93.8%)	89.8% (85.5%, 92.9%)	88.1% (83.2%, 91.6%)
HPV - Male	120	36 (30%)	84 (70%)	NA (42.9, NA)	96.3% (89.6%, 98.7%)	90.3% (81.9%, 94.9%)	83.2% (73.6%, 89.5%)	79.3% (69.1%, 86.5%)	72.9% (61.5%, 81.4%)	70.9% (59.2%, 79.8%)

42 Mo Survival	48 Mo Survival	54 Mo Survival	60 Mo Survival
80.9% (74.0%, 86.2%)	80.9% (74.0%, 86.2%)	78.9% (70.0%, 85.4%)	78.9% (70.0%, 85.4%)

42 Mo Survival	48 Mo Survival	54 Mo Survival	60 Mo Survival
75.6% (64.4%, 83.7%)	75.6% (64.4%, 83.7%)	75.6% (64.4%, 83.7%)	56.4% (29.4%, 76.5%)
87.5% (82.5%, 91.2%)	85.6% (79.4%, 90.1%)	85.6% (79.4%, 90.1%)	85.6% (79.4%, 90.1%)
65.5% (52.5%, 75.7%)	54.5% (39.2%, 67.5%)	54.5% (39.2%, 67.5%)	50.0% (31.0%, 66.4%)

Figure 6: Distribution of Generalized Propensity Scores

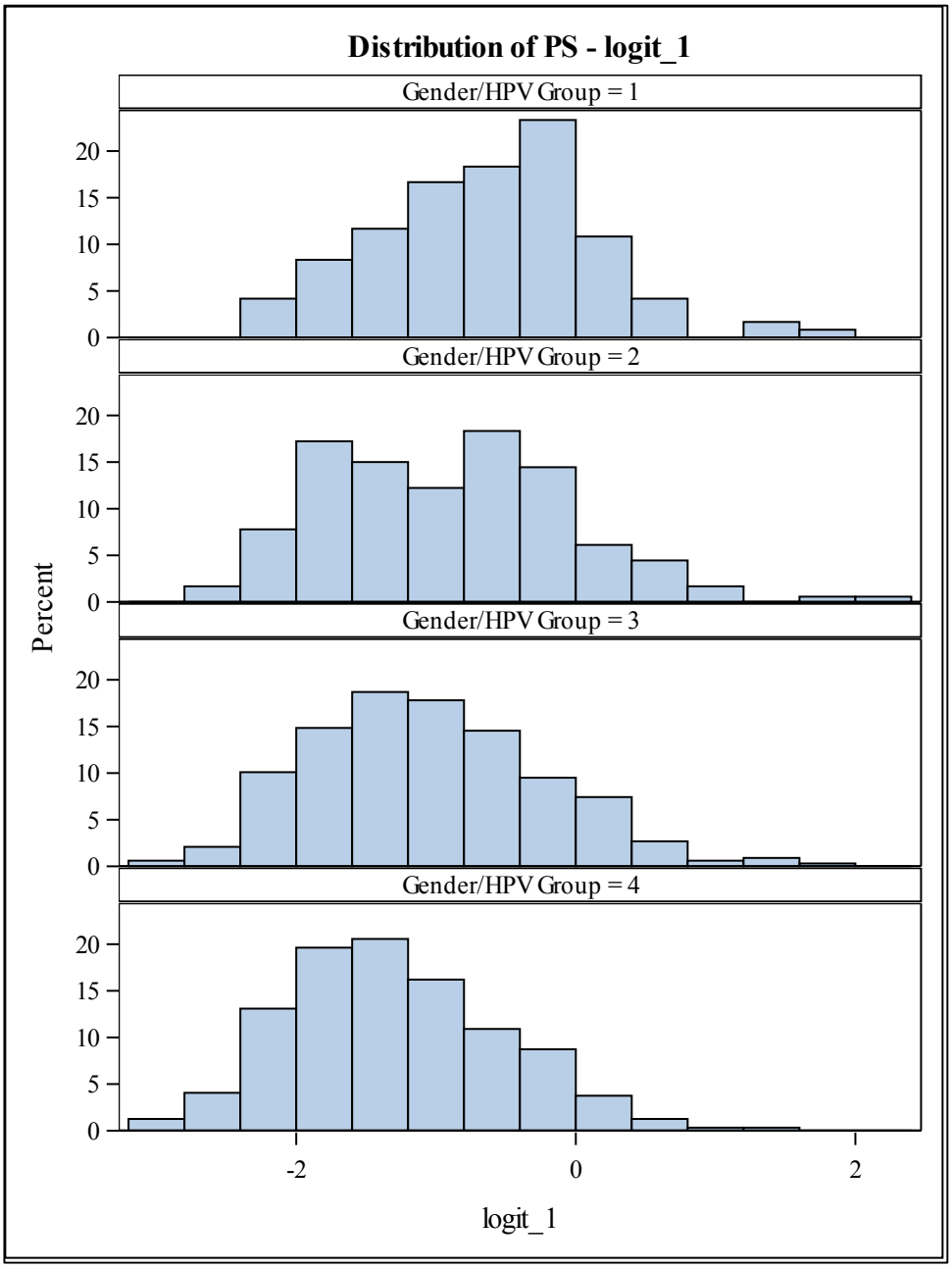


Figure 7: Distribution of Propensity Scores for Males (2-Group PS Analysis)

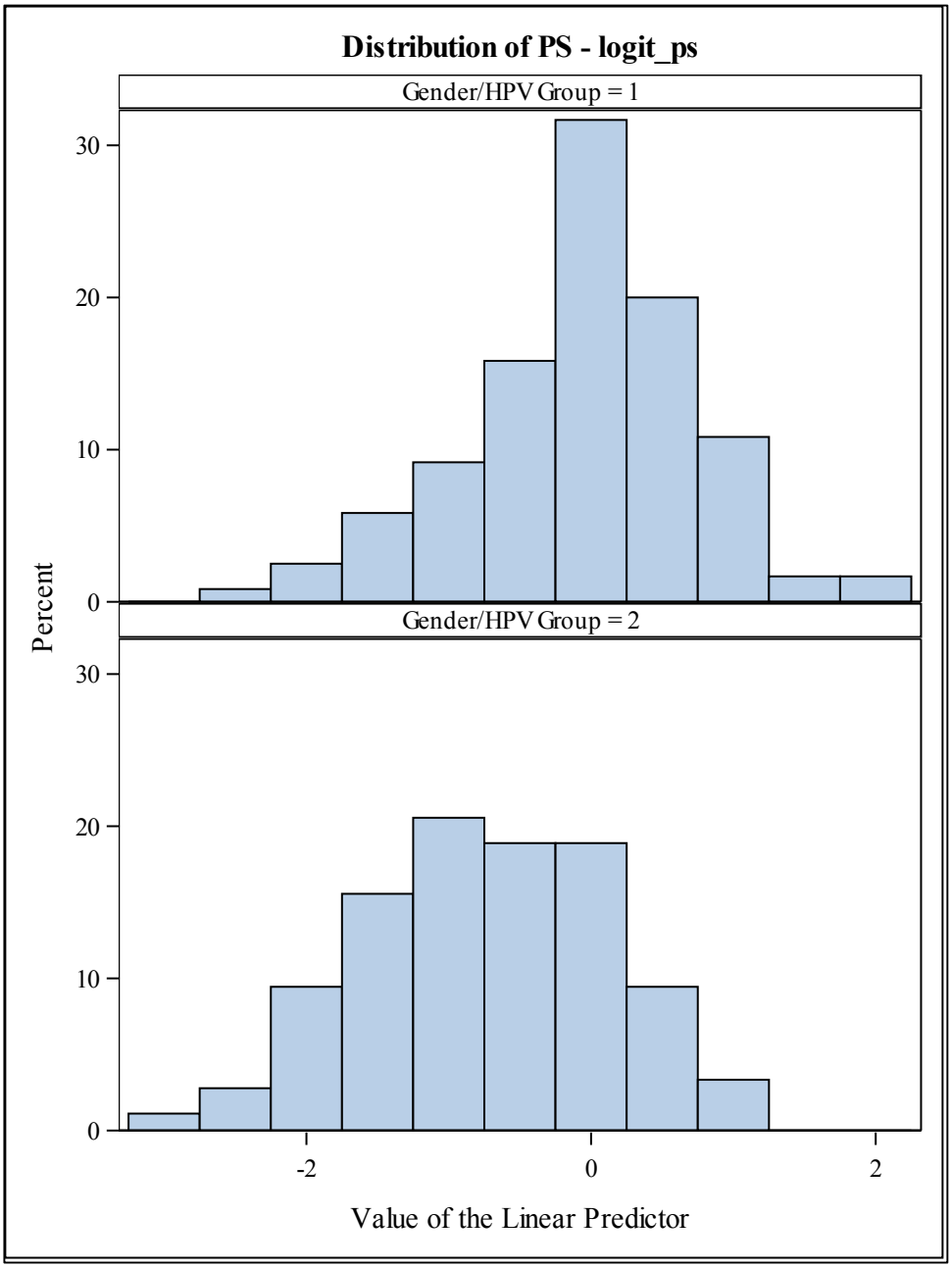


Figure 8: Distribution of Propensity Scores for Females (2-Group PS Analysis)