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Investigation of Multiple Biomarkers in Predicting the Disease Free Survival and Overall Survival among Head and Neck Cancer Patients

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2015

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An abstract of
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Abstract

Investigation of Multiple Biomarkers in Predicting the Disease Free Survival and Overall Survival among Head and Neck Cancer Patients

By Bokai Zhao

Background: Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy worldwide. This paper investigated the clinical factors associated with tumor response by different treatments and identify the biomarkers associated with disease free survival and overall survival among head and neck cancer patients.
Methods: Descriptive analysis was used to describe the demographic and clinical variables. Logistic regression was fitted to determine the risk factor for whether patients went through recurrence. Survival analysis was performed to identify which biomarker(s) would be responsible for patients' OS or DFS. After univariate analysis, hazard ratio and p-value for each potential risk factor was calculated. Forward model selection was applied to determine the final logistic regression model. Kaplan Meier Curves, Supremum test for proportional hazards assumption and the plots of the standardized score process were applied.
Results: The odds of a HNSCC reoccurrence within Age >= 60 PS group was approximately 2.7 times the odds for Age < 60 PS group; the odds of HNSCC reoccurrence for patients whose P16 status were negative was 0.14 times the odds for patients whose P16 status were positive. OS and DFS were also significantly associated with HNSCC recurrence. We didn't find significance in the odds ratio among different levels of any biomarkers with recurrence. For OS, the chance of dying for patients in HER3 < 0.5 was nearly 4 times the chance of dying for patients in HER3 > 0.5. For DFS, the relative risk of PD-L1 in Peritumoral Stroma Level 1 vs Level 4 was approximately 8 with p-value equaling to 0.0291.
Conclusion: In order to improve HNSCC patients' overall survival further, new and less toxic treatment as well as improving patients' general well-being and daily activities were crucial, as P16, PD-L1 and HER3 accounted for a large amount of patients' recurrence and OS. Further studies would be needed to find both new treatment strategies and ways to provide better patients care.

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Table of Contents

1. Introduction

Head and neck cancers are collectively referred to cancers which usually begin with squamous cells on the surface of the mucous membranes in the head and neck (for instance, in the mouth, nose, and throat). These squamous cell cancers above are often referred to as squamous cell carcinomas of the head and neck. Salivary gland cancers are relatively uncommon cases, yet, head and neck cancers can also begin in the salivary glands.

As a common class of cancers, head and neck cancers account for approximately 4% of all cancers in the United States. In 2017, more than 65,000 men and women in this country diagnosed with head and neck cancers[1]. The proportion of these cancers in men is more than twice that of women[2]. Head and neck cancers in people over the age of 50 are also more likely to be diagnosed than younger people.

The causes of head and neck cancers could be various. The use of alcohol and tobacco are the two most important risk factors for head and neck cancers, especially for cancers of the oral cavity, oropharynx, hypopharynx, and larynx[3–5]. At least 75% of head and neck cancers are caused by tobacco and alcohol use[6]. People who are both smoker and drinker are at greater risk of developing these cancers than people who either use tobacco or drink alcohol alone[6–8]. Worth to mention, the use of tobacco and alcohol are not risk factors for salivary gland cancers.

Besides, infection with cancer-causing types of human papillomavirus (HPV), particularly HPV type 16, may result in some types of head and neck cancers, especially oropharyngeal cancers that involve the tonsils or the base of the tongue[9,10].

The potential prognostic biomarkers which are counted important in predicting recurrence, disease free survival and overall survival in this study include EGFR, Her3, Her2, PD-L1 and PD-L2. Among them, EGFR (the abbreviation of epidermal growth factor receptor, also known as ErbB-1; HER1 in humans) is a transmembrane protein. It is a receptor for members of the epidermal growth factor family (EGF family) of extracellular protein ligands. Mutations that cause EGFR overexpression (called up-regulation or amplification) are associated with many cancers, including anal cancer, 40% of cases with lung adenocarcinoma[11,12], 50% of cases with glioblastoma and 80%-100% of cases with epithelium tumors in the head and neck[13]. PD-L1 stands for programmed death-ligand 1. As a scientific validation method to reactivate anti-tumor immune responses, PD-L1 pathway inhibition plays an important role in restoring the cancer immune cycle. Its inhibition is a key step in cancer immunotherapy research[14].

Although from the prior research, we've already known the relationship between biomarkers like EGFR with other tumor cases, the study of biomarkers' mechanism in Head and neck squamous cell carcinoma (HNSCC) is still a blank. In this paper, our objective is to investigate the clinical factors associated with tumor response by different treatments and identify the biomarkers associated with disease free survival and overall

survival among head and neck cancer patients based on a study conducted by Winship Cancer Institution, whose subjects are patients diagnosed with cancers of head and neck.

2. Method

2.1 Data Collection

HNSCC Tissue Samples

The clinical information on the samples was obtained from the surgical pathology reports in the Department of Pathology at Emory University. All of the clinical data analyses were conducted with de-identified records in compliance with the Health Insurance Portability and Accountability Act. Patient tissues (n=108) for this study were obtained from the surgical specimens of patients who were diagnosed with HNSCC at the Emory University Hospital and had no prior treatment with radiation and/or chemotherapy. Patient samples includes a training set to establish a predictive model for HNSCC reoccurrence, which comprised primary SCC samples from 22 patients with reoccurrence and 72 patients without reoccurrence. Erlotinib (1 mM) was used to inhibit the activation of EGFR.

Tissues from the primary tumor were used in the study. Patients' general characteristics are summarized in Table 1.

2.2 Statistical Analysis Method

2.2.1 Descriptive analysis

The descriptive table for patients' characteristics was firstly constructed. For continuous variables, the mean and standard deviation were summarized. For binary or categorical variables, the frequencies and percentage were presented. The descriptive statistics of risk factors were summarized in groups of patients who achieved complete remission and who did not separately.

2.2.2 Logistic Regression

Since the dependent variable had binary outcomes, a linear logistic regression model was fitted. The standard logistic function was used:

$$\sigma(t) = \frac{e^t}{e^t + 1} = \frac{1}{1 + e^{-t}}$$

Because a linear logistic regression was used here, t is a linear function of the independent variable X. The function of t can be expressed as:

$$t = \beta_0 + \beta_1 x$$

Then the logistic function can be written as a function of X:

$$f(x) = \frac{1}{1 + e^{-(\beta_0 + \beta_1 x)}}$$

f(x) is the probability of the dependent variable (outcome) getting a "successful" result. The univariate analysis was performed, the crude odds ratio and 95% confidence interval were calculated for each risk factor to present a general idea of the association between outcome and a single independent variable. The odds for each risk factor is $e^{\beta_0 + \beta_1 x}$, then

the odds ratio can be presented as $\frac{e^{\beta_0+\beta_1 x_1}}{e^{\beta_0+\beta_1 x_0}}$. All categorical variables were reference cell coded.

The multivariable analysis was also performed. Backward selection (or Backward elimination) based on AIC was conducted to select the best linear logistic model to predict the outcome. The general backward selection procedure is as following:

Step 1: Fit a univariate logical model. Calculate the reduction of the from the full model with all independent variables to the current model. The risk factor for the relative reduction in AIC is deleted from the model and all other remained variables will be included in the model of the selection process below.

Step 2: Eliminate another risk factor to the model at a time and calculate the AIC value based on the model selected from step 1. As step 1, the risk factor with the largest AIC reduction is eliminated from the model.

Step 3: Repeat step 2 until the AIC is not reduced when the risk factor is deleted. The model with the smallest AIC is the final model chosen by backward selection.

The adjusted odds ratio of the multivariate model effect is calculated using the final model selected by the backward selection.

2.2.3 Log Rank Test

For survival analysis, the estimated Kaplan Meier survival curves were plotted for each risk factor level to have a general view of the overall survival of patients with different risk factors and as a check for hypotheses of the proportional risk hypothesis. The log rank test is a popular test to test the null hypothesis of no difference in survival between two or more independent groups. The test compares the entire survival experience between groups and can be

thought of as a test of whether the survival curves are identical (overlapping) or not. Survival curves are estimated for each group, considered separately, using the Kaplan-Meier method and compared statistically using the log rank test.

2.2.4 Cox Proportional Hazard model formulation

The Cox proportional hazard model was constructed. The proportional hazard model always consists of two parts: a basic baseline hazard function that describes how the event risk changes at the baseline level of the covariate; and an effect parameter that demonstrates the change in risk based on the covariates. The form of hazard function for the Cox proportional hazard model is:

$$\lambda(t|X_i) = \lambda_0(t)\exp\left(\beta X_{i1} + \cdots + \beta X_{ip}\right) = \lambda_0(t)\exp(\beta X_i)$$

$\lambda(t|X_i)$ is the hazard rate given time t for subject i with covariate X_i. Local Wald test was performed to determine if there were any significant differences between the levels of difference in the covariates. The score test is equivalent to the log rank test here, which gives us some insights from a non-parametric perspective.

Backward model selection was performed again for survival analysis. Adjusted hazard ratio was calculated for each risk factor in multivariable model. Local Wald tests were conducted for each variable in the final model and p-value were output.

2.2.5 Evaluate assumption

When fitting the Cox PH model, we assume that the review time is independent to ensure reliable and unbiased survival estimates. We also assume that the review is non-informative, which means that we believe that the reason for the review is not related to the medical status of the participants. Since the Cox PH model also assumes that the ratio

of the two risk functions is always time-independent, the Supremum test of the proportional hazard hypothesis and the chart of the standardized scoring process are applied.

2.3 Statistical Analysis Plan

Descriptive statistics were first used to summarize the characteristics for each patient. To assess the correlations between categorical clinical factors and numerical biomarker variables, t-test or ANOVA tests were conducted when data followed a normal distribution, otherwise Wilcoxon rank sum test or Kruskal-Wallis test were used instead. Pearson correlation coefficients were calculated to measure the correlation between two numerical variables, and the significance of coefficients were tested using Wald's test. For disease free survival (DFS), disease progression or death from any cause was defined as the event. Time of DFS was calculated as the time from study enrollment to disease progression date, death date, or last contact whichever comes first. For overall survival (OS), death from any cause was defined as the event. Time of OS was calculated as the time from study enrollment to death or last contact. For both DFS and OS, patients were censored at time of last follow-up. OS and DFS rates of two patient groups stratified by each biomarker or other factors were estimated with the Kaplan-Meier method and compared between different groups using the log-rank test, respectively. The DFS and OS of each patient group at specific time points, such as 1 year, 3 years, and 5 years, etc. were also estimated alone with 95% CI. Cox proportional hazards models were further used in the multivariable analyses to assess adjusted effects of biomarkers on the patients' OS and DFS after adjusting for other factors. The proportional hazards assumption was evaluated graphically and analytically with regression diagnostics. All

data management and statistical analysis were conducted using SAS Version 9.4 (SAS Institute, Inc., Cary, North Carolina). The significance level was set to 0.05.

3. Results

3.1 Descriptive analysis

The results of univariate analysis were shown in table 1. From the table, the mean age of the patients was 57.4 with significant difference between patients who suffered HNSCC reoccurrence and who did not (p-value=0.022). Most of the patients' race are White (83.0%), and only 16 patients are from AA, Hispanic or other races (17.0%). The patients from different races also performs significant diverse in expression of reoccurrence (p-value=0.049). For patients who had HNSCC reoccurrence, the average time from treatment to relapse were 28.4. However, for patients who did not who had HNSCC reoccurrence, the average time from treatment to relapse were 64.3. There is a trend of strong association between P16 status and whether suffering reoccurrence which was proved in the following analysis.

3.2 Univariate and multivariable logistic regression

The crude odds ratio and confidence interval for each variable on its own fitted in the model were shown in table 2. For binary risk factors, the reference cell coding was used and 2 was coded as reference group. For categorical risk factors with more 2 levels, the last level was coded as reference group. From the result of crude odds ratio, the odds of a HNSCC reoccurrence within Age >= 60 PS group was approximately 2.7 times the odds

for Age < 60 PS group; the odds of HNSCC reoccurrence for patients whose P16 status were negative was 0.14 times the odds for patients whose P16 status were positive. The odds of HNSCC reoccurrence for patients whose DFS within 24-48 month was 0.011 times the odds of HNSCC reoccurrence for patients whose DFS less than 24 month. The odds of HNSCC reoccurrence for patients whose OS within 24-48 month was 0.215 times the odds of HNSCC reoccurrence for patients whose OS less than 24 month. Age at diagnose, P16 status, OS from date of diagnose (categorical) and time from treatment to relapse (categorical) were significantly associated with HNSCC reoccurrence with p-value equaling to 0.0454, 0.0002, 0.0158 and 0.0002 separately. We didn't find significance in the odds ratio among different levels of any biomarkers with reoccurrence.

After the step I of general backward variable selection procedure, time from treatment to relapse (DFS Month categorical) and p16 status (Negative or Positive) was selected (AIC=82.451). After deleting the other variables to the model selected from step 1, all AIC increased, so the final model only had two risk factors which were (DFS Month categorical) and p16 status.

3.3 Survival Analysis

3.3.1 Kaplan-Meier Method and Stratified Log-rank Test

3.3.1.1 Analysis for whole patients with HNSCC

First, we analyzed HNSCC patients for OS. The Kaplan-Meier Plot of OS for all patients is shown in Figure 1a. According to 1st row of Table 3, we can see that 60.6% out of the

94 patients are censored. The median survival is 109.8 month. The survival probability for 24 months is 79.8% with 95% CI (69.9%, 86.8%).

Then, we analyzed HNSCC patients for DFS. The Kaplan-Meier Plot of DFS for all patients is shown in Figure 2a. Table 4 tells that the survival probability for 24 months is 76.5% and the survival probability for 48 months is 75.0%.

3.3.1.2 Analysis for patients with HNSCC stratified by different biomarkers

In this part, we stratified the HNSCC patients by four different biomarkers, PD_L1 status in tumor, PD_L1 status in peritumoral stroma, EGFR status, HER3 status and HER2 status. we stratify the score of EGFR, HER3 and HER2 at first. For EGFR average overall lies in (0, 1], we set the score=0; when it lies in (1, 2], we set the score=1; when it is greater than 2, we set the score=2. For HER3 average overall lies in (0, 0.5], we set the score=0; when it lies in (0.5, 1], we set the score=1; when it is greater than 2, we set the score=2. For HER2 average overall = 0, we set the score=0; for HER2 average overall > 0, we set the score=1.

For Overall Survival, Kaplan-Meier Plots of the stratified OS were shown in Figure 1b – Figure 1f with each p-value separately. Since only the p-value of log rank test for HER3 <0.05 (p-value=0.0138), we can conclude that there is difference in survival between patients with HER3 less than 0.5 and with HER3 greater than 0.5. According to Table 3, there are total number of 72 subjects for patients with HER3 less than 0.5 and 52.8% of them are censored. The median survival is 95.9 months with 95% CI (61.5, 187.6). The survival probability for 24 months is 76.8% with 95% CI (65.0%, 85.1%). There are total

number of 22 subjects for patients with HER3 greater than 0.5 and 86.4% of them are censored. The median survival is greater than 109.8 month. The survival probability for 24 months is 90.0% with 95% CI (65.6%, 97.4%).

For Disease Free Survival, Kaplan-Meier Plots of the stratified DFS were shown in Figure 2b – Figure 2f with each p-value separately. Only the p-value of log rank test for PD-L1 status in peritumoral stroma <0.05 (p-value=0.0066). We can conclude that there is difference in survival between patients with different status of PD-L1 in peritumoral stroma. According to Table 4, the censored rates are 100.0%, 40.0%, 73.9%, 79.4% and 86.4% separately for each status of PD-L1 in peritumoral stroma from Level 0-4. Except for Level 0 (since the censored rate were 100%), the median survival time increased gradually from Level 1 to Level 4. The same results could also be found in 24 months survival rate.

3.3.2 Cox Proportional Hazard Model

Cox proportional hazard model can show us the difference on the association between the survival time of HNSCC patients and one or more predictor variables. Here we also investigate patients with HNSCC with different PD-L1 status in tumor, PD-L1 status in peritumoral stroma, EGFR status, HER3 status and HER2 status. Here we also used the stratification for EGFR, HER3 and HER2, due to the reason of PH assumption violation, which is listed below.

For Overall Survival, we could see from Table 4 that the chance of dying for patients in HER3 < 0.5 was nearly 4 times the chance of dying for patients in HER3 > 0.5. Another

two risk factors considered associated with OS diagnosed from the univariate analysis were 66 status (p-value<0.0001) and type of treatment received (p-value=0.1147).

Following the general procedure of forward model selection, the minimum AIC model (AIC=273.950) was selected and the variables fitted in this model were HER3 and P16 status. Adjusted hazard ratios and p-values were calculated in Table 5. The final model took the following form:

$$h(t|Z) = h_0(t)\exp(\beta_1 Z_1+\beta_2 Z_2)$$

Where $h_0(t)$ was the baseline hazard function; β_1 was coefficient for HER3 whose level is 0, and Z_1 was covariate for HER3; β_2 was coefficient for P16 status which is negative, and Z_2 was covariate for P16 status.

For Disease Free Survival, we could see from Table 6 that the relative risk of PD-L1 in Peritumoral Stroma (Level 1) vs PD-L1 in Peritumoral Stroma (Level 4) was approximately 8 with p-value equaling to 0.0291, indicating that there was a different Disease Free Survival between different levels of PD-L1 in Peritumoral Stroma, and it is the only biomarkers showed significance in hazard ratio of DFS. P16 was considered associated again with DFS diagnosed from the univariate analysis (p-value<0.0001)

Following the general procedure of forward model selection, the minimum AIC model (AIC=161.641) was selected and the variables fitted in this model were PD-L1 in Peritumoral Stroma and P16 status. Adjusted hazard ratios and p-values were calculated in Table 7. The final model took the following form:

$$h(t|Z) = h_0(t)\exp(\beta_1 Z_1+\beta_2 Z_2+\beta_3 Z_3+\beta_4 Z_4+\beta_5 Z_5)$$

Where $h_0(t)$ was the baseline hazard function; $\beta_1 – \beta_4$ were coefficient for different levels of PD-L1 in Peritumoral Stroma (Level 1 to Level 4), and $Z_1 – Z_4$ were covariate for PD-L1 in Peritumoral Stroma. B_5 was coefficient for P16 status which is negative, and Z_5 was covariate for P16 status.

3.3.3 Assumption checking

The proportional hazard assumption was checked both by Supremum test for proportional hazards assumption and graphically by standardized score process plots. Tests results were shown in table x and none of the risk factors in the final model violated the PH assumption. Figure 3a to Figure 3c were the standardized score process plots for each risk factor. Among them, Figure 3b shows significant violation of the PH assumption for model of HER3 (p-value=0.0410), which means the ratio of the hazards of any two individuals is not always constant over time.

A violations of PH assumption can be resolved by either adding covariate*time interaction or stratification. Here we stratify the model for the three continuous variables EGFR, HER3 and HER2 (for uniformity), since stratification solution is better for confounders than main effects, as stratification prevents interpretation of the stratifying variable.

4. Conclusion and Discussion

In logistic regression analysis, we identified disease free survival and p16 status as the most significant risk factors for the relapse of Head and Neck Cancer. Patients whose time from study enrollment to disease progression date within 25-48 months had a much

lower chance for disease reoccurrence than patients whose time from study enrollment to disease progression date less than 24 months, and patients whose time from study enrollment to disease progression date greater than 48 months had a much lower chance for disease reoccurrence than the two groups above. Patients whose P16 status are positive also show significant lower chance to experience relapse than those whose P16 status are negative.

Another important prognostic risk factor for HNSCC relapse were age and overall survival, so they can be counted as an important assessment for HNSCC relapse. None of the biomarkers or treatments shows significance in reoccurrence, which suggests that the types of treatment did not has a great influence in controlling HNSCC reoccurrence, and survival analysis needed to be conducted to dig more information on association between biomarkers and Head and Neck Cancer.

In the Cox PH model, HER3 was shown to be a prognostic factor for patients' OS; PD-L1 in peritumoral stroma was shown to be a prognostic factor for patients' DFS. Patients with favorable HER3 levels tended to have better Overall Survival outcomes, and with favorable PD-L1 levels in peritumoral stroma tended to have better Disease Free Survival outcomes. To investigate which treatment might improve patients' survival, we also built PH model on Treatment. Different treatments seemed to exert different influence on patients' OS. The hazard ratio of Radiotherapy treatment group is lower than that of Chemoradiotherapy treatment group, and they are both lower than the hazard ratio of patients with no treatment. The results are all significant, which suggested that the

combined treatment of Radiotherapy and Chemotherapy may not work as well as they worked alone.

In this study, our sample size is relatively small (only 94 pieces of useful data), may let the result be precarious and not accurate. We got some odd results, which was different from expectations, might partly also because of small sample size. The most peculiar one was, we found that in logistic regression, patients whose P16 status were positive are less likely to recur, which was contrary to our common sense. To verify this conclusion, we investigated the relationship between OS and patients' P16 status. It showed again that whose P16 status were positive had longer overall survival. Several reasons might result in this conclusion. First, we didn't consider the potential confounders, like age, gender and race, etc. Interactions were not counted in either when the logistic regression and Cox proportional hazard model were constructed. Second, the gravity of illness may be different among the patients. The tumor has different stages. For some of the patients, their tumor could be benign while the others may be severe.

Moreover, missing data is another issue. In Table 1, we have 1 missing value in Smoking, 3 missing values in Tumor Stage, 5 missing values in Treatment and 54 missing values in HPV. If there were not so many missing values in HPV, we might dig more useful information. In spite of these limitations, this study has some strengths. The patient population was diverse and representative.

Generally, the overall survival for HNSCC patients was decent compared with patients with other kinds of tumor. Yet, in order to improve HNSCC patients' overall survival

further, new and less toxic treatment as well as improving patients' general well-being and daily activities were crucial, as P16, PD-L1 and HER3 accounted for a large amount of patients' recurrence OS, and DFS. Further studies would be needed to find both new treatment strategies and ways to provide better patients care.

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6. Tables and Figures

Table 1 Baseline Characteristics of 94 HNSCC Patients

Variable		Overall (n=94)	No Recurrence (n=72)	Recurrence (n=22)	P-value
Age at diagnosis of HNSCC		57.4 (9.7)	56.1 (9.6)	61.5 (9.0)	0.022
Age < or >= 60	<60	56 (59.6%)	47 (65.3%)	9 (40.9%)	0.042
	>=60	38 (40.4%)	25 (34.7%)	13 (59.1%)	
Gender	Male	77 (81.9%)	62 (86.1%)	15 (68.2%)	0.056
	Female	17 (18.1%)	10 (13.9%)	7 (31.8%)	
Race	AA	9 (9.6%)	4 (5.6%)	5 (22.7%)	0.049
	White	78 (83.0%)	63 (87.5%)	15 (68.2%)	
	Hispanic & Unknown	7 (7.4%)	5 (6.9%)	2 (9.1%)	
Smoking*	Never	18 (19.2%)	16 (22.5%)	2 (9.0%)	0.239
	Former	44 (46.8%)	34 (47.9%)	10 (45.5%)	
	Current	31 (33.0%)	21 (29.6%)	10 (45.5%)	
Differentiation	WD	6 (6.4%)	5 (7.0%)	1 (4.5%)	0.201
	MD	36 (38.3%)	24 (33.3%)	12 (54.5%)	
	NK	52 (55.3%)	43 (60.7%)	9 (41.0%)	
Tumor Stage*	1	41 (46.1%)	34 (50%)	7 (33.3%)	0.149
	2	36 (40.4%)	26 (38.2%)	10 (37.6%)	
	3	5 (5.6%)	2 (2.9%)	3 (14.3%)	

	4	7 (7.9%)	6 (8.8%)	1 (4.8%)	
Type of treatment Received*	Radiotherapy	63 (70.8%)	52 (77.6%)	11 (50%)	0.068
	Chemotherapy	1 (1.1%)	1 (1.5%)	0 (0%)	
	Chemoradiotherapy	18 (20.2%)	10 (14.9%)	8 (36.4%)	
	None	7 (7.9%)	4 (6.0%)	3 (13.6%)	
DFS from enrollment to disease progression (continuous)		55.8 (47.4)	64.3 (46.9)	28.4 (38.6)	0.002
DFS from enrollment to disease progression (categorical)	1-24 months	35 (37.2%)	18 (25%)	17 (77.3%)	<.001
	25-48 months	11 (11.7%)	10 (13.9%)	1 (4.5%)	
	>48 months	48 (51.1%)	44 (61.1%)	4 (18.2%)	
OS from date of relapse (continuous)		67.5 (46.4)	70.8 (43.5)	56.7 (54.3)	0.214
OS from date of relapse (categorical)	1-24 months	24 (25.5%)	13 (18.0%)	11 (50%)	<.011
	25-48 months	13 (13.8%)	11 (15.3%)	2 (9.1%)	
	>48 months	57 (60.7%)	48 (66.7%)	9 (40.9%)	
HPV	Positive	30 (75%)	26 (78.8%)	4 (57.1%)	0.230
	Negative	10 (25%)	7 (21.2%)	3 (42.9%)	
P16	Positive	69 (73.4%)	60 (83.3%)	9 (40.9%)	<.001
	Negative	25 (26.6%)	12 (16.7%)	13 (59.1%)	
EGFR		1.18 (1.02)	1.14 (1.00)	1.29 (1.07)	0.561
HER3		0.30 (0.33)	0.29 (0.34)	0.30 (0.30)	0.902
HER2		0.02 (0.05)	0.02 (0.04)	0.02 (0.05)	0.609
PD1+lymphs in Tumor	0	3 (3.4%)	3 (4.4%)	0(0)	0.843
	1	31 (34.8%)	24 (34.8%)	7 (35%)	
	2	18 (20.2%)	13 (18.8%)	5 (25%)	
	3	19 (21.3%)	15 (21.7%)	4 (20%)	
	4	18 (20.2%)	14 (20.3%)	4 (20%)	
	0	2 (2.2%)	2 (2.9%)	0 (0)	0.404

PD1+lymphs in peritumoral stroma	1	9 (10.1%)	5 (7.3%)	4 (20%)
	2	23 (25.8%)	18 (26.1%)	5 (25%)
	3	33 (37.1%)	26 (37.7%)	7 (35%)
	4	22 (24.7%)	18 (26.1%)	4 (20%)

*Missing values are wiped out in certain columns

All the percentages in the table are column percentages

Mean and standard deviation are calculated for continuous variables

p-values is calculated by ANOVA for numerical covariates and chi-square test for categorical covariates

Table 2 Crude Odds Ratio And 95% Confidence Interval for Univariate Model Effect

Variable		Model Effect	
		Crude Odds Ratio (95% CI)	p-value
Age < or >= 60	<60	Reference	0.0454
	>=60	2.716 (1.021, 7.226)	
Gender	Male	Reference	0.0626
	Female	2.893 (0.945, 8.854)	
Race	AA	Reference	0.0724
	White	0.190 (0.046, 2.796)	
	Hispanic & Unknown	0.320 (0.039, 2.618)	
Smoking	Never	Reference	0.2069
	Former	2.353 (0.461, 12.011)	
	Current	3.809 (0.109, 19.866)	
Differentiation	WD	Reference	0.2103
	MD	2.500 (0.262, 23.864)	
	NK	1.067 (0.109, 10.069)	
Tumor Stage	1	Reference	0.2034
	2	1.868 (0.626, 5.571)	

	3	7.258 (1.021, 52.000)	
	4	0.810 (0.084, 7.819)	
Type of treatment received	Radiotherapy	Reference	0.0969
	Chemotherapy	<0.0001(<0.0001,>999.999)	
	Chemoradiotherapy	3.287 (1.216,11.762)	
	None	3.545 (0.693, 18.135)	
HPV	Negative	Reference	0.2414
	Positive	0.309 (0.065,1.992)	
P16	Negative	Reference	0.0002
	Positive	0.138 (0.048,0.396)	
DFS from enrollment to disease progression	1-24 months	Reference	0.0002
	25-48 months	0.0106 (0.005, 0.642)	
	>48 months	0.096 (0.025, 0.301)	
OS from date of relapse	1-24 months	Reference	0.0158
	25-48 months	0.215 (0.029, 1.026)	
	>48 months	0.222 (0.074, 0.641)	

p-values is calculated by ANOVA for numerical covariates and chi-square test for categorical covariates

Table 3 Crude Odds Ratio And 95% Confidence Interval for Multivariate Model Effect

Variable		Model Effect	
		Crude Odds Ratio (95% CI)	p-value
P16	Negative	Reference	0.0047
	Positive	0.181 (0.055, 0.593)	
DFS from enrollment to disease progression	1-24 months	Reference	0.0027
	25-48 months	0.088 (0.009, 0.849)	
	>48 months	0.137 (0.038, 0.492)	

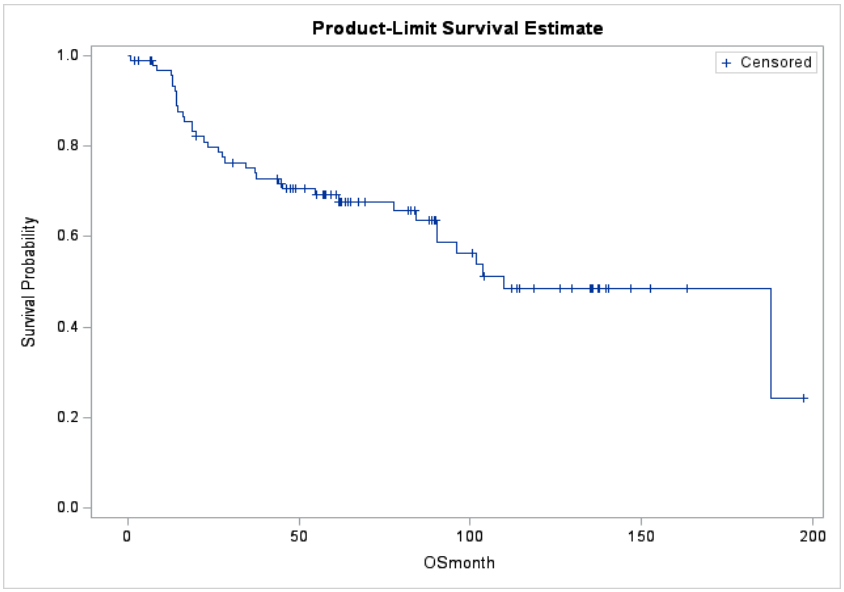


Figure 1a. Kaplan-Meier Plot of OS (in months) for NHSCC

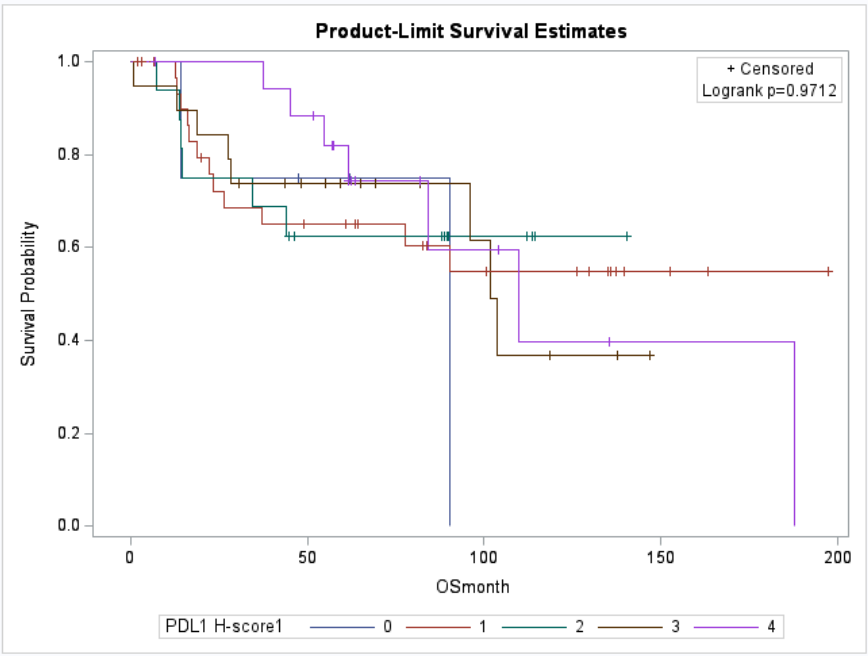


Figure 1b. Kaplan-Meier Plot of OS (in months) for NHSCC stratified by PDL1 H-Score

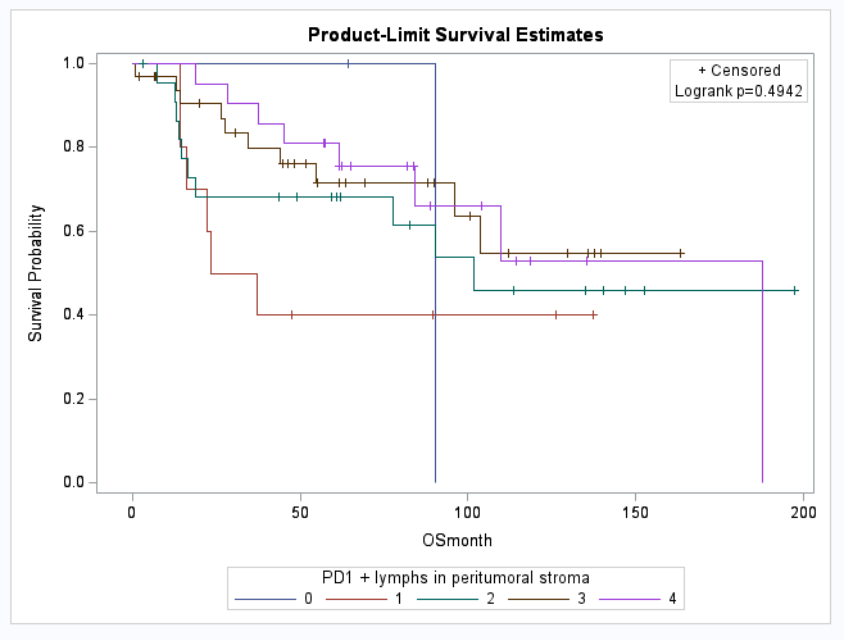


Figure 1c. Kaplan-Meier Plot of OS (in months) for NHSCC stratified by PD1 + Lymphs in Peritumoral Stroma

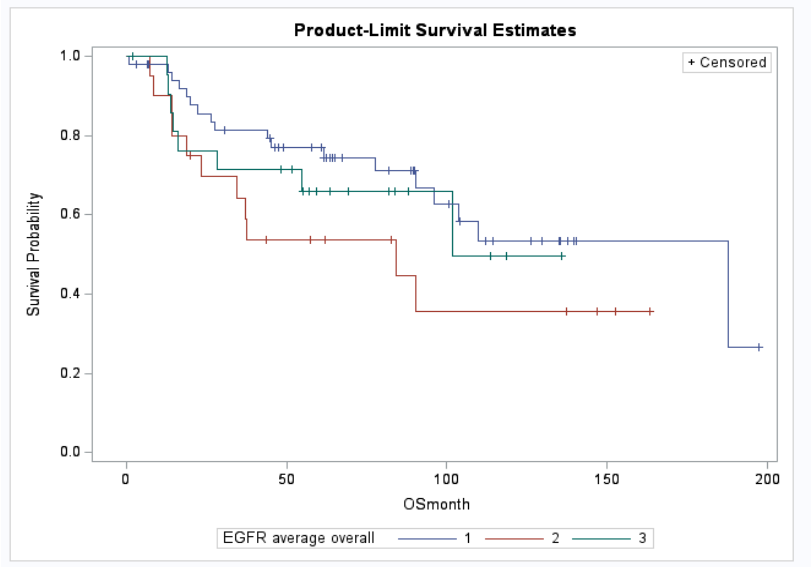


Figure 1d. Kaplan-Meier Plot of OS (in months) for NHSCC stratified by EGFR Average Overall

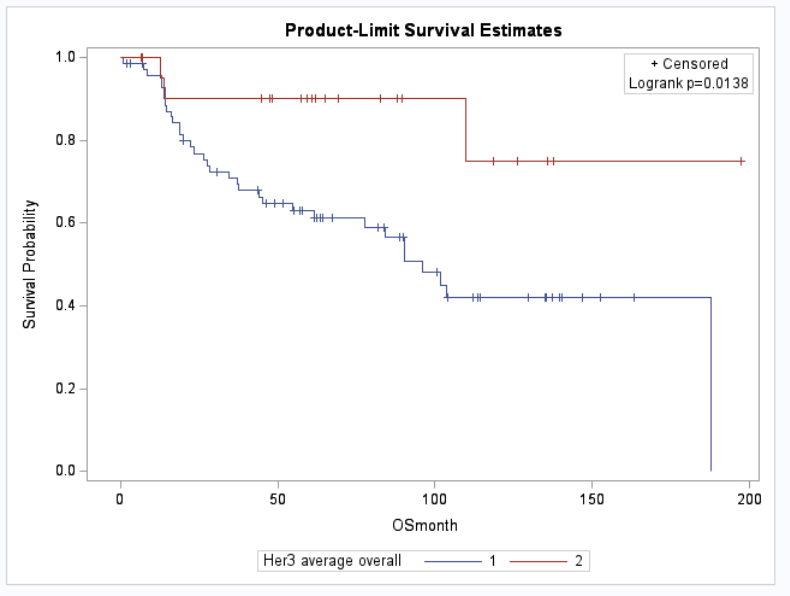


Figure 1e. Kaplan-Meier Plot of OS (in months) for NHSCC stratified by HER3 Average Overall

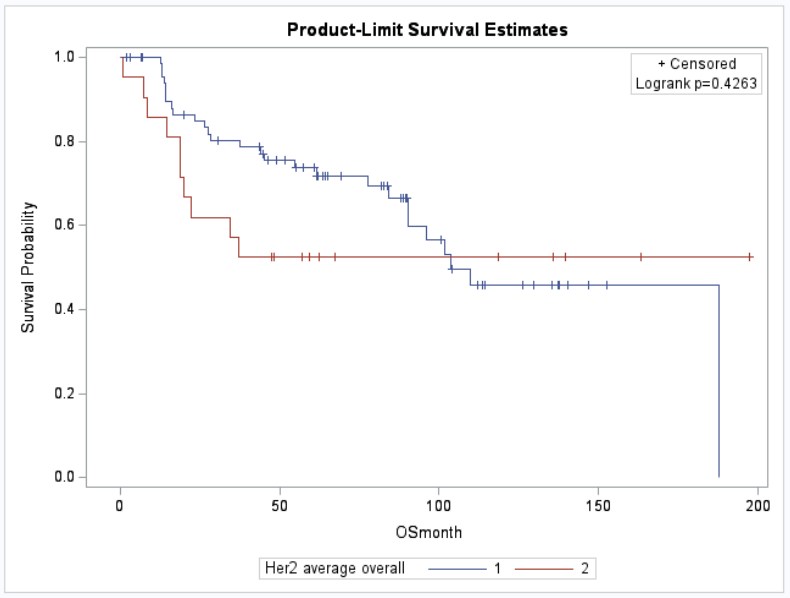


Figure 1f. Kaplan-Meier Plot of OS (in months) for NHSCC stratified by HER2 Average Overall

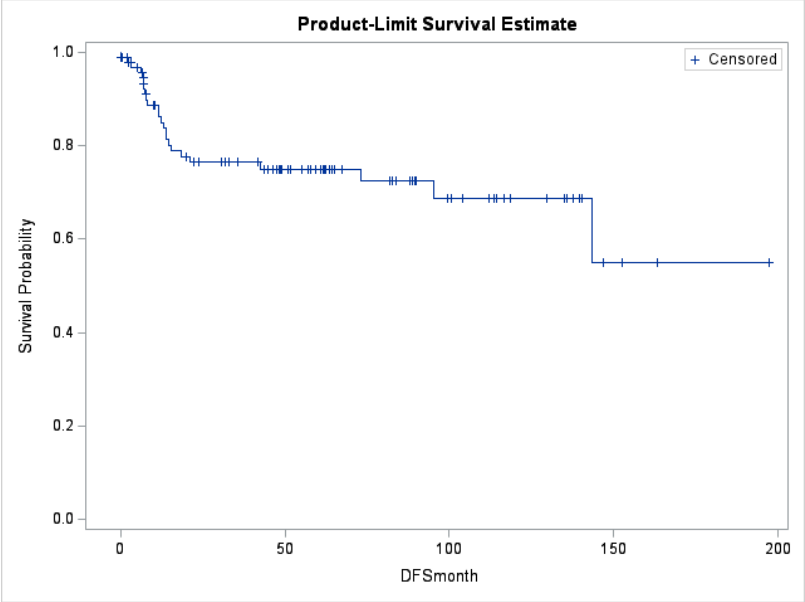


Figure 2a. Kaplan-Meier Plot of DFS (in months) for NHSCC

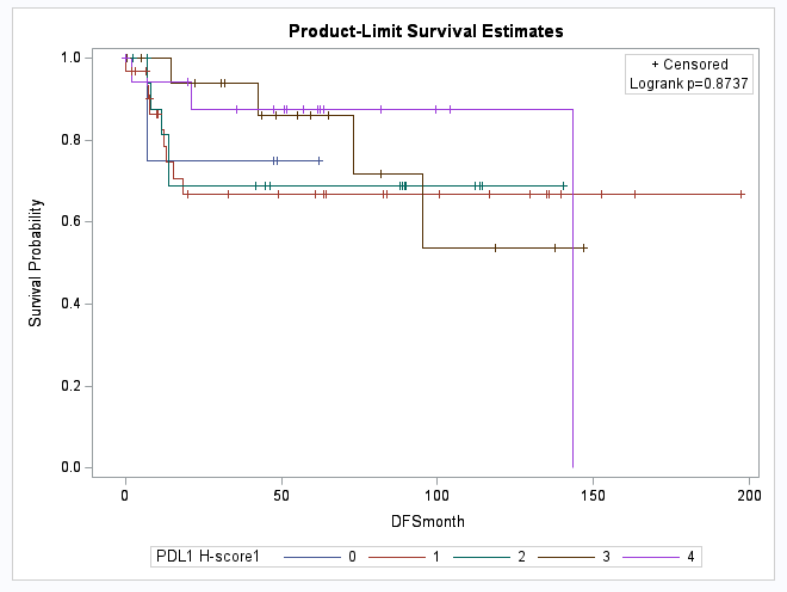


Figure 2b. Kaplan-Meier Plot of DFS (in months) for NHSCC stratified by PD-L1 in Tumor

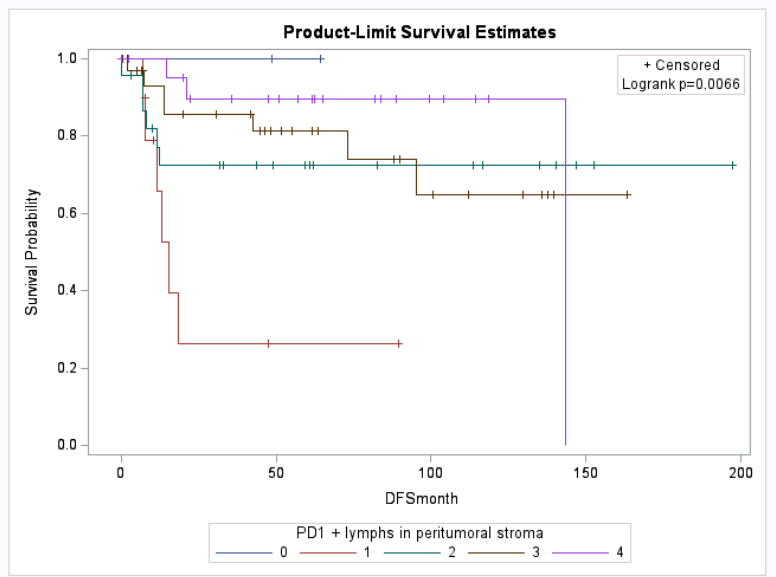


Figure 2c. Kaplan-Meier Plot of DFS (in months) for NHSCC stratified by PD-L1 in Peritumoral Stroma

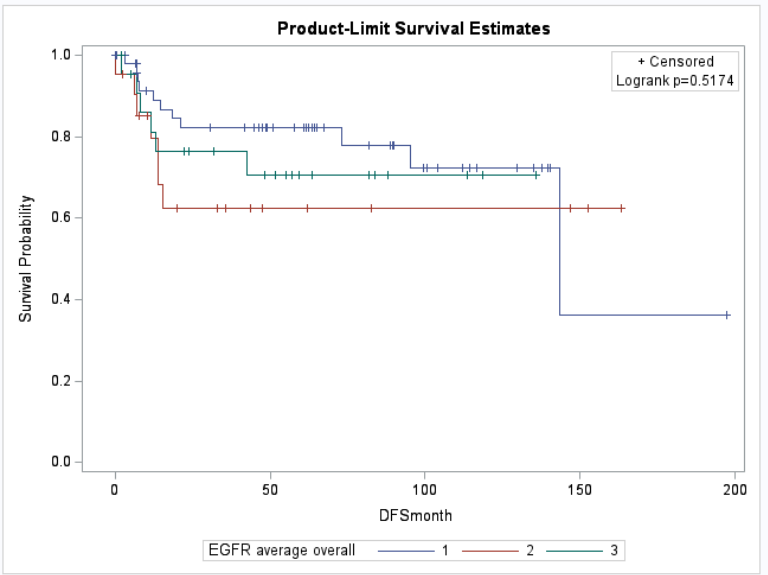


Figure 2d. Kaplan-Meier Plot of DFS (in months) for NHSCC stratified by EGFR Average Overall

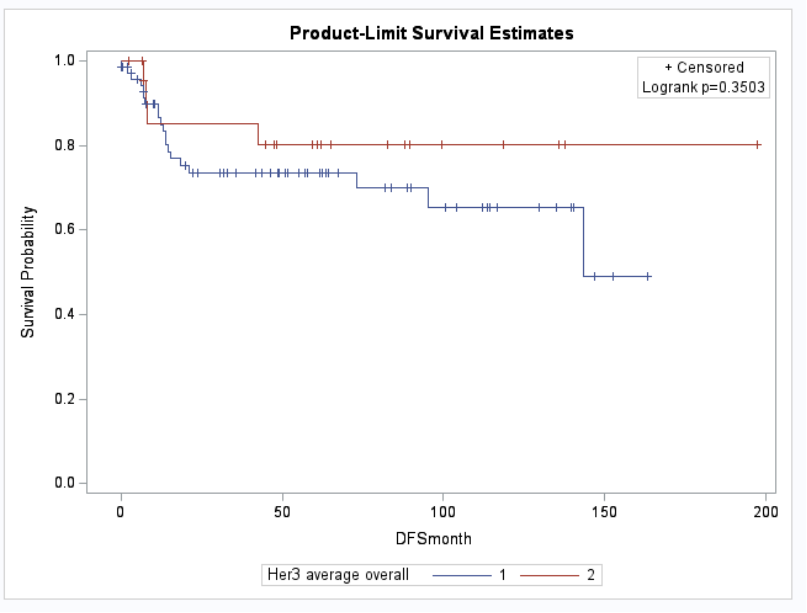


Figure 2e. Kaplan-Meier Plot of DFS (in months) for NHSCC stratified by HER3 Average Overall

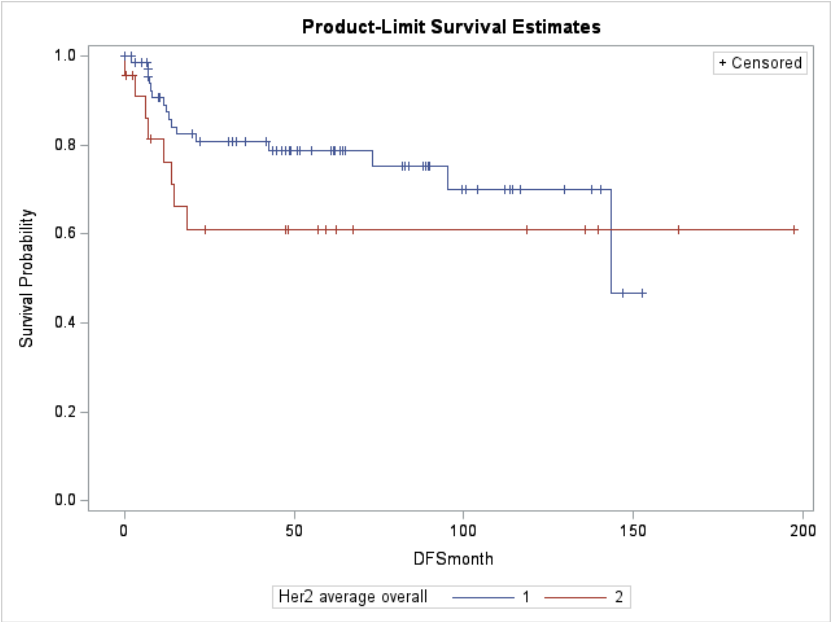


Figure 2f. Kaplan-Meier Plot of DFS (in months) for NHSCC stratified by HER2 Average Overall

Table 4 The Univariate Analysis for Overall Survival

Variable		OS	
		Hazard Ratio (95% CI)	p-value
PD1 + Lymphs in Tumor	0	1.728 (0.355, 8.400)	0.9719
	1	1.124 (0.400, 2.870)	
	2	1.128 (0.375, 3.395)	
	3	1.244 (0.446, 3.467)	
	4	Reference	
PD1 + Lymphs in Peritumoral Stroma	0	1.407 (0.175, 11.327)	0.5172
	1	2.372 (0.815, 6.900)	
	2	1.403 (0.553, 3.560)	
	3	1.041 (0.408, 2.656)	
	4	Reference	
EGFR	0	0.766 (0.330, 1.780)	0.2149
	1	1.513 (0.608, 3.767)	
	2	Reference	
HER3	0	3.970 (1.215, 12.976)	0.0225
	1	Reference	
HER2	0	0.744 (0.358, 1.546)	0.4277
	1	Reference	
P16	Negative	4.533 (2.332, 8.809)	<0.0001
	Positive	Reference	
Type of treatment Received	Radiotherapy	0.394 (0.135, 1.151)	0.1147
	Chemotherapy	1.396 (0.154, 12.674)	
	Chemoradiotherapy	0.816 (0.247, 2.691)	
	None	Reference	

Table 5 Multivariable Analysis with a Best Predictive Model of OS Using Variables Found to be Significant in the Univariate Analysis

Variable		OS	
		Hazard Ratio (95% CI)	p-value
P16	Negative	4.024 (2.009, 8.062)	<0.0001
	Positive	Reference	
HER3	0	2.892 (0.866, 9.655)	0.0843
	1	Reference	

Table 6 The Univariate Analysis for Disease Free Survival

Variable		DFS	
		Hazard Ratio (95% CI)	p-value
PD1 + Lymphs in Tumor	0	1.754 (0.182, 16.926)	0.8785
	1	1.859 (0.500, 6.907)	
	2	1.829 (0.435, 7.696)	
	3	1.248 (0.279, 5.579)	
	4	Reference	
PD1 + Lymphs in Peritumoral Stroma	0	0 (0, NA)	0.0291
	1	7.965 (1.951, 32.512)	
	2	2.057 (0.509, 8.314)	
	3	1.827 (0.472, 7.067)	
	4	Reference	
EGFR	0	0.737 (0.272, 2.000)	0.5237
	1	1.278 (0.420, 3.885)	
	2	Reference	
HER3	0	1.660 (0.567, 4.860)	0.3554
	1	Reference	
HER2	0	0.590 (0.251, 1.384)	0.2249
	1	Reference	

P16	Negative	5.230 (2.311, 11.838)	<0.0001
	Positive	Reference	
Type of treatment Received	Radiotherapy	0.234 (0.065, 0.836)	0.0475
	Chemotherapy	0 (0, NA)	
	Chemoradiotherapy	0.646 (0.169, 2.466)	
	None	Reference	

Table 7 Multivariable Analysis with a Best Predictive Model of DFS Using Variables Found to be Significant in the Univariate Analysis

Variable		OS	
		Hazard Ratio (95% CI)	p-value
P16	Negative	4.988 (2.089, 11.911)	0.0003
	Positive	Reference	
PD1 + Lymphs in Peritumoral Stroma	0	0 (0, NA)	0.0436
	1	7.455 (1.818, 30.575)	
	2	1.766 (0.431, 7.315)	
	3	2.062 (0.530, 8.027)	
	4	Reference	

7. Appendix

Table 8 OS for HNSCC Patients with different Stratifications

Stratification	Levels	Total	Censored	Median Survival	24 Month Survival	48 Month Survival
None	NA	94	57 (60.6%)	109.8 (90.2, NA)	79.8% (69.9%, 86.8%)	70.5% (59.8%, 78.9%)
PD-L1 in tumor	0	4	2 (50.0%)	90.3 (14.2, 90.3)	75.0% (12.8%, 96.0%)	75.0% (12.8%, 96.0%)
	1	32	20 (62.5%)	NA (26.5, NA)	72.1% (51.8%, 85.0%)	64.9% (44.5%, 79.4%)
	2	17	11 (64.7%)	NA (14.8, NA)	75.0% (46.3%, 89.8%)	62.5% (34.9%, 81.1%)
	3	19	11 (57.9%)	101.6 (28.4, NA)	84.2% (58.7%, 94.6%)	73.7% (47.9%, 88.1%)

	4	18	11 (61.1%)	109.8 (61.5, 187.6)	100.0% (100.0%, 100.0%)	88.2% (60.6%, 96.9%)
PD-L1 in Peritumoral Stroma	0	2	1 (50.0%)	90.3 (NA, NA)	100.0% (100.0%, 100.0%)	100.0% (100.0%, 100.0%)
	1	10	4 (40.0%)	30.1 (14.0, NA)	50.0% (18.4%, 75.3%)	40.0% (12.3%, 67.0%)
	2	23	13 (56.5%)	101.6 (16.6, NA)	68.2% (44.6%, 83.4%)	68.2% (44.6%, 83.4%)
	3	34	24 (70.6%)	NA (95.9, NA)	90.4% (73.0%, 96.8%)	76.2% (56.3%, 87.9%)
	4	21	13 (61.9%)	187.6 (84.0, 187.6)	95.2% (70.7%, 99.3%)	81.0% (56.9%, 92.4%)
EGFR	0	52	34 (65.4%)	143.4 (143.4, NA)	85.6% (72.1%, 92.8%)	77.0% (62.4%, 86.6%)
	1	20	9 (45.0%)	NA (13.8, NA)	69.6% (44.5%, 85.1%)	53.6% (29.6%, 72.6%)
	2	22	14 (63.6%)	NA (42.3, NA)	76.2% (51.9%, 89.3%)	71.4% (47.1%, 86.0%)
HER3	0	72	38 (52.8%)	95.9 (61.5, 187.6)	76.8% (65.0%, 85.1%)	64.8% (52.2%, 74.8%)
	1	22	19 (86.4%)	NA (109.8, NA)	90.0% (65.6%, 97.4%)	90.0% (65.6%, 97.4%)
HER2	0	71	44 (62.0%)	103.7 (90.2, 187.6)	84.8% (73.6%, 91.5%)	75.4% (63.0%, 84.1%)
	1	21	11 (52.4%)	NA (18.8, NA)	61.9% (38.1%, 78.8%)	52.4% (29.7%, 70.9%)

Table 9 DFS for HNSCC Patients with different Stratifications

Stratification	**Levels**	**Total**	**Censored**	**Median Survival**	**24 Month Survival**	**48 Month Survival**
None	NA	96	72 (75.0%)	*NA (143.4, NA)	76.5% (65.9%, 84.1%)	75.0% (64.3%, 83.0%)
PD-L1 in tumor	0	4	3 (75.0%)	NA (6.9, NA)	75.0% (12.8%, 96.1%)	75.0% (12.8%, 96.1%)
	1	32	23 (71.8%)	NA (15.5, NA)	66.7% (45.6%, 81.2%)	66.7% (45.6%, 81.2%)

	2	18	13 (72.2%)	NA (13.8, NA)	68.8% (40.5%, 85.6%)	68.8% (40.5%, 85.6%)
	3	19	15 (79.0%)	NA (72.9, NA)	93.8% (63.2%, 99.1%)	86.0% (54.0%, 96.3%)
	4	18	15 (83.3%)	143.4 (NA, NA)	87.4% (58.1%, 96.7%)	87.4% (58.1%, 96.7%)
PD-L1 in Peritumoral Stroma	0	2	2 (100.0%)	NA (NA, NA)	100.0% (100.0%, 100.0%)	100.0% (100.0%, 100.0%)
	1	10	4 (40.0%)	15.5 (6.9, NA)	26.3% (4.0%, 57.5%)	26.3% (4.0%, 57.5%)
	2	23	17 (73.9%)	NA (12.3, NA)	72.3% (48.4%, 86.5%)	72.3% (48.4%, 86.5%)
	3	34	27 (79.4%)	NA (73.0, NA)	85.6% (66.0%, 94.4%)	81.3% (60.6%, 91.8%)
	4	22	19 (86.4%)	143.4 (NA, NA)	89.7% (64.8%, 97.3%)	89.7% (64.8%, 97.3%)
EGFR	0	52	41 (78.9%)	143.4 (143.4, NA)	82.2% (64.8%, 97.3%)	82.2% (64.8%, 97.3%)
	1	21	14 (66.7%)	NA (13.8, NA)	62.5% (36.8%, 80.2%)	62.5% (36.8%, 80.2%)
	2	23	17 (73.9%)	NA (42.3, NA)	76.4% (52.2%, 89.4%)	70.5% (45.4%, 85.6%)
HER3	0	73	53 (72.6%)	143.4 (95.2, NA)	73.6% (60.9%, 82.7%)	73.6% (60.9%, 82.7%)
	1	23	19 (82.6%)	NA (NA, NA)	85.2% (60.8%, 95.0%)	80.2% (55.4%, 92.1%)
HER2	0	71	55 (77.5%)	143.4 (143.4, NA)	80.7% (68.5%, 88.6%)	78.7% (66.1%, 87.1%)
	1	23	15 (65.2%)	NA (14.0, NA)	61.0% (36.8%, 78.3%)	61.0% (36.8%, 78.3%)

*Median survival is the time at which the survivorship function equals 0.5. Since some minimum values appeared to be greater than 0.5, the output would show NA.

TABEL 10 Supremum Test for Proportionals Hazards Assumption

VARIABLE	Maximum Absolute Value	Replications	Seed	Pr > MaxAbsVal
EGFR_AVERAGE_OVERALL	0.9506	1000	19	0.1390
HER3_AVERAGE_OVERALL	1.1782	1000	19	0.0410
HER2_AVERAGE_OVERALL	0.8669	1000	19	0.1610

Figure 3a. Standardized Score Process Plot for EGFR

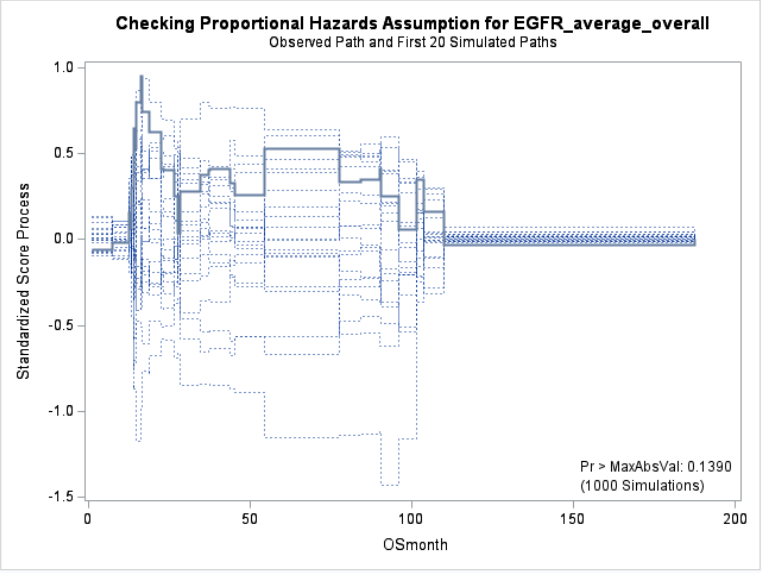


Figure 3b. Standardized Score Process Plot for HER3

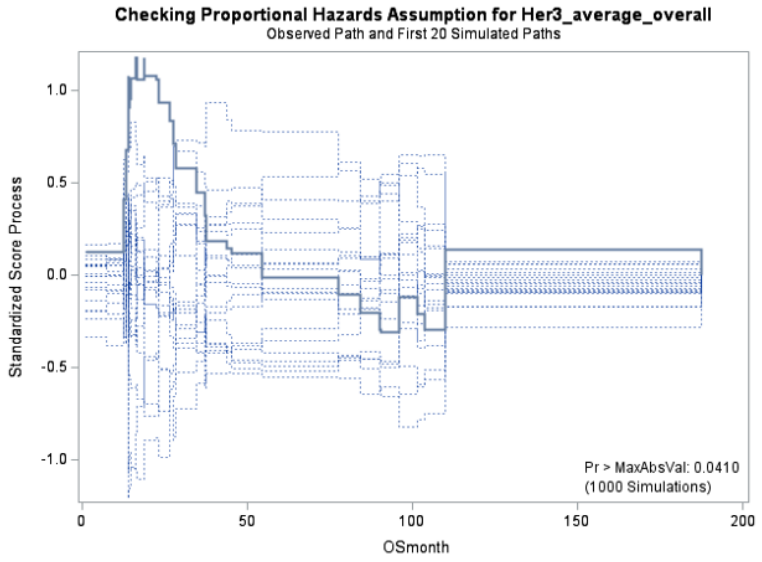
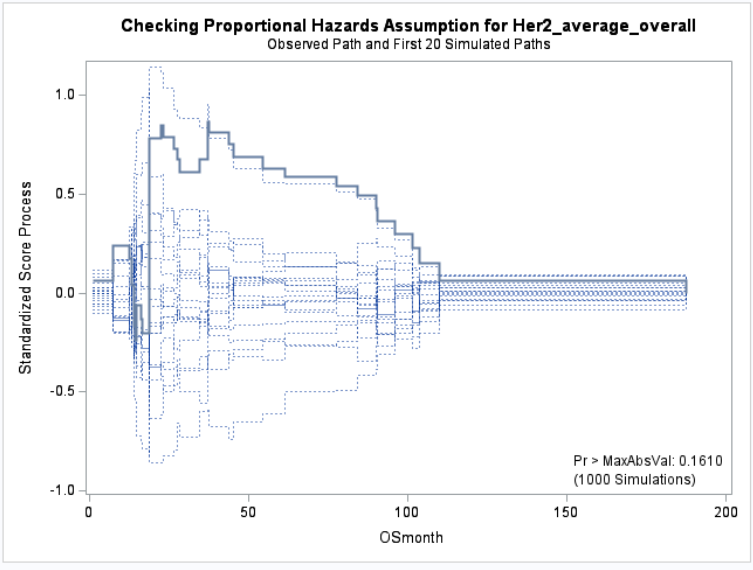


Figure 3c. Standardized Score Process Plot for HER2


Checking Proportional Hazards Assumption for Her2_average_overall
Observed Path and First 20 Simulated Paths
Standardized Score Process
1.0
0.5
0.0
-0.5
-1.0
0
50
100
150
200
OSmonth
Pr > MaxAbsVal: 0.1610
(1000 Simulations)