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Analysis of age, tumor-sidedness, and mismatch repair (MMR) gene with response to immune checkpoint inhibitors (ICIs) in MMR-deficient (dMMR) colorectal cancer (CRC) patients (pts): A multi-institutional study

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2018

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An abstract of

A thesis submitted to the Faculty of the

Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of

Master of Public Health

in Biostatistics and Bioinformatics

2020

Abstract

Analysis of age, tumor-sidedness, and mismatch repair (MMR) gene with response to immune checkpoint inhibitors (ICIs) in MMR-deficient (dMMR) colorectal cancer (CRC) patients (pts): A multi-institutional study

By Weiyi Jiang

Background: ICIs induce durable responses in dMMR CRC pts, overall response rate (ORR) 30-50%. Even though the loss of any MMR gene expression is predictive of responsiveness to ICIs, it is unknown if ORRs are similar across all MMR genes (MLH1, PMS, MSH2, and MSH6). In this study, we analyzed the impact of each specific MMR gene loss and clinical characteristics of patients with best response to ICIs.

Methods: Pts were eligible if they had confirmed dMMR CRC by IHC or microsatellite instability-high (MSI-H) by PCR, and received ICIs between 01/01/2012 and 10/01/2018 at Winship Cancer Institute of Emory University, Mayo Clinic and Vanderbilt University Medical Center. Due to the pattern of frequent concurrent loss and functional dependency, the groups were categorized as MLH1 ±PMS2 vs MSH2 ±MSH6. Cox proportional hazard model and Fisher's exact test were used for the best response and the distribution of variable among the subgroups. The study was approved by IRB.

Results: A total of 66 pts with dMMR CRC were identified. Overall response rates in MLH1 ±PMS2 and MSH2 ±MSH6 groups were 64.1% and 33.33% respectively without statistical difference (Table). Pts who are 50-65 years old had better ORRs compared to pts with age<50 and >65 (41.46%, 34.15%and 24.39% respectively, P=0.277). Left-sided tumors had a trend toward higher ORRs compared to right-sided tumors (68.29% vs 31.71% P=0.157). Gender and BRAF status were not predictors of response. BRAF mutations were more common in right-sided tumors (26.47% vs 8.33% respectively) and in older patients.

Conclusion: Our data suggests that age 50-65 have improved ORR, and there is a trend for improved ORR for left-sided tumors treated with ICIs in MSI-H CRC pts.

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1. Introduction

Cancer is a disease characterized by the unchecked division and survival of abnormal cells. When this type of abnormal growth occurs in the colon or rectum, it is called colorectal cancer (CRC). Colorectal cancer is the third common cause of cancer death in both men and women in the United States [1]. The American Cancer Society's estimates for the number of colorectal cancer cases in the United States for 2019 are 101,420 new cases of colon cancer 44 and 180 new cases of rectal cancer [2]. Although CRC incidence and mortality overall reduce dramatically for several decades, significantly differ by age, race, and tumor subsite remain. CRC incidence, survival, and mortality rates and trends differ by age. From 2000 to 2014, CRC death rates decreased by 34% in individuals aged ≥50 years but increased by 13% in those aged <50 years [3].

Genes and environment are both risk factors for colorectal cancer. The most common syndrome in hereditary colorectal cancer patient population is Lynch syndrome which is attributable to a mutation in DNA mismatch-repair genes including MLH1, MSH2, MSH6, PMS2 and EPCAM [4]. Impaired mismatch repair during replication leads to an accumulation of DNA mutations, which occur in microsatellite DNA fragments particularly, with repetitive nucleotide sequence [5].

Recently, some evidence shows that colorectal cancer is comprised of four distinct molecular subgroups [6]. Some of subtypes more likely to be enriched to either the right or left sides of the colon [7]. Scientists found that right-sided colon cancers have a worse prognosis than left-sided colon cancers, but the reason for this still unclear [8]. Moreover, in 2019, Bencsikova studied the net survival for right-sided colon cancer which was significantly lower than that for left-sided

colon cancer [9]. However, tumor sidedness is not an independent prognostic marker of colorectal cancer patients undergoing curative resection [6].

Immune checkpoint inhibitor therapy has led to dramatic improvements in treatment effects. The discovery of immune checkpoints has opened a new door for tumor immunotherapy. Through the suppression of immune checkpoints, the immune activity of T lymphocytes is enhanced, and finally the killing effect of the body's immune system on tumors is enhanced. Tumor therapy provides a brand-new treatment method and effective treatment methods. Because immune checkpoint therapy is to activate immune cells and enhance the killing efficiency, it is not easy to form tumor mutations and drug resistance, which can achieve long-term treatment of tumors. Application prospects. [10]. Immune checkpoint inhibitors also have primary resistance and acquired resistance in the treatment of colorectal cancer. At the same time, the toxic and side effects of immunosuppressant treatment are serious problems that clinical practice cannot ignore. Therefore, how to effectively screen out patients who may respond well to immunosuppressants is particularly important. Researches on high microsatellite instability (MSI-H), T cell inflammatory gene expression profile (GEP) and mismatch repair (MMR) genes have also been carried out.

In this paper, we used cox proportional hazard model to perform a survival analysis to identify the factors associated with 12&24 months survival and Fisher's exact test for the best response and the distribution of variable among the MLH1 ±PMS2 and MSH2 ±MSH6 subgroups patients who received immune checkpoint inhibitors between 01/01/2012 and 10/01/2018 at Winship Cancer Institute of Emory University, Mayo Clinic and Vanderbilt University Medical Center.

In the Section 2 of this thesis, we showed how patients' data collected and the describe statistical analysis method including descriptive analysis and survival analysis. In Section 3, detailed analysis of univariate association with best response, significance between groups and univariate progression-free analysis were presented. In Section 4, we discussed the overall response rate in each group also considered age, tumor-sidedness as factors.

2. Method

2.1 Data Collection

A multi-institutional study was done in collaboration between Emory's team and Winship Cancer Institute. Patients were eligible if they had confirmed dMMR CRC by IHC or microsatellite instability-high (MSI-H) by PCR, and received ICIs between 01/01/2012 and 10/01/2018 at Winship Cancer Institute of Emory University, Mayo Clinic and Vanderbilt University Medical Center. Related clinical features such as age, gender, each gene mutation, site of tumor, tumor differentiated grade, and treatment response were obtained from the electronic medical records. These variables will go through descriptive analysis and be included in descriptive table (table 1).

After receiving immune checkpoint inhibitors, whether a patient had best responds is recorded. The main drugs in cancer treatment are immune checkpoint inhibitors. For example, these drugs that can block CTLA-4, PD-1 and PD-L1 can mobilize the immune system to attack cancer cells that are trying to avoid immune defenses. In some patients, checkpoint inhibitors can make immune T cells shrink or eliminate tumors and have

long-term therapeutic effects. According to the gene mutations, we recorded each subgroups' responds to the treatment.

Based on the diagnosis of dMMR CRC by IHC or microsatellite instability-high (MSI-H) by PCR, they were given follow up treatments. Treatment start date, treatment stop date, date of continuation sensor by last contact, date of death or last contact and whether a patient survival now all were recorded.

2.2 Statistical Analysis Method

2.2.1 Descriptive analysis

The descriptive table for patients' characteristics was firstly constructed including specific MMR gene loss, tumor's grade and site and ICIs treatment response. Age was categorized into younger than 50, 50-65 and greater than 65. For these binary or categorical variables, the frequencies and percentage were presented (table 1).

2.2.2 Univariate analysis

In this study, the variables that we were interested in were almost all categorical variables. We examined the univariate association and best responds between each covariate. For each categorical covariate, frequencies from a contingency table are reported along with row percentages (col%). The parametric p-value is calculated by chi-square test. The non-parametric p-value is calculated by Fisher's exact test. We examined the univariate association with Best

Response, univariate association with Tumor Grade, univariate association with Age Group, univariate association with Side of Tumor.

2.2.3 Univariate survival analysis

For the univariate survival analysis, the Cox proportional hazard model was used. The hazard ratio with 95% CI is presented along with the log rank test p-value. Kaplan-Meier plots were generated for each covariate. Cox proportional hazard model was constructed. Proportional hazard model always contains two parts: the underlying baseline hazard function, which describes how the risk of the event changes at the baseline level of covariates; and the effect parameter, which demonstrates the change of risk according to covariates. The form of hazard function for the Cox proportional hazard model is:

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

$\lambda(t|X_i)$ is the hazard rate given time t for subject i with covariate Xi. Local Wald test was performed to see if there is any significant difference in difference levels of covariates. The score test is equivalent to the log rank test here, which can give us some insights from nonparametric perspective.

All the analyses were performed using SAS 9.4. The significance level was set to 0.05.

3. Result

3.1 Descriptive analysis

The results of univariate analysis were shown in table 1. From the table, we can see there are 66 patients with dMMR CRC. 37.9% patients have no response to immune checkpoint inhibitors and 62.1% patients have stable, partial or Complete Response. 45.5% patients' tumors are well or moderately differentiated. Others' are moderately to poorly or poorly differentiated. PMS2 has the highest mutation rate which is 54.5%, the second is MLH1 has 51.5% rate of mutation. MSH2 and MSH6 have 25.8% and 27.3% rate of mutation respectively. Patients' tumors' sites are mostly right, the percentage of right tumor site is 74.2%.

3.2 Univariate analysis

None of the gene mutation covariates has significant association with the best response. However, the p-value for BRAF is close to the significant level (table 2). Age group is significantly associated with BRAF mutation (table 3). Patients older than 65 years old have higher chance to have BRAF mutation. All p-value in table 4 are greater than 0.5. therefore, there is no significant association between gene groups and tumor grade. "MLH1 PMS2 without PMS2 only", "MLH1 PMS2 with PMS2 only", and "MSH2 MSH6 with MSH6 only" have significant association with age group (table 5). There is no significant association between gene groups and Side of Tumor (table 6). Overall response rates in MLH1 ±PMS2 and MSH2 ±MSH6 groups were 64.1% and 33.33% respectively without statistical difference (Table). Patients who are 50-65 years old had better ORRs compared to pts with age<50 and >65 (41.46%, 34.15%and 24.39% respectively, P=0.277). Left-sided tumors had a trend toward higher ORRs compared to right- sided tumors (68.29% vs 31.71% P=0.157). Gender and BRAF status were not predictors of response. BRAF mutations were more common in right-sided tumors (26.47% vs 8.33% respectively) and in older patients.

3.3 Univariate survival analysis

In the Cox proportional hazard model, we considered each gene mutations, BRAF mutation and age as risk factors in patients' survival. We find that none of the gene mutations nor the gender is significantly associated with progression free survival (table 1). But the best response is significantly associated with PFS (figure 1). But the best response is significantly associated with PFS. The Age Group is significantly associated with PFS. Other covariates are not significantly associated with PFS (figure2-9).

4. Discussion

In Univariate analysis, none of the gene mutation covariates has significant correlation with the best response to immune checkpoint inhibitors (ICIs). However, age group appears to be the most significant risk factor for complete remission, as expected. Patients who are 50-65 years old had better overall response compared to patients with age<50 and >65. Another important prognostic risk factor for ORR was tumor side, so tumor side can be counted as an important assessment for CRC patients

In the survival analysis, the best respond, BRAF and age group are shown to be factors for patients' survival. Patients with responds to immune checkpoint inhibitors (ICIs) tended to have better overall survival outcomes. No BRAF mutation seemed to exert good influence on patients' OS. Age greater than 65 decreased significantly the patients' chance of survival. It suggests that CRC patients aged 50-65 treated with ICIs, whereas patients > 65 need to consider the risk.

Patients with left-sided tumors have better survival outcomes compared to those with right sided tumors.

The most obvious of limitations of this study are sample size. Although sample size (66 patients) of clinical trial is typically small. The second one is that we did not consider the adverse reactions which are important in the future study of immune checkpoint inhibitors. In spite of these limitations, this study has some strengths. It is a multi-institutional study so we can avoid many subjective factors such as hospital conditions. Also, the patient population was diverse and representative. Other than a diverse patient population, this study had an extended follow-up.

Generally, the overall survival for CRC patients was optimistic. Age and tumor-sidedness infect the response to immune checkpoint inhibitors (ICIs) in MMR-deficient (dMMR) colorectal cancer (CRC) patients (pts). Aged 50-65 treated with ICIs, have improved ORR compared to pts > 65; pts with left-sided tumors have a trend toward improved ORR compared to those with right sided tumors. Further studies would be needed to find both new treatment strategies besides immune checkpoint inhibitors and ways to provide better patients care that make CRC patients live longer.

5. Reference

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6. Appendix

Table 1 Genotypes and tumor characteristics for the 66 study CRC patients with dMMR

Variable	Level	N (%) = 66
MLH1	no mutation	27 (40.9)
	mutation	34 (51.5)
	unknown	5 (7.6)
PMS2	no mutation	25 (37.9)
	mutation	36 (54.5)
	unknown	5 (7.6)
MSH2	no mutation	44 (66.7)
	mutation	17 (25.8)
	unknown	5 (7.6)
MSH6	no mutation	43 (65.2)
	mutation	18 (27.3)
	unknown	5 (7.6)
Tumor Grade	Well or Moderately Differentiated	30 (45.5)
	Moderately to Poorly or Poorly Differentiated	36 (54.5)
Treatment Response	No Response	25 (37.9)
	Stable, Partial or Complete Response	41 (62.1)
Site of tumor	Right	49 (74.2)
	Left	17 (25.8)

Table 2 Univariate Association with Best Response

Covariate		Best Response		P-value
		No response N=25 (%)	Partial response or complete response N=41(%)	
Loss of MLH1 ± PMS2	Not present	10 (47.62)	14 (35.9)	0.377
	Present	11 (52.38)	25 (64.1)	
Loss of MSH2 ± MSH6	Not present	11 (52.38)	26 (66.67)	0.278
	Present	10 (47.62)	13 (33.33)	
Age Group	<50	6 (24)	10 (24.39)	0.277
	50-65	6 (24)	17 (41.46)	
	>65	13 (52)	14 (34.15)	
BRAF	Not present	10 (62.5)	26 (86.67)	0.058
	Present	6 (37.5)	4 (13.33)	
Side of Tumor	Right	21 (84)	28 (68.29)	0.157
	Left	4 (16)	13 (31.71)	

Table 3 Univariate Association with BRAF

			BRAF			
Covariate	**Statistics**	**Level**	**Not present N=36**	**Present N=10**	**Parametric P-value***	**Non-Parametric P-value****
Age Group	N (Row %)	<50	11 (100)	0 (0)	**0.033**	**0.040**
	N (Row %)	50-65	12 (85.71)	2 (14.29)		
	N (Row %)	>65	13 (61.9)	8 (38.1)		
Side of Tumor	N (Row %)	Right	25 (73.53)	9 (26.47)	**0.190**	**0.252**
	N (Row %)	Left	11 (91.67)	1 (8.33)		

BRAF						
Covariate	**Statistics**	**Level**	**Not present N=36**	**Present N=10**	**Parametric P-value***	**Non-Parametric P-value****

* The parametric p-value is calculated by chi-square test.
** The non-parametric p-value is calculated by Fisher's exact test.

Table 4 Univariate Association with Tumor Grade

Tumor Grade							
Covariate	**Statistics**	**Level**	**unknown N=3**	**Well or Moderately Differentiated N=27**	**Moderately to Poorly or Poorly Differentiated N=36**	**Parametric P-value***	**Non-Parametric P-value****
MLH1 PMS2 without PMS2 only	N (Col %)	Not present	1 (33.33)	14 (58.33)	12 (36.36)	**0.237**	**0.237**
	N (Col %)	Present	2 (66.67)	10 (41.67)	21 (63.64)		
MSH2 MSH6 without MSH6 only	N (Col %)	Not present	2 (66.67)	17 (70.83)	24 (72.73)	**0.969**	**1.000**
	N (Col %)	Present	1 (33.33)	7 (29.17)	9 (27.27)		
MLH1 PMS2 with PMS2 only	N (Col %)	Not present	1 (33.33)	11 (45.83)	12 (36.36)	**0.749**	**0.817**
	N (Col %)	Present	2 (66.67)	13 (54.17)	21 (63.64)		
MSH2 MSH6 with MSH6 only	N (Col %)	Not present	2 (66.67)	13 (54.17)	22 (66.67)	**0.621**	**0.737**
	N (Col %)	Present	1 (33.33)	11 (45.83)	11 (33.33)		

* The parametric p-value is calculated by chi-square test.
** The non-parametric p-value is calculated by Fisher's exact test.

Table 5 Univariate Association with Age Group

			Age Group				
Covariate	**Statistics**	**Level**	**<50 N=16**	**50-65 N=23**	**>65 N=27**	**Parametric P-value***	**Non-Parametric P-value****
MLH1 PMS2 without PMS2 only	N (Col %)	Not present	9 (56.25)	15 (75)	3 (12.5)	**<.001**	**<.001**
	N (Col %)	Present	7 (43.75)	5 (25)	21 (87.5)		
MSH2 MSH6 without MSH6 only	N (Col %)	Not present	11 (68.75)	11 (55)	21 (87.5)	**0.056**	**0.053**
	N (Col %)	Present	5 (31.25)	9 (45)	3 (12.5)		
MLH1 PMS2 with PMS2 only	N (Col %)	Not present	8 (50)	12 (60)	4 (16.67)	**0.009**	**0.007**
	N (Col %)	Present	8 (50)	8 (40)	20 (83.33)		
MSH2 MSH6 with MSH6 only	N (Col %)	Not present	8 (50)	8 (40)	21 (87.5)	**0.003**	**0.002**
	N (Col %)	Present	8 (50)	12 (60)	3 (12.5)		

* The parametric p-value is calculated by chi-square test.
** The non-parametric p-value is calculated by Fisher's exact test.

Table 6 Univariate Association with Side of Tumor

			Side of Tumor			
Covariate	**Statistics**	**Level**	**Right N=49**	**Left N=17**	**Parametric P-value***	**Non-Parametric P-value****
MLH1 PMS2 without PMS2 only	N (Col %)	Not present	18 (40.91)	9 (56.25)	**0.291**	**0.382**
	N (Col %)	Present	26 (59.09)	7 (43.75)		

			Side of Tumor			
Covariate	**Statistics**	**Level**	**Right N=49**	**Left N=17**	**Parametric P-value***	**Non-Parametric P-value****
MSH2 MSH6 without MSH6 only	N (Col %)	Not present	34 (77.27)	9 (56.25)	**0.110**	**0.193**
	N (Col %)	Present	10 (22.73)	7 (43.75)		
MLH1 PMS2 with PMS2 only	N (Col %)	Not present	15 (34.09)	9 (56.25)	**0.121**	**0.145**
	N (Col %)	Present	29 (65.91)	7 (43.75)		
MSH2 MSH6 with MSH6 only	N (Col %)	Not present	30 (68.18)	7 (43.75)	**0.085**	**0.133**
	N (Col %)	Present	14 (31.82)	9 (56.25)		

* The parametric p-value is calculated by chi-square test.
** The non-parametric p-value is calculated by Fisher's exact test.

Table 7 Univariate Survival Analysis

				pfs	
Covariate	**Level**	**N**	**Hazard Ratio (95% CI)**	**HR P-value**	**Log-rank P-value**
MLH1 PMS2 without PMS2 only	Present	32	1.35 (0.79-2.30)	0.278	**0.276**
	Not present	25	-	-	
MSH2 MSH6 without MSH6 only	Present	15	1.14 (0.63-2.07)	0.669	**0.669**
	Not present	42	-	-	
MLH1 PMS2 with PMS2 only	Present	35	1.04 (0.60-1.80)	0.882	**0.881**
	Not present	22	-	-	
MSH2 MSH6 with MSH6 only	Present	21	0.90 (0.52-1.56)	0.699	**0.699**
	Not present	36	-	-	
BRAF	Present	10	1.78 (0.83-3.80)	0.137	**0.132**

			pfs		
Covariate	**Level**	**N**	**Hazard Ratio (95% CI)**	**HR P-value**	**Log-rank P-value**
	Not present	35	-	-	
Gender	Male	33	0.79 (0.47-1.32)	0.369	**0.367**
	Female	30	-	-	

Figure 1 Kaplan Meier Survival Curves Stratified by Best Responds

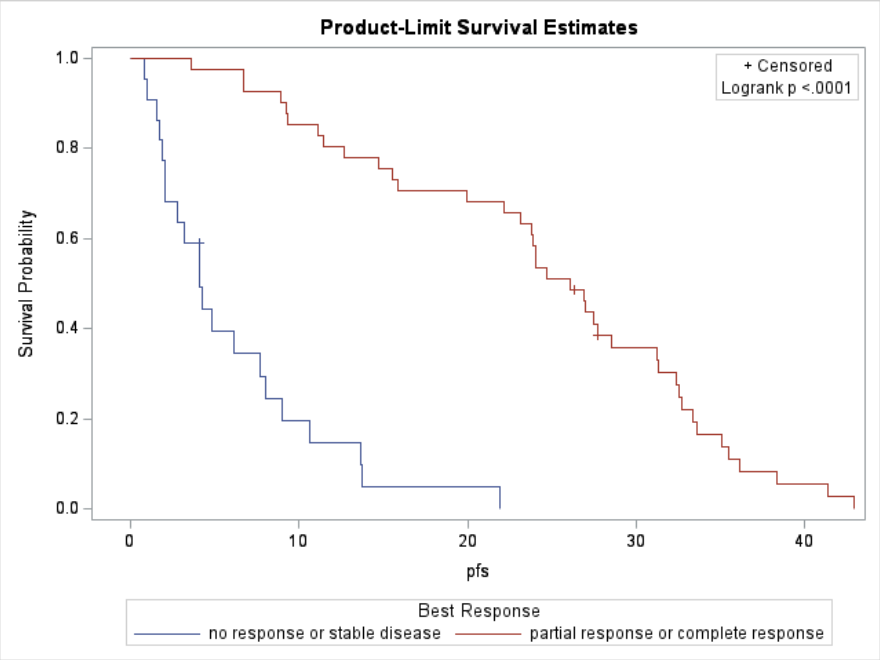

Figure 2 Kaplan Meier Survival Curves Stratified by MLH1 PMS2 without PMS2 only

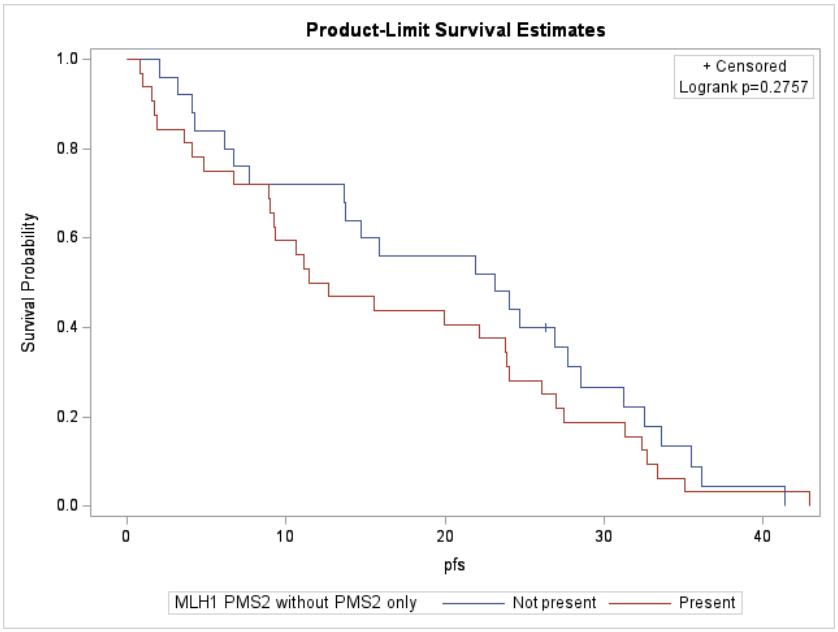

Figure 3 Kaplan Meier Survival Curves Stratified by MSH2 MSH6 without MSH6 only

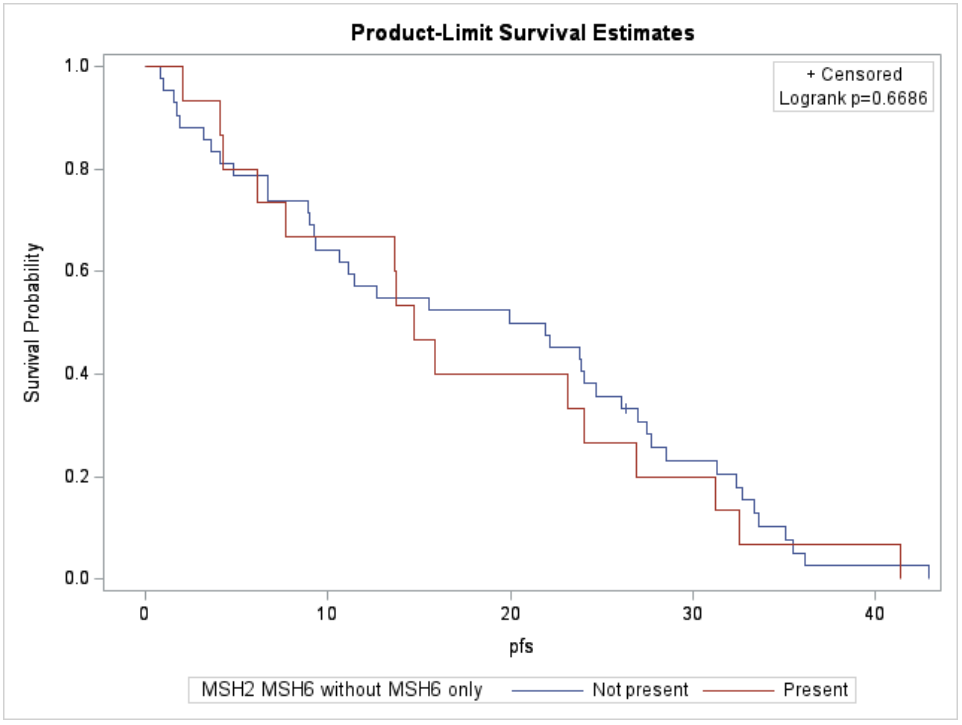

Figure 4 Kaplan Meier Survival Curves Stratified by MLH1 PMS2 with PMS2 only

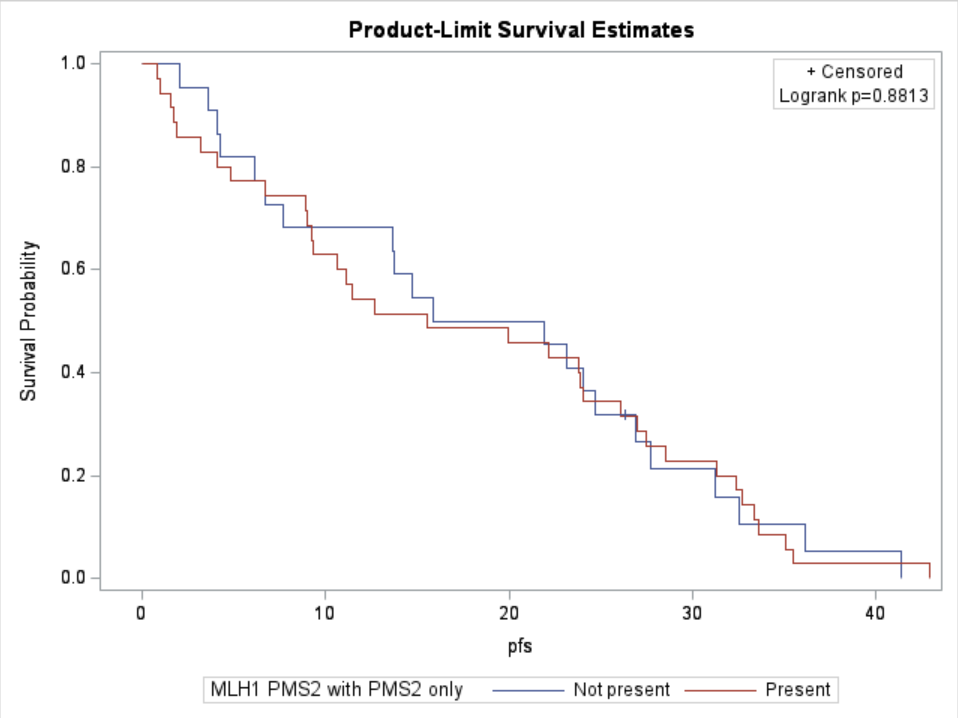

Figure 5 Kaplan Meier Survival Curves Stratified by MSH2 MSH6 with MSH6 only

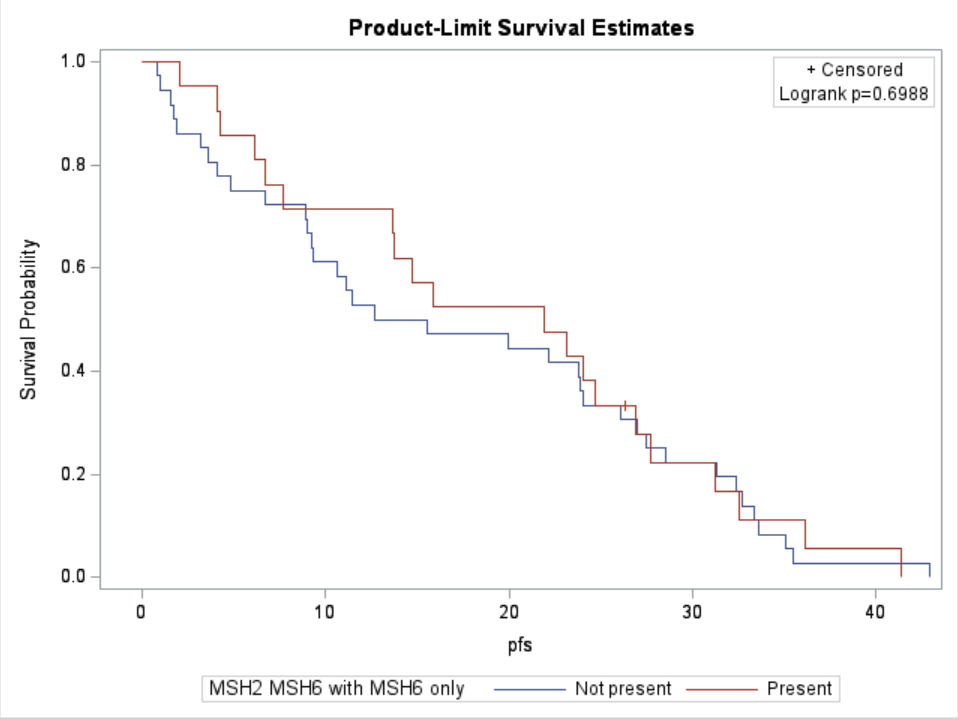

Figure 6 Kaplan Meier Survival Curves Stratified by BRAF

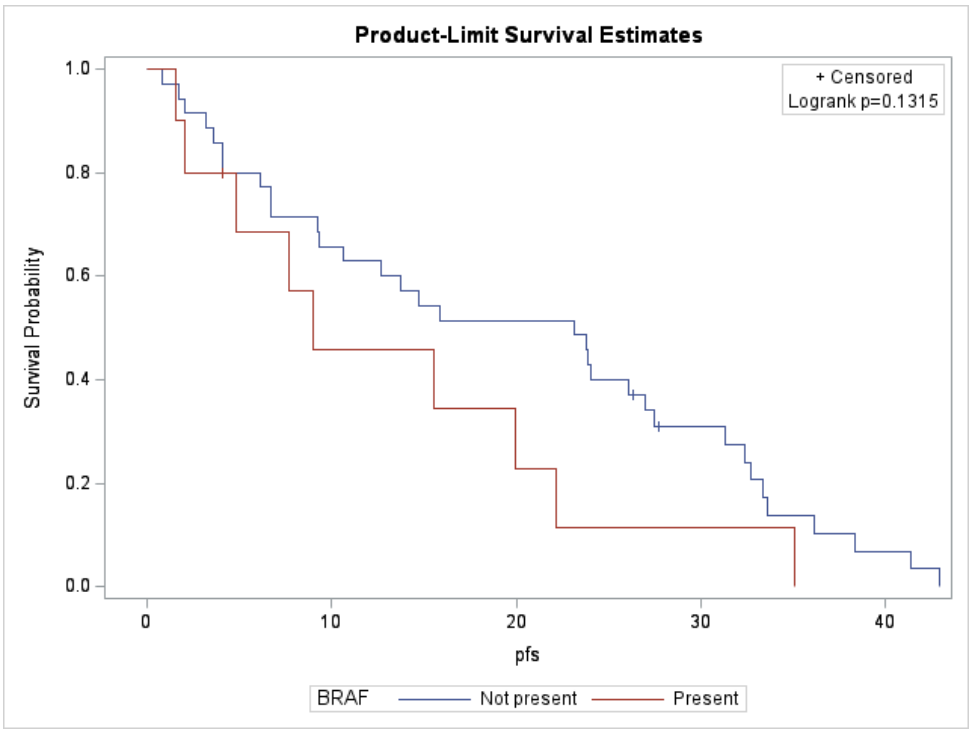

Figure 7 Kaplan Meier Survival Curves Stratified by Gender

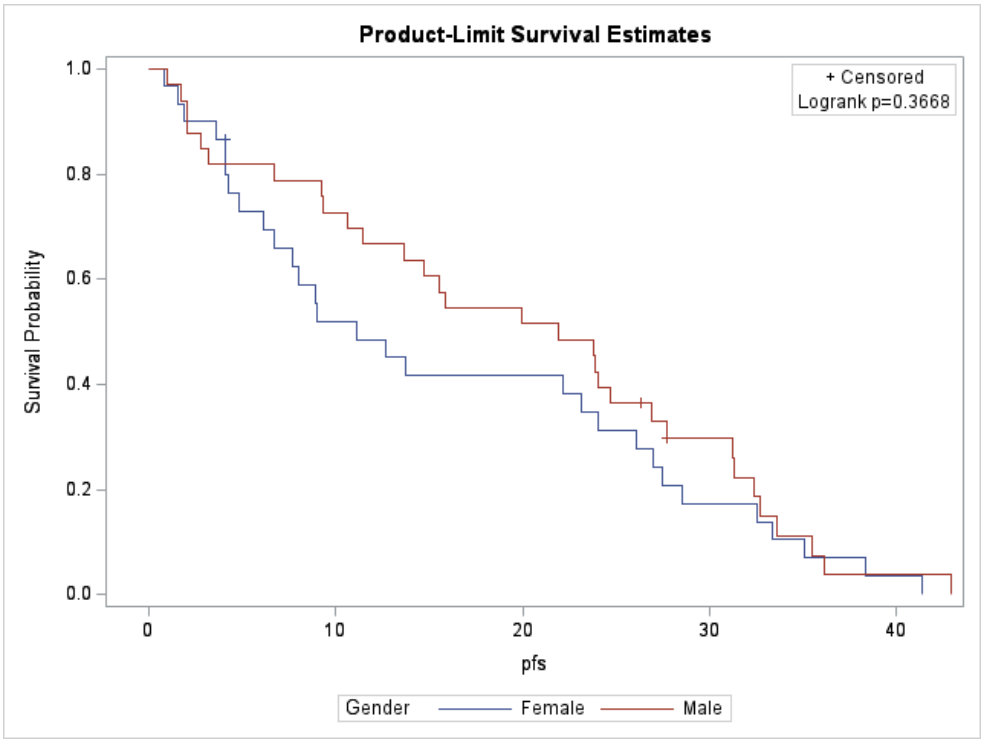

Figure 8 Kaplan Meier Survival Curves Stratified by Age Group

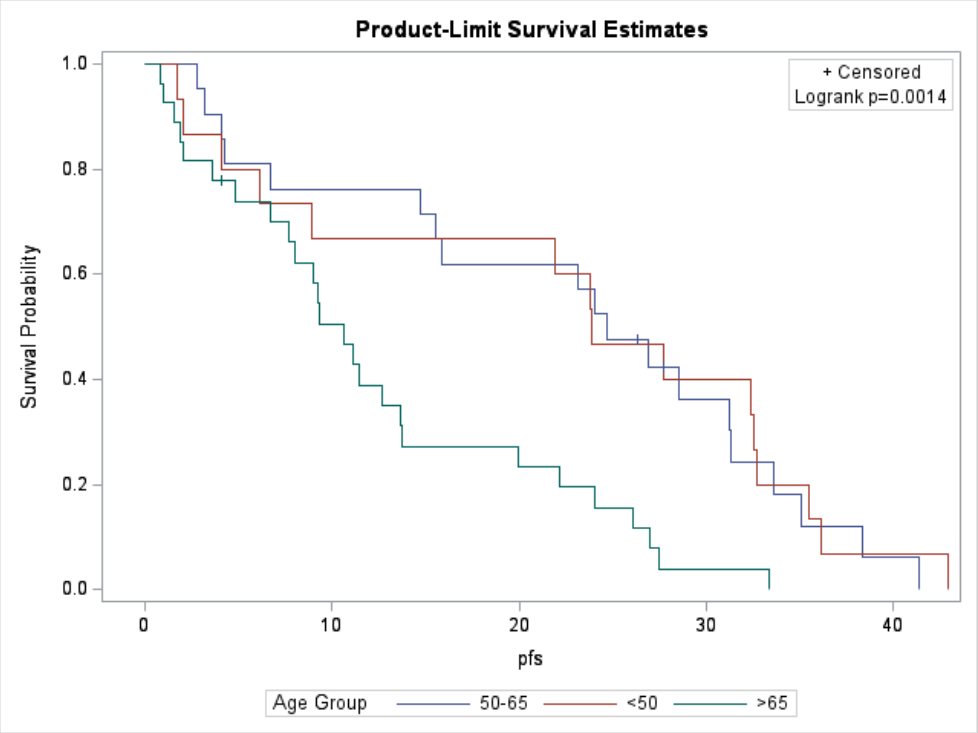

Figure 9 Kaplan Meier Survival Curves Stratified by Side of Tumor

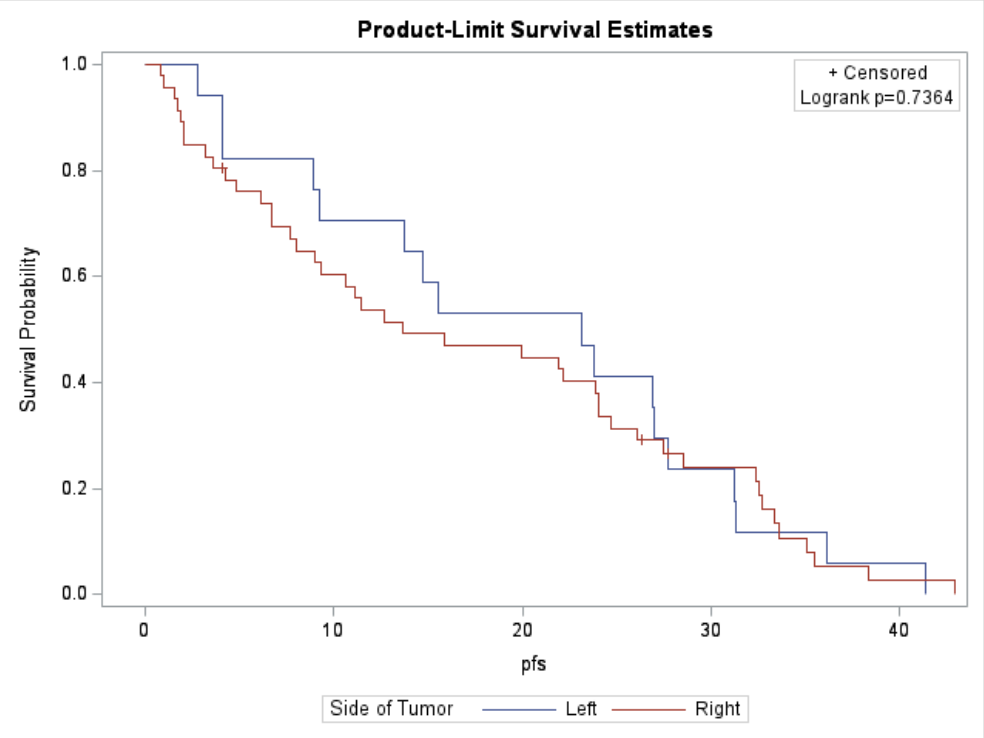

Table 8 12&24 months Survival Analysis

BRAF	No. of Subject	Event	Censored	Median Survival (95% CI)	12 Mo Survival	24 Mo Survival
Not present	35	33 (94%)	2 (6%)	23.1 (9.4, 27)	62.9% (44.8%, 76.5%)	40.0% (24.0%, 55.5%)
Present	10	9 (90%)	1 (10%)	9 (1.6, 22.1)	45.7% (14.3%, 73.0%)	11.4% (0.6%, 39.5%)
Age Group	**No. of Subject**	**Event**	**Censored**	**Median Survival (95% CI)**	**12 Mo Survival**	**24 Mo Survival**
50-65	21	19 (90%)	2 (10%)	24.7 (14.7, 31.3)	76.2% (51.9%, 89.3%)	52.4% (29.7%, 70.9%)
<50	15	15 (100%)	0 (0%)	23.9 (4.1, 32.6)	66.7% (37.5%, 84.6%)	46.7% (21.2%, 68.7%)
>65	27	26 (96%)	1 (4%)	10.6 (6.7, 13.7)	38.9% (20.7%, 56.7%)	15.6% (4.9%, 31.7%)

Side of Tumor	No. of Subject	Event	Censored	Median Survival (95% CI)	12 Mo Survival	24 Mo Survival
Left	17	17 (100%)	0 (0%)	23.1 (8.9, 27.7)	70.6% (43.1%, 86.6%)	41.2% (18.6%, 62.6%)
Right	46	43 (93%)	3 (7%)	13.7 (8, 24)	53.6% (38.2%, 66.8%)	33.5% (20.3%, 47.2%)