Microsatellite instability in stage III colon cancer patients receiving fluoropyrimidine ± oxaliplatin: an

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Microsatellite instability in stage III colon cancer patients receiving fluoropyrimidine ± oxaliplatin: an ACCENT pooled analysis of 12 adjuvant trials

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Microsatellite instability in stage III colon cancer

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Key Words

Colon cancer; microsatellite instability; deficient mismatch repair; prognosis; oxaliplatin

Conflict of Interest

Dr. André reports consulting/advisory role and or received honoraria from, Amgen, Bristol-Myers Squibb, Chugai, Clovis, Gritstone Oncology, Haliodx, MSD Oncology, Pierre Fabre, Roche/Ventana, Sanofi, Servier and Tesaro/GSK and has received travel, accommodations, and expenses from

Roche/Genentech, MSD Oncology, and Bristol-Myers Squibb.

Dr. Goldberg reports consulting/advisory role and or honoraria and has received travel, accommodations, and expenses from Amgen, Genentech, Merck, Novartis, and Taiho from Amgen.

Dr. Shi reports consulting/advisory role from Yiviva Inc. (myself), stock from Johnson & Johnson, Merk and Amgen (myself), research funds from Celgene and Roche/Genentech (to institution)

Dr. Taieb reports consulting/advisory role and or received honoraria from, Amgen, Haliodx, MSD Oncology, Astra-Zeneca, Pierre Fabre, Roche, Sanofi, Lilly, Servier and Merck KGAA and has received travel, accommodations, and expenses from Roche/Genentech, Celgene, Pierre Fabre, Servier and Merck KGAA.

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combined analyses that answered questions previously believed to be impossible. The world of oncology statistics and analysis will not be the same without him, but his legacy continues.

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Context summary

Key objective:

What is the effect of fluoropyrimidine ± oxaliplatin among patients with MSI stage III colon cancer, and what are the main prognosticators of patients with MSI stage III colon cancer patients treated with fluoropyrimidine plus oxaliplatin?

Knowledge generated:

Adding oxaliplatin to fluoropyrimidine significantly improves disease-free survival and overall survival of patients with MSI stage III colon cancer. T-stage and N-stage are the strongest prognosticators of MSI patients treated with oxaliplatin plus fluoropyrimidine.

Relevance:

Fluoropyrimidine plus oxaliplatin should be the standard of care adjuvant treatment for patients with resected stage III MSI colon cancer. With one third of T4 and/or N2 stage III MSI/dMMR CC patients experiencing disease recurrence or death within 2 years after curative tumor resection, innovative therapeutic strategies should be sought for this population.

ABSTRACT

Purpose

In patients with stage III colon cancer (CC) whose tumors demonstrate MSI (microsatellite instability) the efficacy of adjuvant fluoropyrimidine (FP) ± oxaliplatin has not been clearly demonstrated and the prognostic value of MSI remains uncertain.

Material and Methods

Individual patient data from the ACCENT database were used to evaluate the effect of FP ± oxaliplatin on disease-free survival (DFS) and overall survival (OS) among MSI stage III CC and the prognostic value of MSI in patients treated with FP+oxaliplatin, by stratified Cox models adjusted for demographic and clinicopathological factors.

Results

MSI status was available for 5457 patients (609 MSI, 11.2%; 4848 MSS [microsatellite stable], 88.8%) from 12 randomized clinical trials (RCT). Oxaliplatin significantly improved OS of MSI patients from the 2 RCTs testing FP±oxaliplatin (N=185; aHR=0.52, 95%CI 0.28-0.93). Among the 4250 patients treated with FP + oxaliplatin (461 MSI, 3789 MSS), MSI was associated with better OS in the N1 group compared to MSS (aHR=0.66, 95%CI 0.46-0.95) but similar survival in the N2 population (aHR=1.13, 95%CI 0.86-1.48; P-interaction=0.029). The main independent prognosticators of MSI patients treated with FP+oxaliplatin were T-stage (aHR=2.09, 95%CI 1.29-3.38) and N-stage (aHR=3.57, 95%CI 2.32-5.48). Similar results were observed for DFS in all analyses.

Conclusion

Adding oxaliplatin to FP improves OS and DFS in MSI stage III CC patients. Compared to MSS, MSI patients experienced better outcomes in the N1 group but similar survival in the N2 group.

INTRODUCTION

Microsatellite instability (MSI) is a molecular phenotype related to a deficient DNA mismatch repair system (MMR). MMR deficiency (dMMR) results from MMR gene germline mutations (i.e. Lynch syndrome) or from an epigenetic silencing of the MMR system (i.e. sporadic), the latter being frequently associated with the *BRAF*^{V600E} mutation [1].

MSI/dMMR phenotype is observed in approximately 15-18% of stage II colon cancers (CC), 9-10% of stage III CC, and 4-5% of metastatic colorectal cancers (mCRC) [2–6]. MSI/dMMR has been demonstrated as a favorable prognostic marker in stage II CC. Data regarding its value as a prognostic marker are more controversial in stage III and mCRC patients, suggesting a possible shift in the prognostic impact of MSI/dMMR across disease stages and an heterogeneity in MSI/dMMR patients' outcomes [4–17]. However, in all these works, patient numbers were limited and not all patients were treated with the current standard adjuvant treatment for stage III CC patients combining FP plus oxaliplatin.

Previous works have reported that adjuvant FP may be ineffective or even detrimental in MSI/dMMR CC patients with localized diseases, especially in those CC patients with stage II disease [2, 3, 18]. However, more recent data showed the superiority of FP plus oxaliplatin over FP alone in stage III MSI/dMMR CC patients [4, 19]. Importantly, clinical trials evaluating immune checkpoint inhibitors for MSI/dMMR mCRC patients have demonstrated impressive results, raising the interest for evaluating these agents for adjuvant treatment of patients with stage III MSI/dMMR CC [20–24]. Therefore, there is a need to understand the effectiveness of adjuvant treatments and what are the main prognostic factors in MSI/dMMR stage III CC patients.

In this individual patient data analysis, we aimed at evaluating the prognostic value of MSI status and the effect of oxaliplatin-based adjuvant treatments on overall survival (OS) and disease-

free survival (DFS) among MSI stage III CC patients. Prognosticators of MSI/dMMR stage III CC patients treated with oxaliplatin-based adjuvant therapy were also studied.

MATERIAL AND METHODS

Patients

All stage III CC patients with available microsatellite and/or MMR status from randomized trials included in the ACCENT database testing surgery ± adjuvant FP or FP ± oxaliplatin, or from oxaliplatin-based adjuvant treatment arms, were included. Exclusion criteria were: stage I-II CC, unknown tumor stage, unknown MSI and MMR status, discrepant results from polymerase chain reaction and immunochemistry assays (MSI but pMMR or MSS but dMMR), and patients who were assigned to treatment arms other than those prescribing FP or FP plus oxaliplatin (i.e. irinotecan or targeted therapies).

Microsatellite / MMR status determination

MSI/dMMR status was determined by immunohistochemistry or PCR testing. Tumors showing loss of MMR protein expression by immunohistochemistry and/or exhibiting high-level of MSI by PCR testing were defined as MSI/dMMR (loss of MMR protein expression). Tumors with no loss of MMR protein expression and/or MSS or low-level of MSI were defined as MSS/pMMR. Patients tested with both methods and exhibiting opposite results were excluded.

Objectives

The objectives of this work were (i) to evaluate the added value of oxaliplatin in addition to FP treatment in relation with MSI/dMMR status and (ii) to evaluate the prognostic value of MSI status in stage III CC patients treated with a standard oxaliplatin-based adjuvant therapy. The analysis if the added value of FP adjuvant chemotherapy to surgery alone in relation with MSI/dMMR status was a secondary objective.

Statistical analysis

The outcomes included OS and DFS. T stage and N stage were combined in a TN variable (T1-3 and N1, low risk; T4 and/or N2, high risk) as defined in the pooled analysis of the IDEA project [25]. The ratio of positive to examined lymph nodes was calculated (LNR) and analyzed with a cutoff defined at 0.3 [26].

Because of potential stage migration over time [27], only trials in which there was a direct comparison of surgery ± FP adjuvant therapy were analyzed for the evaluation of the MSI/dMMR predictive effect on the efficacy of FP adjuvant treatment (secondary objective). Randomized trials evaluating a direct comparison of FP adjuvant therapy ± oxaliplatin were analyzed to assess MSI/dMMR predictive value on oxaliplatin-based adjuvant treatment efficacy (primary objective). Treatment arms of trials evaluating FP plus oxaliplatin were analyzed for the evaluation of the prognostic value of MSI/dMMR status and for the determination of prognostic factors among MSI/dMMR patients treated with FP plus oxaliplatin.

The direct adjusted Kaplan-Meier curve [28] was used to estimate the distributions of primary and secondary endpoints by treatment and MSI/MMR status, adjusting for age, gender, performance score, T stage and N stage. Multivariable Cox models stratified by studies with interaction terms were used to evaluate the predictive value of MSI/MMR status. Within patients treated with FP plus oxaliplatin, association of baseline factors with OS was assessed using univariate Cox analyses, and then parameters with P-values of less than 0.05 were entered into a multivariable Cox regression model, after considering the amount of missing values and collinearity among variables with a correlation matrix. The proportional hazard (PH) assumption for the stratified Cox model was examined using the scaled Schoenfield residuals, or testing the interaction with time for covariates [29]. Analyses were carried out using SAS software (version 9.4; SAS Institute Inc).

RESULTS

Population characteristics

5457 patients with stage III CC who were enrolled in 12 randomized trials were included in this study [supplementary figure 1 and supplementary table 1], including 609 MSI/dMMR (11.8%) and 4848 MSS/pMMR (88.8%) patients. Of those studies there were (i) 6 randomized trials testing surgery ± FP (49 MSI/dMMR, 357 MSS/pMMR), (ii) 2 trials testing FP ± oxaliplatin (185 MSI/dMMR, 1440 MSS/pMMR) and (iii) 4 additional trials with at least one treatment arm consisting of oxaliplatin plus FP (375 MSI/dMMR, 3051 MSS/pMMR).

Overall, MSI/dMMR CC patients were more frequently female, T1-3 tumor stage, with a larger number of lymph nodes examined and moderate or poor tumor differentiation. They had more frequently tumors arising from the right colon and harboring *BRAF*^{V600E} mutation [supplementary table 2]. The overall median follow-up was 7.2 years (95% confidence interval (95%CI) 7.2-7.3).

Effect of fluoropyrimidine adjuvant therapy on survival

Individual patient data from 6 randomized trials testing surgery ± FP as adjuvant treatment were pooled. 1750 of 3270 patients enrolled in these studies had stage III CC, of which 23% had known MSI/dMMR status, leading to 406 patients (49 MSI/dMMR, 357 MSS/pMMR) available for analysis [supplementary table 1]. Adjuvant treatment with FP alone was associated with better outcomes in the MSS/pMMR group but not in the MSI/dMMR population [supplementary tables 3 and 4 and supplementary figure 2].

Effect of fluoropyrimidine plus oxaliplatin on survival: pooled analysis of the C-07 and MOSAIC trials

Individual data from patients with available data for MSI/dMMR status enrolled in the C-07 and MOSAIC trials (FP-based adjuvant therapy ± oxaliplatin) were analyzed. 185 patients were MSI/dMMR (11.4%) and 1440 MSS/pMMR (88.6%) [supplementary table 5]. Kaplan-Meyer curves for OS and DFS are displayed in figure 1. The adjusted hazard ratios for OS comparing FP plus oxaliplatin to FP alone were 0.52 (95%CI 0.28-0.93) and 0.89 (95%CI 0.74-1.06) in the MSI/dMMR and MSS/pMMR populations, respectively. The interaction effect between MSI/dMMR status and oxaliplatin effect did not reach statistical significance (interaction test P-value = .11). Similar results were observed for DFS (HR = 0.47, 95%CI 0.27-0.82 and HR = 0.82, 95%CI 0.70-0.97 in MSI/dMMR and MSS/pMMR groups; interaction test P-value = .14). The efficacy of oxaliplatin combined with 5-fluorouracil plus leucovorin by subgroups of the MSI/dMMR population is displayed in supplementary figure 3. No violation to proportional hazards assumption regarding treatment variable (p=0.75) was detected.

Prognostic value of MSI status in patients treated with oxaliplatin plus fluoropyrimidine combination

4250 patients (461 MSI/dMMR, 3789 MSS/pMMR) treated with combination of FP and oxaliplatin in MOSAIC, C-07, C-08, PETACC-8, N0147 and AVANT trials were included in this analysis [supplementary table 1]. *BRAF*^{V600E} mutational status was available for 93.4% of this population. Baseline characteristics are summarized in supplementary table 6.

No interaction was observed between MSI/dMMR and T stage, primary tumor sidedness or *BRAF*^{V600E} mutational status (interaction P > 0.12). However, MSI/dMMR had different prognostic effects depending on the N stage category (interaction test P-value for OS = 0.029). Compared to MSS/pMMR, MSI/dMMR was associated with better OS in the N1 population (HR = 0.66, 95%CI 0.46-0.95) but a similar OS in the N2 population (HR = 1.13, 95%CI 0.86-1.48) [figure 2, figure 3A]. This significant interaction was confirmed for DFS, with an excess of events in the first two years of follow-up in the MSI/dMMR N2 population compared with MSS/pMMR N2 patients, though the log-rank test remained not significant [figure 3B]. This interaction was also observed between

MSI/dMMR and TN stage grouped as high and low risk stage III patients (T4 and/or N2 versus T1-3 and N1; interaction test P-value for OS = 0.004) [figure 3C and 3D]. The significant departure of proportional hazards assumption was detected regarding MSI variable for OS in N1 (P-value = 0.002) and high risk subgroups (P-value = 0.005) after Bonferroni multiplicity adjustment. Further analyses were performed by modeling the varying HR over time. For patients with N1 disease, the improved survival associated with MSI/dMMR status was strengthened if a patient can survive beyond 3 to 4 years. For high risk subgroup, the detrimental survivorship in patients with MSI/dMMR status was likely during the early time after treatment (before 3 to 4 years).

Prognosticators of the MSI/dMMR population treated with oxaliplatin plus FP

Table 1 summarizes results from the univariate and multivariable analyses among the 461 MSI/dMMR patients treated with oxaliplatin plus FP. In univariate analysis, gender, T stage, N stage, TN stage and lymph node ratio were found prognostic for OS. *BRAF*^{V600E} mutation was not associated with poorer outcomes in the MSI/dMMR population (HR = 1.18, 95%CI 0.77-1.81), nor the proximal location of the tumor. Lymph node ratio and TN stage were excluded of the multivariable model due to collinearity with N stage. The prognosticators for MSI/dMMR stage III CC patients in the multivariable model were N stage (N2 versus N1, HR = 3.10, 95%CI 2.13-4.50), T stage (T4 versus T1-3, HR = 2.39, 95%CI 1.56-3.66) and gender (male versus female, HR = 1.71, 95%CI 1.14-2.58). Kaplan-Meyer 3-year DFS estimates were respectively 65.0% (95%CI 6-70.0%) versus 87.0% (95%CI 84.3-89.9) for N2 and N1 MSI/dMMR groups, 60.4% (95%CI 52.9-68.9%) versus 82.1% (95%CI 79.5-84.9) for T4 and T1-3 MSI/dMMR groups, and 64.5% (95%CI 60.1-69.2) versus 90.1% (87.5-92.8) for high-risk and low-risk MSI/dMMR CC patients.

DISCUSSION AND CONCLUSION

We report here an evaluation of prognostic and predictive values of MSI/dMMR among stage III CC patients. We showed that (i) there is no benefit nor detrimental effect of adjuvant FP in pts with MSI/dMMR tumors, (ii) the combination of FP plus oxaliplatin significantly improves OS for MSI/dMMR stage III CC patients, (iii) MSI/dMMR is a positive prognostic factor for N1 CC patients and is not prognostic for N2 CC patients and (iv) N stage and T stage are the main prognosticators for MSI/dMMR stage III CC patients treated with FP plus oxaliplatin adjuvant therapy.

The structure of the ACCENT database enabled robust analyses of MSI/dMMR data from multiple phase III randomized trials at an individual patient level, especially for this relatively rare subpopulation in stage III CC. Thanks to the ACCENT database has trials that enrolled patients over a time period spanning more than three decades (1977 to 2009 for this study) and because therapeutic strategies should be taken into account for biomarker studies, we explored MSI/dMMR along with the type of adjuvant therapy (no adjuvant therapy, FP, or FP plus oxaliplatin). First, we evaluated the predictive value of MSI/dMMR for the efficacy of FP as adjuvant treatment. FP in MSI/dMMR CC has been a matter of controversy for decades with some studies showing lack of efficacy of this therapeutic class and other studies evoking a detrimental effect of FP among stage II MSI/dMMR CC patients [2, 18, 30–32]. A previous analysis of the ACCENT database led by Sargent and colleagues on FP-based adjuvant treatment showed a consistent prognostic impact of MSI/dMMR but did not evaluate the effect of FP-based regimens for the MSI/dMMR stage III population [33]. Here we decided to limit our analysis to trials that randomized patients with resected stage III CC between follow-up with no adjuvant chemotherapy and FP adjuvant treatment. In taking this approach, we limited potential biases, such as unbalanced factors with potential prognostic impact, by doing an analysis stratified by study. Whereas FP-based adjuvant treatment significantly improves the outcome of MSS/pMMR patients, it was not found effective for the MSI/dMMR group. Importantly, even if no significant detrimental effect of FP-based adjuvant treatment in the MSI/dMMR population was observed in our

study, the limited percentage of patients tested for MSI/dMMR in the adjuvant trials assessing surgery ± adjuvant FP preclude drawing definitive conclusions. As the current work is the largest ever published on FP adjuvant treatment for MSI stage III CC patients, it suggests that FP alone should not be recommended as adjuvant treatment for these patients.

To investigate the efficacy of oxaliplatin-based adjuvant chemotherapy for MSI/dMMR stage III CC patients, we pooled data of the C-07 and MOSAIC trials, 2 randomized phase III trials that established oxaliplatin plus FP as standard of care adjuvant treatment for stage III CC patients [34, 35]. Post hoc analyses of each study failed to demonstrate the added value of oxaliplatin efficacy for MSI/dMMR CC patients, but (i) they were underpowered, and (ii) in these analyses the cohorts with stage II and stage III diseases were combined [4, 8, 36]. In addition, some patients from the C-07 trial were excluded from our analysis due to potential misdiagnosis for MSI/dMMR status, having discrepant results between PCR and immunohistochemistry tests [37]. Here we show that patients with stage III MSI/dMMR CC significantly benefit from oxaliplatin-based adjuvant treatment (HR = 0.52, 95%CI 0.28-0.93). Interestingly, even if the interaction test did not reach statistical significance, the added-value of oxaliplatin seemed to have more impact on DFS and OS for MSI/dMMR patients than it did for the MSS/pMMR population (absolute change in 5-year OS rates: +9.4% and +2.0% respectively). These results bring contrast to the negative results of the FoxTrot trial, which showed a very low rate of pathological responses (73.6% with no regression) and no survival benefit from neoadjuvant FOLFOX in the 106 MSI/dMMR patients treated in that trial [38].

Among the MSI/dMMR population, older patients represent a clinically meaningful, and challenging situation. MSI/dMMR tumors are more frequent in patients older than 70. Several clinical trials suggest that this population may have attenuated benefit from the addition of oxaliplatin to fluoropyrimidine-based adjuvant treatment [39–41]. Given the lack of efficacy of FP as a single agent for MSI stage III CC, it would have been of interesting to evaluate the effect of oxaliplatin in patients with MSI tumors older than 70. Unfortunately, with only 37 MSI CC patients older than 70 randomized

between FP alone and FP plus oxaliplatin, we could not address this important question due to small patient numbers. Nonetheless, we did not detect any significant interaction between age and the addition of oxaliplatin to FP-based adjuvant treatment [supplementary figure 3].

It is of interest to evaluate MSI/dMMR prognostic value in patients treated with standard of care adjuvant treatment, namely a combination of FP and oxaliplatin (as we confirmed here). Towards this end, we pooled individual patient data from the oxaliplatin plus FP treatment arms of trials included in the ACCENT database (i.e. MOSAIC, C-07, C-08, AVANT, N0147, PETACC8[4, 8, 42–45]). In the overall population, MSI/dMMR was not associated with better outcomes (HR = 0.94, 95%CI 0.76-1.17). As observed in a combined analysis of NCCTG N0147 and PETACC-8 trials [15, 46], the prognostic impact of MSI/dMMR showed a significant interaction with the lymph node stage. Compared with MSS/pMMR, MSI/dMMR was indeed associated with better outcomes for N1 patients (P = 0.027), but poorer survival for N2 patients. This last result did not reached significance, though (P = 0.374). More precisely, for the N2 population, MSI/dMMR was associated with an excess risk of death or disease relapse in the first two years of follow-up, but afterwards, the survival curves crossed. This interaction was also observed when pooling T and N stages in low- and high-risk groups as defined in the IDEA collaboration (T1-3 and N1 versus T4 and/or N2) and MSI/dMMR status. We did not detect any interaction between primary tumor sidedness and MSI/dMMR status (data not shown) as it has been previously reported for patients treated with FOLFOX ± cetuximab in the NCCTG N0147 trial [15]. Unfortunately, we did not have the necessary data elements to properly dichotomize MSI tumors between Lynch syndrome-related tumors and sporadic cases. Nonetheless, no statistically significant difference in patients outcomes was observed for *BRAF*^{V600E}-mutated as compared with *BRAF* wild-type MSI/dMMR patients, confirming results from the PETACC-8 and NCCTG N0147 trials [47, 48]. It is noteworthy that some classic prognostic factors such as lymphovascular invasion, carcinoembryonic antigen level [49] or obstruction were not available in the database. T stage and N stage were the strongest prognosticators of MSI stage III patients treated with oxaliplatin plus FP adjuvant

treatment, with a 3-year DFS rates of 65.0% (95%CI 6-70.0%) for N2 patients and 60.4% (95%CI 52.9-68.9%) for T4 stage III CC patients.

MSI/dMMR has become a major theranostic biomarker harboring a high discrimination capacity for the efficacy of immune checkpoint inhibitors among CRC patients. Given their impressive activity in the metastatic setting [20–22, 50], this justifies evaluating these antibodies in the adjuvant setting. Two phases III randomized trials have been launched specifically for patients with resected MSI/dMMR stage III CC: the ATOMIC trial which evaluates FOLFOX ± atezolizumab (NCT02912559) and the POLEM trial (NCT03827044; 24 weeks of FP or 12 weeks of FP plus oxaliplatin, ± avelumab for MSI/dMMR or polymerase epsilon-mutated patients). Here, in the T4 and/or N2 MSI/dMMR groups, we were able to identify populations of MSI/dMMR stage III CC patients with a high risk of disease recurrence. Indeed, the estimated 3-year DFS rates of MSI/dMMR T4 stage III patients and N2 patients were respectively 60.4% (95%CI 52.9-68.9) and 64.9% (95%CI 60.2-70.0), the latter experiencing poorer survival than the MSS/pMMR N2 population. With one third of T4 and/or N2 high risk stage III MSI/dMMR CC patients experiencing disease recurrence or death within 2 years after curative tumor resection, therapeutic innovations should be sought for this patient population.

To conclude, our individual patient data meta-analysis shows that the combination of oxaliplatin plus FP should be the standard of care adjuvant treatment for MSI/dMMR CC patients and that N stage should be at least a stratification parameter in future trials dedicated to the MSI/dMMR population.

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TABLES

Table 1: Prognosticators for MSI patients treated with standard of care oxaliplatin plus fluoropyrimidine

	Events/Total	Hazard Ratio (95% CI)	P-value[1]	Events/Total	Hazard Ratio (95% CI)	P-value[1]
Age (Step Size: 1)	97/461	1.01 (1.00-1.03)	0.1262			
Gender	97/461		0.0079			0.0098
Female	41/245	Reference		41/245	Reference	
Male	56/216	1.73 (1.15-2.61)		55/215	1.71 (1.14-2.58)	
T-stage	96/460		0.0013			0.0045
T1-3	71/382	Reference		71/382	Reference	
T4	25/78	2.29 (1.43-3.66)		25/78	2.09 (1.29-3.38)	
Grouped Calculated Ratio of Positive to Examined Lymph Nodes	76/332		<.0001			
<=0.3	38/256	Reference				
>0.3	38/76	4.27 (2.71-6.73)				
Number of Lymph Nodes Examined Grouped	77/366		0.8315			
<12	17/74	Reference				
12+	60/292	0.94 (0.55-1.63)				
N-stage (calculated)	97/461		<.0001			<.0001
N1	32/282	Reference		32/282	Reference	
N2	65/179	3.81 (2.49-5.83)		64/178	3.57 (2.32-5.48)	
TN Stage	97/461		<.0001			
Low Risk (T1-3 and N1)	25/244	Reference				
High Risk (T4 or N2)	72/217	3.89 (2.46-6.15)				
Primary Tumor Sidedness	89/400		0.6427			
Left Colon	12/59	0.87 (0.47-1.60)				
Right Colon	77/341	Reference				
BRAF Status	90/429		0.4426			
WT	53/273	Reference				
MT	37/156	1.18 (0.77-1.81)				
Performance Score	96/454		0.1157			
0	67/342	Reference				
1+	29/112	1.45 (0.92-2.27)				
Differential Grade	35/210		0.1224			
Grade I-II	17/121	Reference				
Grade III-IV	18/89	1.69 (0.87-3.30)				
KRAS Status (calculated)	74/363		0.6839			
WT	60/283	Reference				
MT	14/80	0.89 (0.49-1.59)				

[1]Stratified type 3 likelihood-ratio p-value;
*Stratified by Trial;

FIGURE LEGENDS

Figure 1: Effect of fluoropyridimine-based and oxaliplatin-based adjuvant treatment according to the MSI/dMMR status

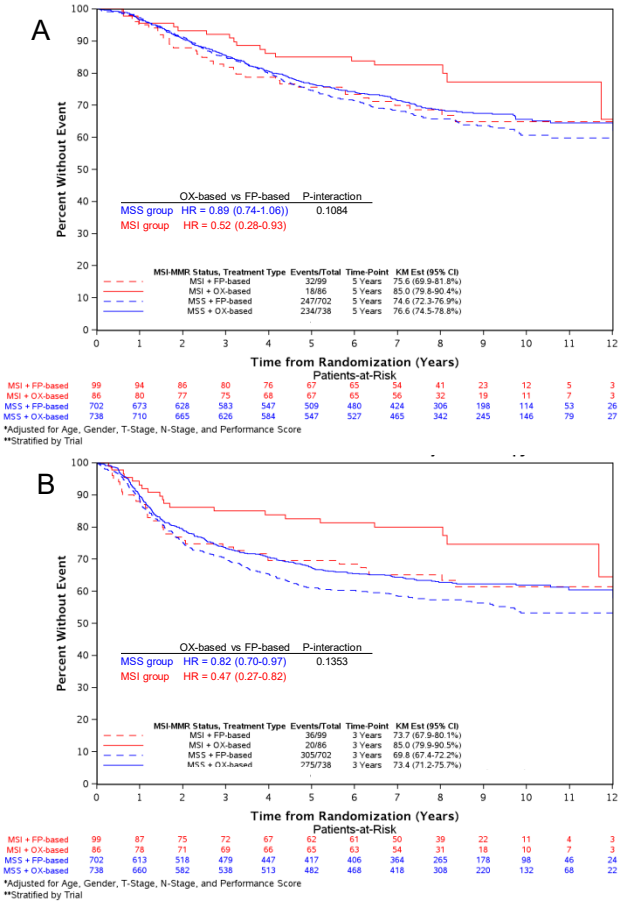
Overall survival (C) and disease-free survival (D) of patients treated with fluoropyrimidine or fluoropyrimidine plus oxaliplatin therapy.

Figure 2: Forest plot for MSI effect on overall survival by baseline factors in the population treated with oxaliplatin and fluoropyrimidine

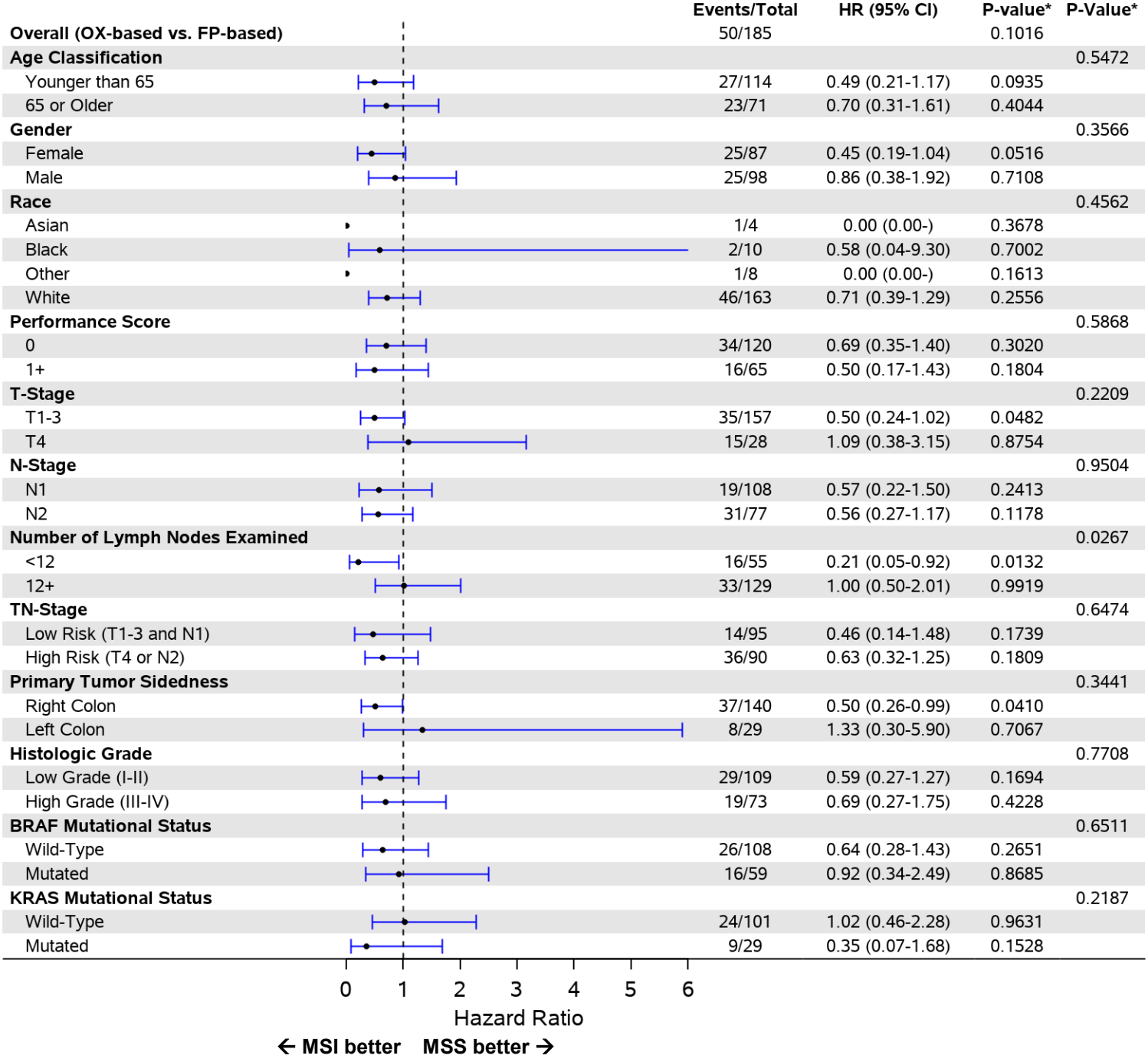
Figure 3: Outcomes of patients treated with oxaliplatin plus fluoropyrimidine according to MSI status and N stage

Overall survival (A) and disease-free survival (B) of patients treated with fluoropyrimidine plus oxaliplatin therapy by MSI/MSS status and N stage;

Overall survival (C) and disease-free survival (D) of patients treated with fluoropyrimidine plus oxaliplatin therapy by MSI/MSS status and TN stage

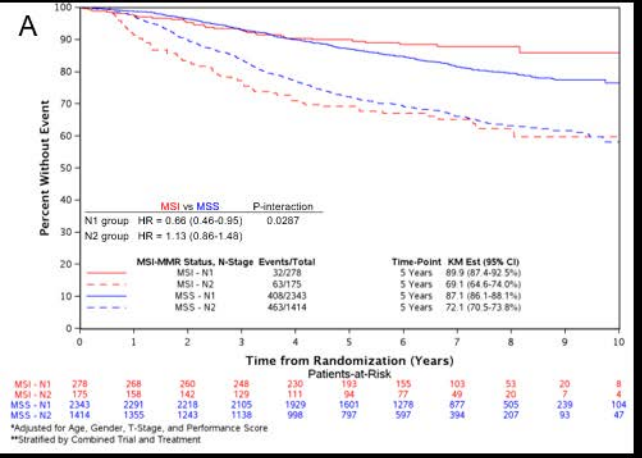

A
Percent Without Event
OX-based vs FP-based P-interaction
MSS group HR = 0.89 (0.74-1.06)) 0.1084
MSI group HR = 0.52 (0.28-0.93)
MSI-MMR Status, Treatment Type Events/Total Time-Point KM Est (95% CI)
MSI + FP-based 32/99 5 Years 75.6 (69.9-81.8%)
MSI + OX-based 18/86 5 Years 85.0 (79.8-90.4%)
MSS + FP-based 247/702 5 Years 74.6 (72.3-76.9%)
MSS + OX-based 234/738 5 Years 76.6 (74.5-78.8%)
Time from Randomization (Years)
Patients-at-Risk
MSI + FP-based 99 94 86 80 76 67 65 54 41 23 12 5 3
MSI + OX-based 86 80 77 75 66 67 65 56 32 19 11 7 3
MSS + FP-based 702 673 628 583 547 509 480 424 306 198 114 53 26
MSS + OX-based 738 710 665 626 584 547 527 465 342 245 146 79 27
*Adjusted for Age, Gender, T-Stage, N-Stage, and Performance Score
**Stratified by Trial
B
Percent Without Event
OX-based vs FP-based P-interaction
MSS group HR = 0.82 (0.70-0.97) 0.1353
MSI group HR = 0.47 (0.27-0.82)
MSI-MMR Status, Treatment Type Events/Total Time-Point KM Est (95% CI)
MSI + FP-based 36/99 3 Years 73.7 (67.9-80.1%)
MSI + OX-based 20/86 3 Years 85.0 (79.9-90.5%)
MSS + FP-based 305/702 3 Years 69.8 (67.4-72.2%)
MSS + OX-based 275/738 3 Years 73.4 (71.2-75.7%)
Time from Randomization (Years)
Patients-at-Risk
MSI + FP-based 99 87 75 72 67 62 61 50 39 22 11 4 3
MSI + OX-based 86 78 71 69 66 65 63 54 31 18 10 7 3
MSS + FP-based 702 613 518 479 447 417 406 364 265 178 98 46 24
MSS + OX-based 738 660 582 538 513 482 468 418 308 220 132 68 22
*Adjusted for Age, Gender, T-Stage, N-Stage, and Performance Score
**Stratified by Trial

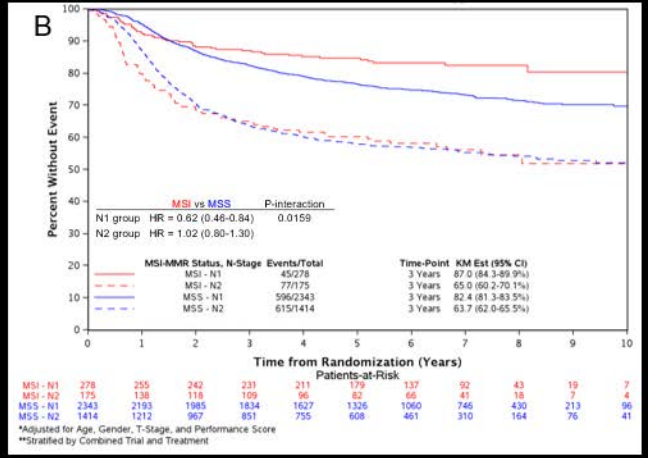
Overall Survival: Forest Plot for Treatment Effect in MSI patients by Baseline Factors

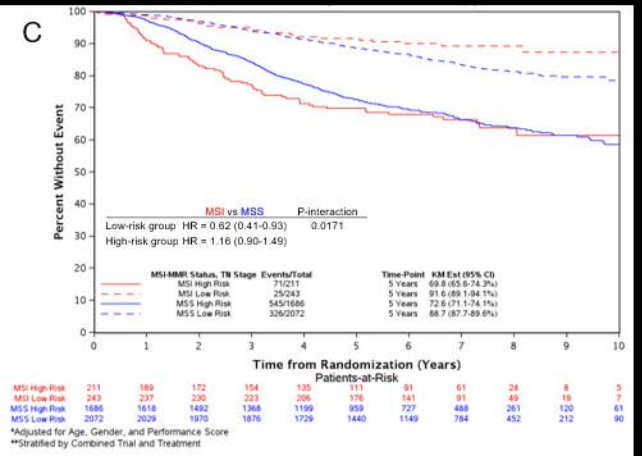


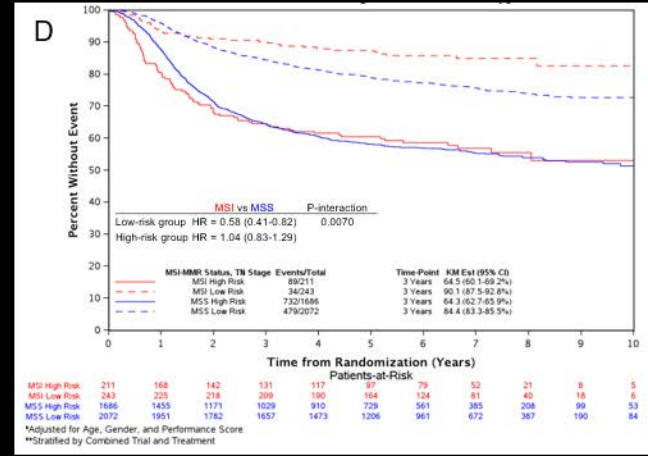
	Events/Total	HR (95% CI)	P-value*	Interaction P-Value*
Overall (OX-based vs. FP-based)	50/185		0.1016	
Age Classification				0.5472
Younger than 65	27/114	0.49 (0.21-1.17)	0.0935	
65 or Older	23/71	0.70 (0.31-1.61)	0.4044	
Gender				0.3566
Female	25/87	0.45 (0.19-1.04)	0.0516	
Male	25/98	0.86 (0.38-1.92)	0.7108	
Race				0.4562
Asian	1/4	0.00 (0.00-)	0.3678	
Black	2/10	0.58 (0.04-9.30)	0.7002	
Other	1/8	0.00 (0.00-)	0.1613	
White	46/163	0.71 (0.39-1.29)	0.2556	
Performance Score				0.5868
0	34/120	0.69 (0.35-1.40)	0.3020	
1+	16/65	0.50 (0.17-1.43)	0.1804	
T-Stage				0.2209
T1-3	35/157	0.50 (0.24-1.02)	0.0482	
T4	15/28	1.09 (0.38-3.15)	0.8754	
N-Stage				0.9504
N1	19/108	0.57 (0.22-1.50)	0.2413	
N2	31/77	0.56 (0.27-1.17)	0.1178	
Number of Lymph Nodes Examined				0.0267
<12	16/55	0.21 (0.05-0.92)	0.0132	
12+	33/129	1.00 (0.50-2.01)	0.9919	
TN-Stage				0.6474
Low Risk (T1-3 and N1)	14/95	0.46 (0.14-1.48)	0.1739	
High Risk (T4 or N2)	36/90	0.63 (0.32-1.25)	0.1809	
Primary Tumor Sidedness				0.3441
Right Colon	37/140	0.50 (0.26-0.99)	0.0410	
Left Colon	8/29	1.33 (0.30-5.90)	0.7067	
Histologic Grade				0.7708
Low Grade (I-II)	29/109	0.59 (0.27-1.27)	0.1694	
High Grade (III-IV)	19/73	0.69 (0.27-1.75)	0.4228	
BRAF Mutational Status				0.6511
Wild-Type	26/108	0.64 (0.28-1.43)	0.2651	
Mutated	16/59	0.92 (0.34-2.49)	0.8685	
KRAS Mutational Status				0.2187
Wild-Type	24/101	1.02 (0.46-2.28)	0.9631	
Mutated	9/29	0.35 (0.07-1.68)	0.1528	

*Stratified Type-3 Likelihood-Ratio P-value;
**Stratified by Trial;


A
Percent Without Event
MSI vs MSS P-interaction
N1 group HR = 0.66 (0.46-0.95) 0.0287
N2 group HR = 1.13 (0.86-1.48)
MSI-MMR Status, N-Stage Events/Total
MSI - N1 32/278
MSI - N2 63/175
MSS - N1 408/2343
MSS - N2 463/1414
Time-Point KM Est (95% CI)
5 Years 89.9 (87.4-92.5%)
5 Years 69.1 (64.6-74.0%)
5 Years 87.1 (86.1-88.1%)
5 Years 72.1 (70.5-73.8%)
Time from Randomization (Years)
Patients-at-Risk
MSI - N1 278 268 260 248 230 193 155 103 53 20 8
MSI - N2 175 158 142 129 111 94 77 49 20 7 4
MSS - N1 2343 2291 2218 2105 1929 1601 1278 877 505 239 104
MSS - N2 1414 1355 1243 1138 998 797 597 394 207 93 47
*Adjusted for Age, Gender, T-Stage, and Performance Score
**Stratified by Combined Trial and Treatment


B
Percent Without Event
MSI vs MSS P-interaction
N1 group HR = 0.62 (0.46-0.84) 0.0159
N2 group HR = 1.02 (0.80-1.30)
MSI-MMR Status, N-Stage Events/Total
MSI - N1 45/278
MSI - N2 77/175
MSS - N1 596/2343
MSS - N2 615/1414
Time-Point KM Est (95% CI)
3 Years 87.0 (84.3-89.9%)
3 Years 65.0 (60.2-70.1%)
3 Years 82.4 (81.3-83.5%)
3 Years 63.7 (62.0-65.5%)
Time from Randomization (Years)
Patients-at-Risk
MSI - N1 278 255 242 231 211 179 137 92 43 19 7
MSI - N2 175 138 118 109 96 82 66 41 18 7 4
MSS - N1 2343 2193 1985 1834 1627 1326 1060 746 430 213 96
MSS - N2 1414 1212 967 851 755 608 461 310 164 76 41
*Adjusted for Age, Gender, T-Stage, and Performance Score
**Stratified by Combined Trial and Treatment


C
Percent Without Event
MSI vs MSS P-interaction
Low-risk group HR = 0.62 (0.41-0.93) 0.0171
High-risk group HR = 1.16 (0.90-1.49)
MSI-MMR Status, TN Stage Events/Total
MSI High Risk 71/211
MSI Low Risk 25/243
MSS High Risk 545/1686
MSS Low Risk 326/2072
Time-Point KM Est (95% CI)
5 Years 69.8 (65.6-74.3%)
5 Years 91.6 (89.1-94.1%)
5 Years 72.6 (71.1-74.1%)
5 Years 88.7 (87.7-89.6%)
Time from Randomization (Years)
Patients-at-Risk
MSI High Risk 211 189 172 154 135 111 91 61 24 8 5
MSI Low Risk 243 237 230 223 206 176 141 91 49 19 7
MSS High Risk 1686 1618 1492 1368 1199 959 727 488 261 120 61
MSS Low Risk 2072 2029 1970 1876 1729 1440 1149 784 452 212 90
*Adjusted for Age, Gender, and Performance Score
**Stratified by Combined Trial and Treatment


D
Percent Without Event
MSI vs MSS P-interaction
Low-risk group HR = 0.58 (0.41-0.82) 0.0070
High-risk group HR = 1.04 (0.83-1.29)
MSI-MMR Status, TN Stage Events/Total
MSI High Risk 89/211
MSI Low Risk 34/243
MSS High Risk 732/1686
MSS Low Risk 479/2072
Time-Point KM Est (95% CI)
3 Years 64.5 (60.1-69.2%)
3 Years 90.1 (87.5-92.8%)
3 Years 64.3 (62.7-65.9%)
3 Years 84.4 (83.3-85.5%)
Time from Randomization (Years)
Patients-at-Risk
MSI High Risk 211 168 142 131 117 97 79 52 21 8 5
MSI Low Risk 243 225 218 209 190 164 124 81 40 18 6
MSS High Risk 1686 1455 1171 1029 910 729 561 385 208 99 53
MSS Low Risk 2072 1951 1782 1657 1473 1206 961 672 387 190 84
*Adjusted for Age, Gender, and Performance Score
**Stratified by Combined Trial and Treatment