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Efficacy of Anti-EGFR in Microsatellite Instability Metastatic Colorectal Cancer Depending on Sporadic or Familial Origin

Aziz Zaanan, MD, PhD,[1,2,*] Julie Henriques, MSc,[3,4] Romain Cohen, MD, PhD,[5] David Sefrioui, MD,[6] Camille Evrard, MD,[7] Christelle de la Fouchardiere, MD,[8] Thierry Lecomte, MD, PhD,[9] Thomas Aparicio, MD, PhD,[10,11] Magali Svrcek, MD, PhD,[12] Julien Taieb, MD, PhD,[1,2] Thierry André, MD, PhD,[5] Dewi Vernerey, PhD,[3,4] David Tougeron, MD, PhD, [13]; for the Association des Gastro-entérologues Oncologues (AGEO)

[1]Department of Gastroenterology and Digestive Oncology, European Georges Pompidou Hospital, AP-HP Centre, Paris, France; [2]Centre de Recherche des Cordeliers, INSERM, Sorbonne Université, Université de Paris, MEPPOT, F-75006 Paris, France; [3]Bourgogne Franche-Comté University, INSERM, Etablissement Français du Sang Bourgogne Franche-Comté, UMR1098, Interactions Hôte-Greffon-Tumeur/Ingénierie Cellulaire et Génique, Besançon, France; [4]Methodology and Quality of Life Unit in Oncology, University Hospital of Besançon, Besançon, France; [5]Medical Oncology Department, Sorbonne University, Saint-Antoine Hospital, AP-HP, F-75012 Paris, France; [6]Digestive Oncology Unit, Department of Hepatogastroenterology, Rouen University Hospital, Normandie Univ, UNIROUEN, Inserm, 1245, IRON Group, Rouen, France; [7]Medical Oncology Department, Poitiers University Hospital, Poitiers, France; [8]Medical Oncology Department, Léon Bérard Center, Lyon, France; [9]Department of Hepato-Gastroenterology and Digestive Oncology, Tours University Hospital, EA, 7501 GICC, University of Tours, Tours, France; [10]Gastroenterology Department, Saint Louis Hospital, AP-HP, Université de Paris, Paris, France; [11]Gastroenterology Department, Avicenne Hospital, AP-HP, Bobigny, France; [12]Sorbonne University, Department of Pathology, Saint-Antoine Hospital, AP-HP, F-75012 Paris, France and [13]Gastroenterology Department, Poitiers University Hospital and University of Poitiers, Poitiers, France

*Correspondence to: Aziz Zaanan, MD, PhD, Department of Gastroenterology and Digestive Oncology, European Georges Pompidou Hospital, Université de Paris, APHP Centre, 20 rue Leblanc, 75015 Paris, France (e-mail: aziz.zaanan@aphp.fr).

Abstract

Anti-epidermal growth factor receptor (EGFR) efficacy in patients with microsatellite instability (MSI) metastatic colorectal cancer (mCRC) according to sporadic vs familial origin is unknown. We retrospectively analyzed 128 patients with MSI mCRC treated with first-line chemotherapy ± anti-EGFR. Among them, 61 and 67 patients were respectively categorized as familial and sporadic based on mismatch repair protein immunostaining, *BRAF* mutational status, and *MLH1* promoter methylation status. We observed that addition of anti-EGFR to chemotherapy was associated with a statistically significant improvement of progression-free survival for familial (median = 5.0 vs 10.2 months, hazard ratio [HR] = 0.47, 95% confidence interval [CI] = 0.23 to 0.94; P = .03) but not for sporadic (median = 4.4 vs 5.4 months, HR = 0.80, 95% CI = 0.39 to 1.60; P = .52) MSI mCRC patients. In multivariate analysis, the survival benefit of adding anti-EGFR to chemotherapy remained statistically significant for familial MSI cases (P = .04). These findings deserve to be confirmed in a prospective study and could help decision making in MSI mCRC without access or resistant to immunotherapy.

In colorectal cancer (CRC), DNA microsatellite instability (MSI) or deficient mismatch repair (dMMR) occurs in a familial context from a germline mutation in one of MMR genes (*MLH1, MSH2, MSH6,* or *PMS2*) known as Lynch syndrome, or in sporadic cases, by an epigenetic inactivation of *MLH1* gene because of the hypermethylation of its promoter (1). The activating somatic V600E mutation in *BRAF* is frequent (50%-70%) in sporadic MSI tumors but absent (or exceptional) in familial MSI tumors (2).

In metastatic CRC (mCRC), dMMR/MSI is relatively uncommon (<5%) (3,4) but has been shown to be a predictive marker of response to immune checkpoint inhibitors (ICI) (anti-PD-1 alone or combined with anti-CTLA-4) (5,6). However, approximately 30%-40% of MSI mCRC patients do not respond to ICI or will develop resistance to ICI and need alternative treatment options (5,6). The data regarding the effect of chemotherapy +/- targeted therapies in MSI CRC are very scarce, but anti–epidermal growth factor receptor (EGFR) therapy appeared to be ineffective in some studies (7,8). In this work, we aimed to examine the

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Table 1. Clinical and pathological characteristics of patients with MSI metastatic colorectal cancer treated in first-line with chemotherapy alone or combined with anti-EGFR monoclonal antibody[a]

Characteristic	All MSI patients			MSI sporadic			MSI familial		
	Sporadic MSI (n=67) No. (%)	Familial MSI (n=61) No. (%)	p[b]	Chemotherapy alone (n=52) No. (%)	Chemotherapy + anti-EGFR (n=15) No. (%)	p[b]	Chemotherapy alone (n=44) No. (%)	Chemotherapy + anti-EGFR (n=17) No. (%)	p[b]
Gender	67	61	<.001	52	15	.33	44	17	.79
Male	19 (28.4)	41 (67.2)		13 (25.0)	6 (40.0)		30 (68.2)	11 (64.7)	
Female	48 (71.6)	20 (32.8)		39 (75.0)	9 (60.0)		14 (31.8)	6 (35.3)	
Age, y	67	61	<.001	52	15	.32	44	17	.13
Median (range)	71.1 (44.7-90.2)	49.2 (17.3-78.3)		71.3 (44.7-90.2)	68.3 (49.5-85.2)		46.7 (17.3-78.3)	51.1 (39.1-74.5)	
ECOG PS	42	36	.36	34	8	.91	25	11	.81
0-1	33 (78.6)	25 (59.4)		27 (79.4)	6 (75.0)		17 (68.0)	8 (72.7)	
≥2	9 (21.4)	11 (30.6)		7 (20.6)	2 (25.0)		8 (32.0)	3 (27.3)	
Primary tumor localization	67	61	<.001	52	15	.74	44	17	.22
Right	58 (86.6)	33 (54.1)		44 (84.6)	14 (93.3)		21 (47.7)	12 (70.6)	
Left	8 (11.9)	18 (29.5)		7 (13.5)	1 (6.7)		14 (31.8)	4 (23.5)	
Rectum	1 (1.5)	10 (16.4)		1 (1.9)	0 (0.0)		9 (20.5)	1 (5.9)	
Surgery of primary tumor	67	61	.17	52	15	.03	44	17	1.0
Yes	63 (94.0)	53 (86.9)		51 (98.1)	12 (80.0)		38 (86.4)	15 (88.2)	
Synchronicity of metastases	67	61	.93	52	15	.66	44	17	<.01
Synchronous	39 (58.2)	36 (59.0)		31 (59.6)	8 (53.3)		31 (70.4)	5 (29.4)	
Metachronous	28 (41.8)	25 (41.0)		21 (40.4)	7 (46.7)		13 (29.6)	12 (70.6)	
No. metastatic sites	67	61	.26	52	15	.82	44	17	.79
1	46 (68.7)	36 (59.0)		36 (69.2)	10 (66.7)		25 (56.8)	11 (64.7)	
≥2	21 (31.3)	25 (41.0)		16 (30.8)	5 (33.3)		19 (43.2)	6 (35.3)	
Metastases site	67	61		52	15		44	17	
Liver	18 (26.9)	31 (50.8)	.01	14 (26.9)	4 (26.7)	1.0	25 (56.8)	6 (35.3)	.13
Lung	11 (16.4)	6 (9.8)	.27	9 (17.3)	2 (13.3)	1.0	4 (9.1)	2 (11.8)	1.0
Peritoneal	29 (43.3)	29 (47.5)	.63	23 (44.2)	6 (40.0)	1.0	21 (47.7)	8 (47.1)	.96
Lymph nodes	26 (38.8)	18 (29.5)	.27	19 (36.5)	7 (46.7)	.48	10 (22.7)	8 (47.1)	.06
Differentiation	57	54	.27	46	11	.06	39	15	.12
Well/moderate	30 (52.6)	34 (63.0)		27 (58.7)	3 (27.3)		12 (30.8)	8 (53.3)	
Poor	27 (47.4)	20 (37.0)		19 (41.3)	8 (72.7)		27 (69.2)	7 (46.7)	
BRAF status	67	57	<.001	52	15	1.0	40	17	.57
Wild-type	17 (25.4)	53 (93.0)		13 (25.0)	4 (26.7)		38 (95.0)	15 (88.2)	
Mutated	50 (74.6)	4 (7.0)		39 (75.0)	11 (73.3)		2 (5.0)	2 (11.8)	
KRAS exon 2 status	67	55	<.001	52	15	.58	39	16	<.001
Wild-type	62 (92.5)	36 (65.4)		47 (90.4)	15 (100)		20 (51.3)	16 (100)	
Mutated	5 (7.5)	19 (34.6)		5 (9.6)	0 (0.0)		19 (48.7)	0 (0.0)	
Complete RAS status	61	37	<.001	49	12	.59	26	11	<.001
Wild-type	55 (90.2)	16 (43.2)		43 (87.8)	12 (100)		6 (23.1)	10 (90.9)	
Mutated	5 (8.8)	21 (56.8)		6 (12.2)	0 (0.0)		20 (76.9)	1 (9.1)	
Chemotherapy regimen in first-line	67	61	<.001	52	15	<.001	44	17	<.001
5FU monotherapy-based	14 (20.9)	2 (3.3)		14 (26.9)	0 (0.0)		2 (4.6)	0 (0.0)	
FOLFIRI-based	14 (20.9)	19 (31.1)		6 (11.6)	8 (53.3)		7 (15.9)	12 (70.6)	

(continued)

Table 1. (continued)

Characteristic	All MSI patients			MSI sporadic			MSI familial		
	Sporadic MSI (n=67) No. (%)	Familial MSI (n=61) No. (%)	P^b	Chemotherapy alone (n=52) No. (%)	Chemotherapy + anti-EGFR (n=15) No. (%)	P^b	Chemotherapy alone (n=44) No. (%)	Chemotherapy + anti-EGFR (n=17) No. (%)	P^b
FOLFOX-based	38 (56.7)	32 (52.5)		31 (59.6)	7 (46.7)		28 (63.6)	4 (23.5)	
FOLFIRINOX-based	1 (1.5)	8 (13.1)		1 (1.9)	0 (0.0)		7 (15.9)	1 (5.9)	

a Clinicopathological characteristics at baseline are described as continuous variables with median and range and qualitative variables with frequency and percentages. ECOG PS = Eastern Cooperative Oncology Group performance status; EGFR = epidermal growth factor receptor; 5FU = 5-fluorouracil; MSI = microsatellite instability.
b Comparisons of characteristics in MSI patients between sporadic and familial and between first-line treatment with or without anti-EGFR were performed with Wilcoxon test for continuous variables and χ^2 test or Fisher exact test for qualitative variables.

survival outcomes with chemotherapy alone or combined with anti-EGFR of patients with MSI mCRC according to sporadic or familial origin.

We retrospectively included all consecutive patients with MSI/dMMR mCRC treated in first-line by chemotherapy alone or combined with anti-EGFR monoclonal antibody between 2007 and 2017 in 18 French centers. Demographic data, tumor characteristics, treatment responses, and survival were collected. The study was approved by the ethics committee Comité de Protection des Personnes Ouest III, and because of its retrospective nature with a majority of deceased patients, no patient consent was required. MMR tumor status was determined in each center by immunohistochemistry and/or by DNA MSI testing, as recommended (9,10). Methylation of MLH1 promoter and testing for RAS and BRAF (V600E) mutations were performed in genomic DNA extracted from formalin-fixed, paraffin-embedded tumor tissue, as previously described (8,11). The determination of sporadic and familial MSI tumors was based on the MMR protein immunostaining profile, BRAF mutational status, and MLH1 promoter methylation status, as recommended (12).

The efficacy of treatment was assessed by the progression-free survival (PFS) defined as the time elapsed from the beginning of first-line chemotherapy until the date of first progression or death, whichever came first. We also evaluated the overall survival (OS) defined as the time elapsed until death (all causes). Survival curves were drawn with the Kaplan-Meier method. Univariate and multivariate analyses (Cox proportional hazards model) were used to calculate hazard ratios (HRs) with 95% confidence intervals (CIs). Proportional hazards assumptions were examined graphically by plotting log-minus-log of survival. Variables with P values of no more than 0.10 in univariate analyses were included in a multivariate model, and stepwise selection was then performed. To limit potential bias because of confounding parameters unbalanced between treatment arms in sporadic and familial MSI groups, the inverse probability of treatment weighting method was applied in the Cox regression model using the propensity score derived from multivariate logistic regression (Supplementary Table 2, available online). A P value of less than .05 was considered statistically significant. All statistical tests were 2-sided. All analyses were performed using SAS software version 9.3 (SAS Institute, Cary, NC).

Among the 342 patients diagnosed with MSI mCRC, we have excluded those who were not treated with first-line chemotherapy or treated with an anti-angiogenic agent in first-line and those for whom sporadic or familial MSI status was unknown (13). A total of 128 patients with MSI mCRC treated by first-line chemotherapy +/- anti-EGFR monoclonal antibody were analyzed. Among them, 67 patients were categorized as sporadic cases (BRAF mutated and/or with methylation of MLH1 promoter), including 15 treated with anti-EGFR inhibitors and 61 patients as familial cases (BRAF wild-type and unmethylated MLH1 or loss of MSH2, MSH6, and PMS2 protein expression), including 17 treated with anti-EGFR inhibitors (12). The median follow-up was 33.5 months (95% CI = 20.7 to 45). Demographic and pathological characteristics between first-line treatment (chemotherapy alone or with anti-EGFR) and dMMR mechanism (sporadic and familial MSI mCRC) are listed in Table 1. As compared with familial origin, patients with sporadic MSI mCRC were statistically significantly more likely to be older, female, and treated with 5FU alone and to have a cancer localized in the right side, with less liver metastasis, BRAF mutated, and RAS wild-type (Table 1).
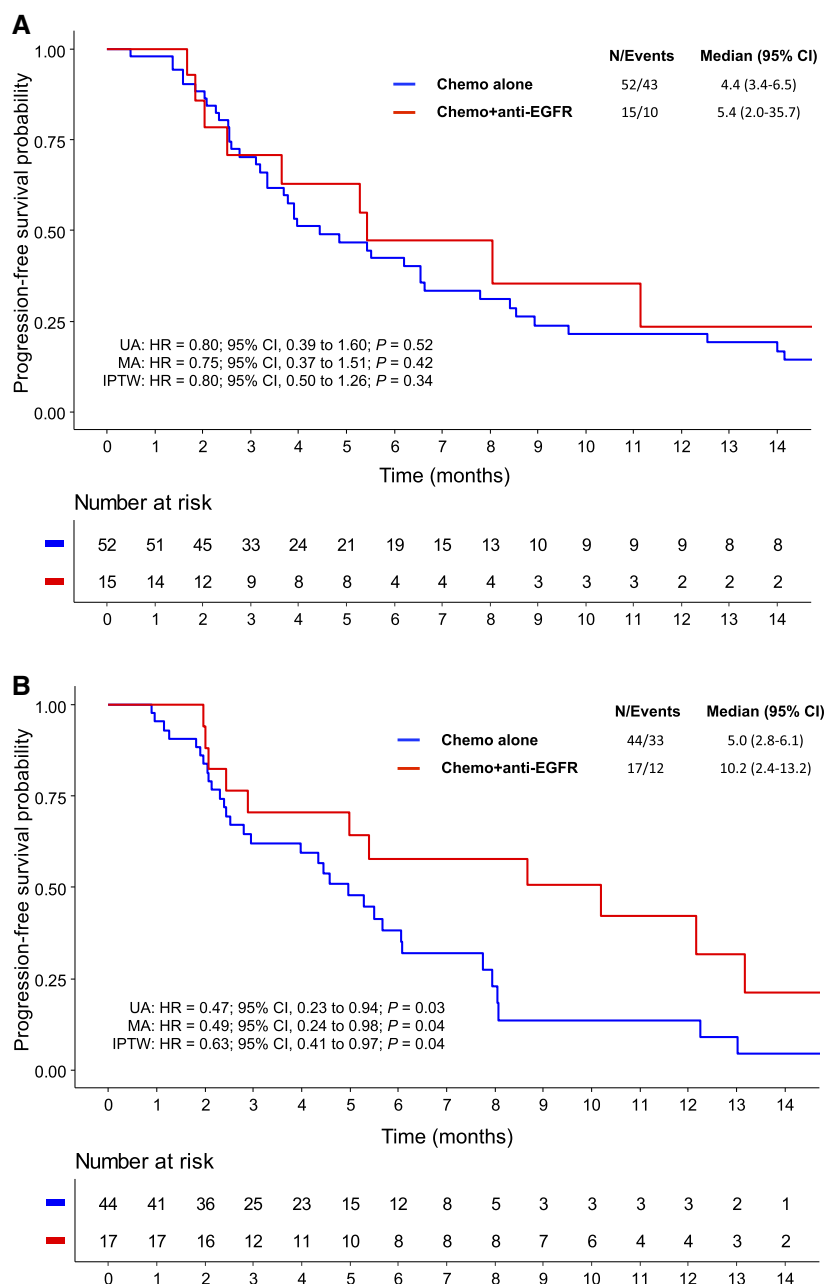
Figure 1. Progression-free survival in patients with sporadic or familial MSI metastatic colorectal cancer treated with first-line by chemotherapy alone or combined with anti-EGFR monoclonal antibody. For sporadic MSI cases **(A)**, multivariate analysis was adjusted on variables with P values of no more than .10 in univariate analyses (ie, number of metastatic sites and peritoneal metastases). The IPTW was applied in the Cox regression model using the propensity score based on tumor differentiation and chemotherapy regimen. For familial MSI cases **(B)**, multivariate analysis was adjusted on variables with P values of no more than .10 in univariate analyses (ie, gender and surgery of primary tumor). The IPTW was applied in the Cox regression model using the propensity score based on synchronicity of metastases, lymph nodes metastases, and chemotherapy regimen. All statistical tests were 2-sided. Anti-EGFR = anti-epidermal growth factor receptor; chemo = chemotherapy; CI = confidence interval; HR = hazard ratio; IPTW = inverse probability of treatment weighting; MA = multivariate analysis; MSI = microsatellite instability; UA = univariate analysis.

The median PFS of sporadic and familial MSI mCRC patients were 5.3 and 5.4 months, respectively (HR = 1.10, 95% CI = 0.73 to 1.64; P = .65). In patients with sporadic MSI mCRC, there was no statistically significant difference in terms of PFS between chemotherapy alone and anti-EGFR–based treatment (median = 4.4 vs 5.4 months, HR = 0.80, 95% CI = 0.39 to 1.60; P = .52) (Figure 1A). However, in patients with familial MSI mCRC, the addition of anti-EGFR to chemotherapy was associated with a

statistically significant improvement of PFS (median = 5.0 vs 10.2 months, HR = 0.47, 95% CI = 0.23 to 0.94; P = .03) (Figure 1B). The survival benefit of adding anti-EGFR remained statistically significant for familial MSI cases in both multivariate analysis (HR = 0.49, 95% CI = 0.24 to 0.98; P = .04) (Supplementary Table 1, available online) and inverse probability of treatment weighting Cox analysis (HR = 0.63, 95% CI = 0.41 to 0.97; P = .04) (Supplementary Table 2, available online). For OS, the addition

of anti-EGFR to first-line chemotherapy did not statistically significantly improve outcomes for patients with sporadic (HR = 0.88, 95% CI = 0.42 to 1.84; P = .73) and familial (HR = 0.93, 95% CI = 0.37 to 2.34; P = .88) MSI tumors. These OS results should be interpreted with caution because of different treatments received beyond the first-line chemotherapy, including immunotherapy.

A post hoc analysis of the randomized CALGB/SWOG 80405 trial showed that OS from the first-line chemotherapy (FOLFOX or FOLFIRI) for MSI mCRC patients (n = 37) was shorter for those receiving cetuximab as compared with bevacizumab (median OS = 11.9 vs 30.0 months). In contrast, median OS was similar between cetuximab and bevacizumab treatment arms for MSS mCRC patients (n = 586; median OS = 30.7 vs 30.3 months) (7). The low number of MSI mCRC patients included in this study, without classification between sporadic and familial cases, did not help to evaluate the efficacy of anti-EGFR–based chemotherapy according to the dMMR mechanism. More recently, the post hoc analysis of PETACC8 and N0147 trials showed in patients with sporadic MSI stage III colon cancer (n = 255) that the addition of anti-EGFR to FOLFOX was associated with shorter DFS, whatever the *BRAF* mutational status (8). In our study, we observed that adding anti-EGFR to chemotherapy in sporadic MSI cases was not associated with a statistically significant improvement of PFS in *BRAF* wild-type (HR = 1.63, 95% CI = 0.42 to 6.38; P = .48) or in *BRAF* mutated (HR = 0.63, 95% CI = 0.28 to 1.44; P = .26) tumors. Some studies have suggested that the resistance to anti-EGFR could be linked to genomic alterations involved in sporadic MSI CRC, such as *HER2* or *MET* amplification, *PTEN* or *PIK3CA* mutations, and methylator phenotype (CIMP) (14–16).

To our knowledge, our report is the largest series of patients with MSI mCRC evaluating anti-EGFR efficacy according to the dMMR mechanism. Study limitations include first the retrospective nature but with few missing data, and second the categorization of dMMR/MSI tumors into suspected familial origin that was not systematically confirmed by germline MMR mutation testing but was done using already validated algorithms (12). Our findings deserve to be confirmed in a prospective study and could help decision making in MSI mCRC patients without access or resistant to ICI.

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Notes

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