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Precision Medicine and Imaging

Clinical Cancer Research

Prognostic Value of Methylator Phenotype in Stage III Colon Cancer Treated with Oxaliplatin-based Adjuvant Chemotherapy

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Abstract

Purpose: There are conflicting results concerning the prognostic value of the CpG island methylator phenotype (CIMP) in patients with nonmetastatic colon cancer. We studied this phenotype in stage III colon cancer characterized for mismatch repair (MMR), *RAS*, and *BRAF* status, and treated with adjuvant FOLFOX-based regimen.

Experimental Design: Tumor samples of 1,907 patients enrolled in the PETACC-8 adjuvant phase III trial were analyzed. The method used was methylation-specific PCR, where CIMP$^+$ status was defined by methylation of at least 3 of 5 following genes: *IGF2*, *CACNA1G*, *NEUROG1*, *SOCS1*, and *RUNX3*. Association between CIMP status and overall survival (OS), disease-free survival (DFS), and survival after recurrence (SAR), was assessed by Cox model adjusted for prognostic factors and treatment arm (FOLFOX4 ± cetuximab).

Results: CIMP status was successfully determined in 1,867 patients (97.9%): 275 (14.7%) tumors were CIMP$^+$. Compared with CIMP$^-$ patients, CIMP$^+$ patients were more frequently older ($P = 0.002$), females ($P = 0.04$), with right-sided ($P < 0.0001$), grade 3–4 ($P < 0.0001$), pN2 ($P = 0.001$), dMMR ($P < 0.0001$), BRAF mutated ($P < 0.0001$), and RAS wild-type ($P < 0.0001$) tumors. In multivariate analysis, CIMP$^+$ status was associated with shorter OS [HR, 1.46; 95% confidence interval (CI), 1.02–1.94; $P = 0.04$] and SAR [HR, 1.76; 95% CI, 1.20–2.56; $P < 0.0004$]; but not DFS [HR, 1.15; 95% CI, 0.86–1.54; $P = 0.34$]. A nonsignificant trend of detrimental effect of cetuximab was observed in patients with CIMP$^+$ tumors for OS, DFS, and SAR.

Conclusions: In a large cohort of well-defined patients with stage III colon cancer, CIMP$^+$ phenotype is associated with a shorter OS and SAR but not to DFS. *Clin Cancer Res; 24(19); 4745–53. ©2018 AACR.*

Introduction

Three major molecular phenotypes have been recognized in colon cancer: chromosomal instability (1), microsatellite instability owing to deficient mismatch repair system (dMMR; ref. 2), and methylator phenotype (3). This latter one is associated with hypermethylation of the CpG islands localized in the gene enhancer regions and is named "CIMP" for "CpG island methylator phenotype." It represents about 10% to 20% of colon cancer (3–5). Clinical features more frequently associated with this phenotype are female gender, older age, and proximal location of the tumor; in addition, these tumors are more frequently *BRAF*-mutated, *KRAS*, and *TP53* wild-type, and associated with a dMMR phenotype due to hypermethylation of the enhancer region of *MLH1* gene.

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Please see the Supplementary Appendix for a list of the Investigators/Collaborators.

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Translational Relevance

We evaluated the prognostic value of CIMP+ ("CpG island methylator phenotype") in stage III colon cancer from 1,907 patients enrolled in the PETACC-8 trial, which compared FOLFOX4 with FOLFOX4 plus cetuximab regimen. In multivariate analysis, $CIMP^+$ status was associated with shorter overall survival and survival after recurrence, but not disease-free survival, suggesting that $CIMP^+$ is a poor prognostic marker in the metastatic setting. Furthermore, in patients with *RAS* and *BRAF* wild-type and pMMR tumor, a marginal significant interaction was observed between CIMP status and treatment arm in disfavor of FOLFOX4 + cetuximab arm for patients with a $CIMP^+$ tumors. Altogether, our data suggest that $CIMP^+$ should be an important stratification factor in metastatic colon cancer future clinical trials and that anti-EGFRs being a main class of targeted agents in the metastatic setting, the potential deleterious effect of anti-EGFRs in $CIMP^+$ patients in this setting should be further investigated.

Nonmetastatic colon cancer prognosis is primarily based on the TNM classification that guides adjuvant treatment indications (6). Several markers have been suggested to refine the prognostic classification of nonmetastatic colon cancer, such as dMMR, *KRAS*, and *BRAF* mutation status (2, 7). The prognostic impact of $CIMP^+$ phenotype in nonmetastatic colon cancer is controversial with several studies showing a pejorative impact of this phenotype (8–13), whereas several others failed to demonstrate such impact (14–16). In addition, the efficacy of 5-fluorouracil (5FU) or oxaliplatin (FOLFOX)-based adjuvant chemotherapy regimen in this tumor phenotype is a matter of debate (8, 12, 14–20). To clarify the prognostic role of the $CIMP^+$ phenotype, we characterized the tumor methylation status of patients included in the PETACC-8 randomized clinical trial, which compared FOLFOX4 with FOLFOX4 plus cetuximab regimen in patients with stage III colon cancer (21). We examined the relationship between $CIMP^+$ phenotype and disease-free survival (DFS), overall survival (OS), and survival after recurrence (SAR).

Materials and Methods

PETACC-8 trial is a phase III randomized trial that included 2,559 patients with stage III colon cancer and compared FOLFOX4 (oxaliplatin, leucovorin, and 5-FU) regimen with FOLFOX4 + cetuximab in the adjuvant setting (21). Among these patients, 2,043 have signed an informed consent for translational research study, and tumor DNA extracted from formalin-fixed, paraffin-embedded sections after macrodissection was available for 1,907 of them in this study. Microsatellite instability, *KRAS*, *NRAS*, and *BRAF* status were reported previously (22).

Analysis of DNA methylation

To characterize CIMP status, the panel of five markers described by Weisenberger and colleagues was used: *IGF2*, *CACNA1G*, *NEUROG1*, *RUNX3* and *SOCS1* (5). $CIMP^+$ phenotype was defined by methylation-positive status of at least 3 markers out of the 5 tested (5). In the literature, the definition of $CIMP^+$ status is not standardized regarding the gene panel used and marker threshold (13). We decided to use this definition because of its better diagnostic performance compared with the classic panel (methylated in tumors (MINT)1, MINT2, MINT31, and *MLH1*; ref. 23) as demonstrated by Weisenberger and colleagues (5) and its frequent use in many recent studies (10, 13, 16, 18, 19, 24).

Sodium bisulfite conversion

Bisulfite conversion was carried out with EZ-96 DNA Methylation-Lightning Kit (Zymo Research). The amount of DNA used was approximately 200 ng in an elution volume of 20 μL to obtain a final concentration of bisulfite-treated DNA of 10 ng/μL.

Methylation-specific PCR

Primers used for the methylation-specific PCR (25) are given in Supplementary Table S1. Three PCRs were performed. First, we performed a multiplex PCR of the three following markers *IGF2*/*CACNA1G*/*NEUROG1* using the corresponding 12 primers for the determination of the methylation status. A mix of 500 μL the 12 primers were done using 10 μL of each primer at 100 μmol/L, and 380 μL Tris–EDTA buffer. Briefly, the multiplex PCR was performed in 25 μL reaction volume using the Multiplex PCR Kit, (Qiagen Multiplex PCR Kit), with 2.5 μL of the primer mix, 12.5 μL of the multiplex PCR Master Mix, 2.5 μL of the Q Solution, 4 μL of the bisulfite-converted DNA, and 3.5 μL of RNAse-free water. PCR was performed as follows: 15 minutes at 95°C, followed by 40 cycles (30 seconds at 94°C, 90 seconds at 59°C, and 90 seconds at 72°C), and 10 minutes at 72°C. Second, MSP was performed in two separate reactions for the two remaining markers, owing to different annealing temperatures. The same protocol was used except for the primer mix, which contained 4 specific primers for the annealing temperature, which was 55°C for *RUNX3*, and 60°C for *SOCS1*.

Capillary electrophoresis of PCR amplification products

PCR amplification products were diluted (1:150 for the triplex PCR reaction, 1:50 for *RUNX3* PCR reaction, and 1:200 for *SOCS1* PCR reaction). A total of 1.5 μL of this diluted solution was mixed with 18.5 μL of formamide (HIDI Formamide, Applied Biosystems) and 0.1 μL of a size marker [Genescan 400 HD (ROX) size standard, Applied Biosystems). Capillary electrophoresis was carried out on a 3730xl DNA Analyzer (Thermo Fisher Scientific). Electrophoresis data were interpreted using the software GeneMapper (Thermo Fisher Scientific). For each gene, the peak height ratio of the methylated/unmethylated amplicon was measured. If the ratio was greater than 0.1 or if the peak height of methylated amplicon was greater than 500, the marker was considered methylated.

Because of the definition of CIMP status (methylation of at least three markers of the five tested), for each sample, the analysis was initially carried out on the triplex *IGF2*/*CACNA1G*/*NEUROG1*. If the three markers or none of these markers were methylated, the status was, respectively, $CIMP^+$ and CIMP−; therefore, the analysis was stopped. If one or two markers were considered methylated, the analysis of *SOCS1* and *RUNX3* was carried out to further characterize CIMP status.

Statistical analysis

Categorical variables were compared using the χ^2 test. Continuous variables were compared using *t* test. OS was defined as the time from randomization to death. DFS was defined as the interval from the randomization to locoregional or metastatic recurrence, the appearance of a secondary colon or rectal cancer, or

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death, whichever occurred first. SAR was defined as the interval from the first locoregional or metastatic recurrence or the appearance of a secondary colon or rectal cancer to death. OS, DFS, and SAR curves were estimated with Kaplan–Meier method and were compared by a two-sided log-rank test.

A Cox proportional hazard model was achieved by adjusting for known prognostic factors: age, gender, WHO performance status (PS), bowel obstruction and/or perforation, tumor stage pT and pN, histopathology grade, MMR status, *BRAF* and *RAS* mutation status, and also treatment arm. Analyses were carried out according to a two-sided statistical significance level of 5%.

Results

CIMP status

Among the 2,043 that signed the informed consent for translational research in PETACC8 trial, CIMP status was tested on 1,907 samples. In 40 cases, CIMP status could not be determined due to poor DNA quality and CIMP status determination could be achieved in 1,867 samples (97.9%) and was positive in 275 cases (14.7%; see Supplementary Fig. S1). The CIMP-analyzed group was well matched to those for whom we cannot obtain the CIMP status owing to the absence of tumor block or failure of CIMP determination (full details in Supplementary Table S2).

Patients in CIMP$^+$ group were slightly older (60.8 years vs. 58.9 years; $P = 0.002$) and more frequently women than patients in CIMP$^-$ group [48.7% vs. 42.1%, respectively; OR, 1.3; 95% confidence interval (CI), 1–1.7; $P = 0.04$]. CIMP$^+$ tumors were more frequently located in the proximal part of the colon than CIMP$^-$ tumors (75.3% vs. 32.4%, respectively; OR, 6.35; 95% CI, 4.7–8.7; $P < 0.001$), more frequently poorly differentiated (grade 3–4; 36.3% vs. 15.9%, respectively; OR, 3; 95% CI, 2.2–4.0; $P < 0.001$), with a greater proportion of pT3-4 stage (94.9% vs. 89.6%, respectively; OR, 2.2; 95% CI, 1.2–4.1; $P = 0.005$), and pN2 stage (46.2% vs. 36.1%, respectively; OR, 1.51; 95% CI, 1.2–2.0; $P = 0.001$). On a molecular level, CIMP$^+$ tumors were more frequently dMMR than CIMP$^-$ tumors (34.2% vs. 5.7%, respectively; OR, 8.6; 95% CI, 6.0–12.3; $P < 0.001$), *BRAF* mutated (50.4% vs. 4.3%, respectively; OR, 22.5; 95% CI,15.8–32.3; $P < 0.001$), and less frequently *RAS* mutated (36.3% vs. 50.5%, respectively; OR, 0.55; 95% CI, 0.4–0.7; $P < 0.001$). There was no significant difference for the remaining tested clinical variables (Table 1). Because these clinical variables and these genetic

Table 1. Comparison of the two groups CIMP$^+$ and CIMP$^-$

	CIMP$^+$ ($n = 275$)	CIMP$^-$ ($n = 1592$)	OR (95% CI)	*P*
Sex				
Men	141 (51.3%)	922 (57.9%)	1.3 (1.0–1.7)	0.04
Women	134 (48.7%)	670 (42.1%)		
Age				
≤70 years	241 (87.6%)	1,429 (89.8%)	1.2 (0.81–1.85)	0.29
>70 years	34 (12.4%)	163 (10.2%)		
Bowel obstruction and/or perforation				
No	219 (79.6%)	1,295 (81.3%)	0.90 (0.6–1.25)	0.50
Yes	56 (20.4%)	297 (18.7%)		
Missing	0	0		
pT (TNM stage)				
pT1-pT2	14 (5.1%)	166 (10.4%)	2.2 (1.2–4.1)	0.005
pT3-pT4	261 (94.9%)	1,424 (89.6%)		
pT3	196	1,104		
pT4	65	320		
Missing	0	2		
pN (TNM stage)				
pN1	148 (53.8%)	1,017 (63.9%)	1.5 (1.2–2.0)	0.001
pN2	127 (46.2%)	575 (36.1%)		
Missing	0	0		
Tumor localization				
Left	67 (24.7%)	1,059 (67.6%)	6.35 (4.7–8.7)	<0.0001
Right	204 (75.3%)	507 (32.4%)		
Both	3	18		
Missing	1	8		
Histopathology grade				
G1–2	174 (63.7%)	1,322 (84.1%)	3.0 (2.2–4.0)	<0.0001
G3–4	99 (36.3%)	250 (15.9%)		
Missing	2	20		
Microsatellite status				
pMMR	169 (65.8%)	1,391 (94.3%)	8.6 (6.0–12.3)	<0.0001
dMMR	88 (34.2%)	84 (5.7%)		
Missing	18	117		
BRAF mutation status				
BRAF wild-type	133 (49.6%)	1,467 (95.7%)	22.5 (15.8–32.3)	<0.0001
BRAF mutated	135 (50.4%)	66 (4.3%)		
Missing	7	59		
RAS mutation status				
RAS wild-type	167 (63.7%)	724 (49.5%)	0.55 (0.4–0.7)	<0.0001
RAS mutated	95 (36.3%)	738 (50.5%)		
Missing	13	130		

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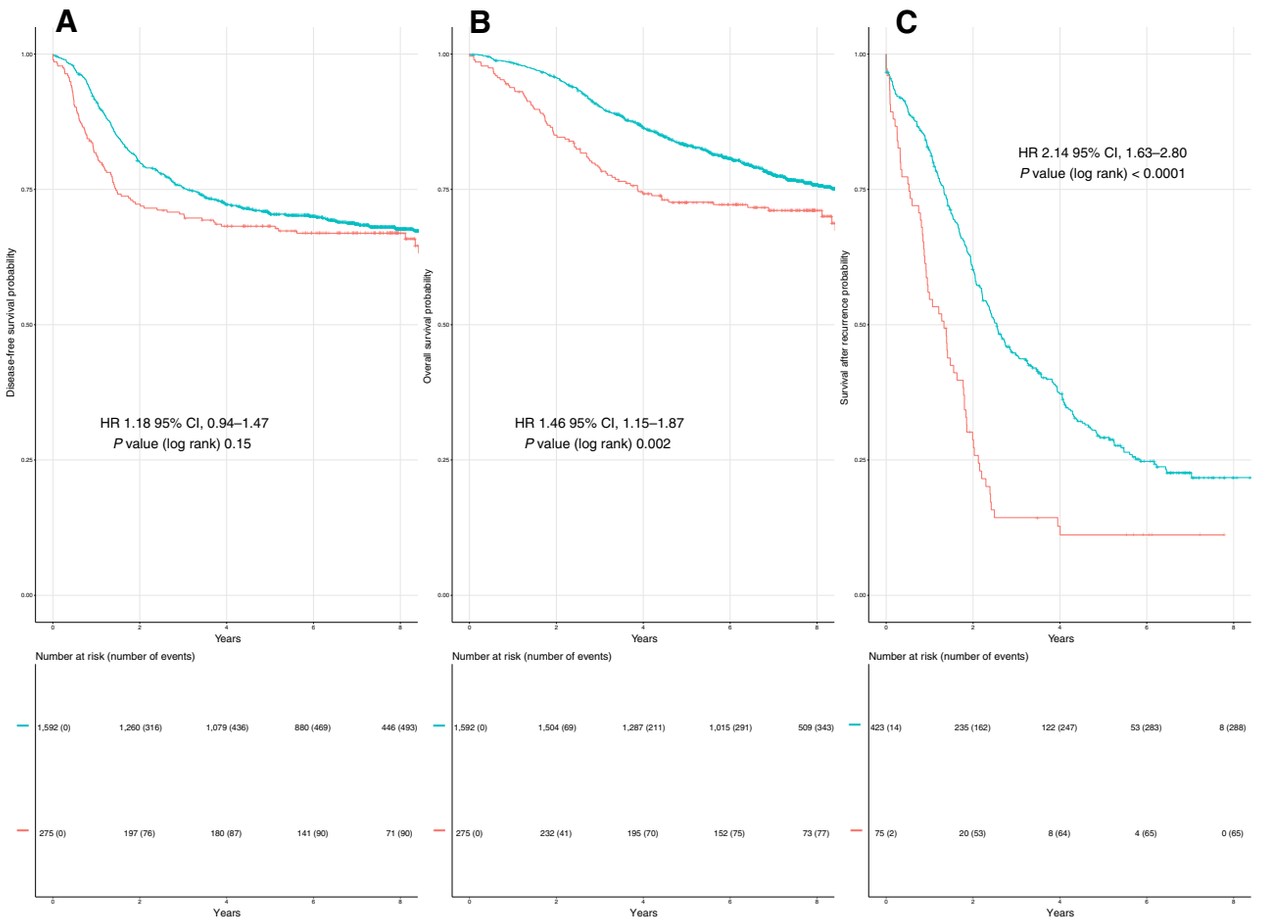

Figure 1.

Kaplan–Meier curves according to CIMP status in the overall population. **A,** DFS in the overall population according to CIMP status. **B,** OS in the overall population according to CIMP status. **C,** SAR in the overall population according to CIMP status. Curves in blue color: CIMP$^-$; curves in red color, CIMP$^+$.

alterations are associated, we observed that dMMR- and *BRAF*-mutated right-sided tumors were 100 times more frequent in the CIMP$^+$ tumor group than in the CIMP$^-$ tumor group (20.6% vs. 0.2%, respectively) and that pMMR and *BRAF*-mutated right-sided tumors were 10 times more frequent in the CIMP$^+$ tumor group as compared with CIMP$^-$ tumor group (20.2% vs. 2.7%, respectively). Finally, pMMR and *BRAF* wild-type left-sided tumors were 6 times more frequent in CIMP$^-$ tumor group than in CIMP$^+$ tumor group (60% vs. 10.1%, respectively; see Supplementary Fig. S2).

Survival analysis

Univariate analysis. Median follow-up durations were 6.1 years (range 0–10.1) and 6.6 years (range 0–10.3) for patients with CIMP$^+$ tumors and CIMP$^-$ tumors, respectively.

In the overall population, DFS was not significantly different between the two groups of patients with CIMP$^+$ tumors and CIMP$^-$ tumors (HR, 1.18; 95% CI, 0.94–1.47; $P = 0.15$) with 3-year DFS at 70.4% and 75.4%, respectively. OS was significantly shorter in patients with CIMP$^+$ tumors than CIMP$^-$ tumors (HR, 1.46; 95% CI, 1.15–1.87; $P = 0.002$) with 5-year OS at 72.6% and 83.2%, respectively. SAR was significantly shorter in patients with CIMP$^+$ tumors as compared with patients with CIMP$^-$ tumors (HR, 2.14; 95% CI, 1.63–2.80; $P < 0.0001$) with 1-year SAR at 54.7% and 82.2%, respectively (Fig. 1).

Because CIMP$^+$ tumors are strongly associated with dMMR phenotype and *BRAF* mutations, we analyzed the survival data separately in patients with pMMR and dMMR tumors and in patients with *BRAF* wild-type and mutated tumors. In patients with pMMR tumor: DFS was significantly shorter in patients with CIMP$^+$ tumors than CIMP$^-$ tumors (HR, 1.44; 95% CI, 1.12–1.87; $P = 0.005$) with 3-year DFS at 63.7% and 75.3%, respectively. OS was significantly shorter in patients with CIMP$^+$ tumors than CIMP$^-$ tumors (HR, 1.92; 95% CI, 1.46–2.51; $P < 0.0001$) with 5-year OS at 64.5% and 83.4%, respectively. SAR was significantly shorter in patients with CIMP$^+$ tumors than CIMP$^-$ tumors (HR, 2.80; 95% CI, 2.06–3.80; $P < 0.0001$) with 1-year SAR at 55.4% and 82.3%, respectively (Fig. 2). Similar results were observed when the analysis was performed independently for proximal and distal tumors (Table 2). In patients with dMMR tumors, no significant difference was observed between patients with CIMP$^+$ and CIMP$^-$ tumors for DFS, OS, and SAR (respectively, $P = 0.75$; $P = 0.85$; and $P = 0.53$; see Supplementary Fig. S3).

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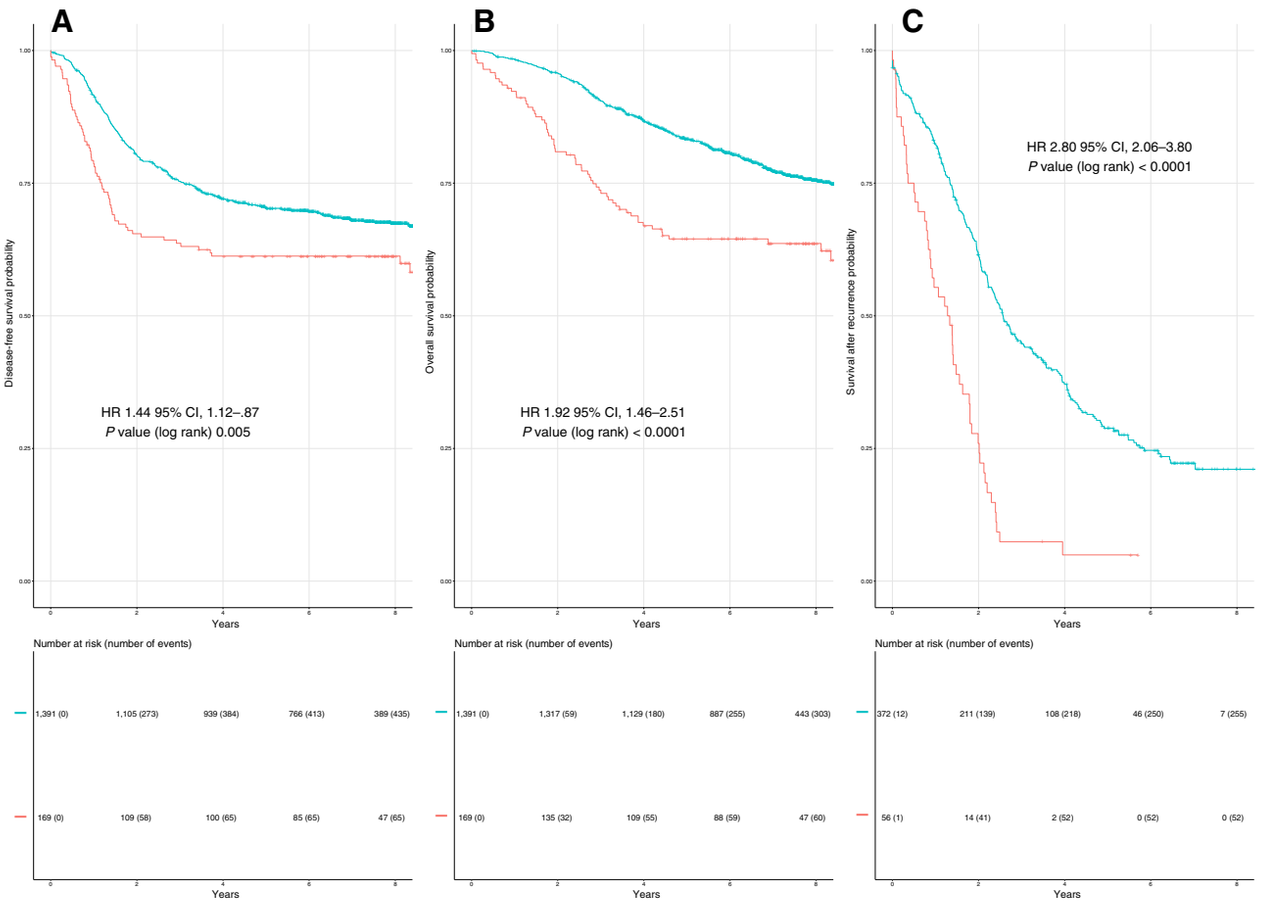

Figure 2.

Kaplan–Meier curves according to CIMP status in pMMR group. **A,** DFS in pMMR group according to CIMP status. **B,** OS in pMMR group according to CIMP status. **C,** SAR in pMMR group according to CIMP status. Curves in blue color: $CIMP^-$; curves in red color: $CIMP^+$.

In patients with BRAF wild-type tumors, DFS, OS, and SAR were shorter in patients with $CIMP^+$ than in $CIMP^-$ tumors (HR, 1.26; 95% CI, 0.93–1.70; $P = 0.13$; HR, 1.57; 95% CI, 1.4–2.18; $P < 0.007$; and HR, 1.90; 95% CI, 1.30–2.78; $P < 0.001$, respectively). No significant difference was observed in the *BRAF*-mutated tumors.

Multivariate analysis. Cox proportional hazard model was performed including the following variables: age, gender, WHO PS, bowel obstruction and/or perforation, treatment arm, tumor stage pT and pN, grade, MMR status, and *BRAF* and *RAS* mutation status. In the overall population, OS and SAR were significantly shorter in patients with a $CIMP^+$ tumor than in patients with a $CIMP^-$ tumor (respectively, HR, 1.46; 95% CI, 1.02–1.94; $P < 0.04$ and HR, 1.76; 95% CI, 1.20–2.56; $P < 0.004$; see Table 3), in contrast to DFS, which was not significantly different between the two groups (HR, 1.15; 95% CI, 0.86–1.54; $P = 0.34$).

In multivariate analysis, by adjusting on the same prognostic factors apart from MMR status, in the pMMR population, OS and SAR remained shorter in patients with $CIMP^+$ tumor as compared with patients with $CIMP^-$ tumor (HR, 1.40; 95% CI, 1.00–1.96; $P = 0.05$ and HR, 1.98; 95% CI, 1.33–2.95; $P < 0.001$, respectively).

Analysis of treatment efficacy according to CIMP status

We investigated the impact of treatment (FOLFOX4 vs. FOLFOX4 plus cetuximab) according to CIMP status of the patients' tumors. In univariate analysis, nonsignificant trends in DFS, OS, and SAR differences in disfavor of the FOLFOX4 +

Table 2. Survival according to CIMP status and tumor localization in pMMR tumors

	Proximal and pMMR tumors[a]			Distal and pMMR tumors[b]		
	$CIMP^+$	$CIMP^-$	*P*	$CIMP^+$	$CIMP^-$	*P*
3-year DFS	64.0% (95% CI) [55.7–73.4]	74.3% (95% CI) [70.2–78.6]	<0.04	62.0% (95% CI) [49.9–77.0]	75.7% (95% CI) [73.1–78.5]	0.054
5-year OS	63.6% (95% CI) [55.2–73.1]	79.4% (95% CI) [75.6–83.5]	0.004	65.6% (95% CI) [53.6–80.3]	85.1% (95% CI) [82.8–87.4]	0.002
1-year SAR	59.5% (95% CI) [45.6–77.6]	72.9% (95% CI) [64.9–81.8]	<0.002	50.0% (95% CI) [31.5–79.4]	86.3% (95% CI) [82.1–90.6]	<0.001

[a]3-year DFS, 5-year OS, and 1-year SAR for patients with a proximal and pMMR tumor according to CIMP status.
[b]3-year DFS, 5-year OS, and 1-year SAR for patients with a distal and pMMR tumor according to CIMP status. The *P* values correspond to the log-rank test.

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Table 3. Multivariate analysis for OS and survival after recurrence in the whole population

	OS		SAR	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
CIMP				
CIMP$^-$	1		1	
CIMP$^+$	1.46 (1.02–1.94)	<0.04	1.76 (1.20–2.56)	<0.004
Age				
≤70 years	1		1	
>70 years	1.52 (1.13–2.05)	0.006	1.55 (1.07–2.24)	0.02
Gender				
Female	1		1	
Male	1.26 (1.02–1.57)	<0.04	1.07 (0.84–1.37)	0.58
Performance status				
0	1			
≥1	1.39 (1.09–1.77)	<0.009	1.23 (0.93–1.64)	0.15
Stage				
pT1-T2	1		1	
pT3-T4	2.30 (1.31–4.03)	0.003	1.27 (0.65–2.49)	0.49
pN1	1		1	
pN2	2.15 (1.74–2.67)	<0.001	1.38 (1.07–1.78)	0.01
Histologic grading				
G1–G2	1		1	
G3–G4	1.50 (1.16–1.96)	<0.002	1.48 (1.11–1.96)	0.007
Bowel obstruction and/or perforation				
No	1		1	
Yes	1.26 (0.98–1.62)	0.06	0.91 (0.68–1.21)	0.53
Treatment arm				
FOLFOX4	1		1	
FOLFOX4 + cetuximab	1.16 (0.94–1.44)	0.15	1.22 (0.96–1.56)	0.11
RAS status				
Wild-type	1		1	
Mutated	1.44 (1.14–1.81)	<0.002	1.20 (0.92–1.57)	0.17
BRAF status				
Wild-type	1		1	
Mutated	1.22 (0.82–1.81)	0.32	1.50 (0.98–2.13)	<0.07
MMR status				
dMMR	1		1	
pMMR	1.80 (1.16–2.81)	<0.009	1.42 (0.83–2.43)	0.20

cetuximab arm were observed in the group of patients with CIMP$^+$ tumors (Fig. 3). These marginal differences are retained after adjustment. The respective adjusted HR for the experimental arm (FOLFOX4 + cetuximab) was 1.35 (95% CI, 0.87–2.10), $P = 0.19$; 1.49 (95% CI, 0.92–2.41), $P = 0.10$ and 1.23 (95% CI, 0.66–2.29), $P = 0.52$ for DFS, OS, and SAR. On the contrary, in patients with CIMP$^-$ tumors, DFS and OS were very similar between the two treatment arms. The respective adjusted HR of DFS and OS were 0.97 (95% CI, 0.80–1.18), $P = 0.80$; 1.13 (95% CI, 0.90–1.42) $P = 0.30$. The HR for SAR was of the same magnitude than that observed in CIMP$^+$ tumors: 1.30 (95% CI, 1.00–1.68), $P = 0.05$.

Finally, we tested an interaction between the CIMP status and the treatment arm in the group of patients with *RAS* and *BRAF* wild-type pMMR tumors (601 patients), to eliminate the possible interactions with *BRAF* and dMMR status. After adjustment on age, gender, PS, tumor stage (pT and pN), tumor grade, and tumor location, we observed a nonsignificant trend for interaction ($P = 0.10$) in disfavor of FOLFOX4 + cetuximab arm for patients with a CIMP$^+$ tumors.

Discussion

Using the most recent described panel of markers to determine the CIMP status, the prevalence of the CIMP$^+$ phenotype in this large series of stage III colon cancer was 14.7% (95% CI, 13.1%–16.3%). This is in accordance with the prevalence observed in the literature, which ranges between 9.6% and 23.1% (9, 16, 18, 20, 25). In addition, we observed similar associations between CIMP status, clinical variables, and tumor characteristics that have been shown in other studies (3, 5, 26, 27). Indeed, the CIMP$^+$ phenotype seems more frequently associated with a female gender, a right-sided, poorly differentiated, *BRAF* mutated, and dMMR tumor. These associations reflect the overlap between CIMP$^+$ and dMMR phenotype due to the methylation of the enhancer region of *MLH1* gene. More than 50% of the tumors with a dMMR phenotype are CIMP$^+$ and in the CIMP$^+$ tumors those that shared a proximal location, a *BRAF* mutation and a dMMR-positive status are 100 times more frequent than in CIMP$^-$ tumors.

These associations could explain the discrepant result in the literature on the prognostic impact of the CIMP status. In our whole series, the CIMP$^+$ status is significantly associated with a shorter OS and SAR after adjustment on the main confounding factors. It is important to note that among the studies limited to stage III colon cancer, this study is the only one that have adjusted the prognostic value of CIMP$^+$ phenotype for all main clinical, pathologic but also extensive molecular prognostic markers' characterization (9, 17). Subgroup analyses showed on the one hand that the prognostic impact of the CIMP status is limited to the group of tumors with a pMMR status in univariate and multivariate models. On the other hand, the prognostic impact of the CIMP status is observed both in right- and left-sided pMMR

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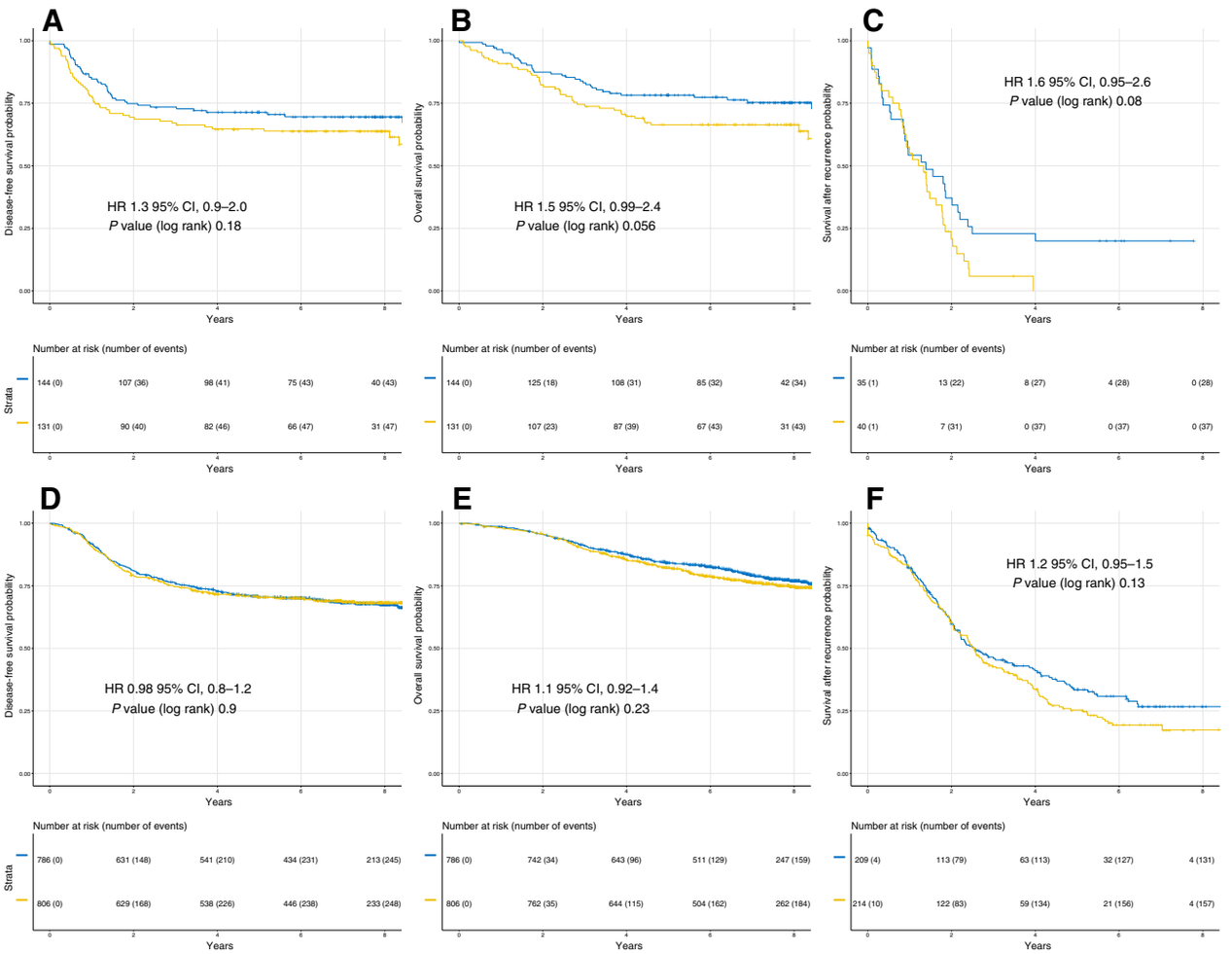

Figure 3.

Kaplan–Meier curves according to study treatment (FOLFOX4 vs. FOLFOX4 plus cetuximab) in CIMP$^+$ and CIMP$^-$ groups. **A,** DFS in patients with CIMP$^+$ tumor according to treatment arm. **B,** OS in patients with CIMP$^+$ tumor according to treatment arm. **C,** SAR in patients with CIMP$^+$ tumor according to treatment arm. **D,** DFS in patients with CIMP$^-$ tumor according to treatment arm. **E,** OS in patients with CIMP$^-$ tumor according to treatment arm. **F,** SAR in patients with CIMP$^-$ tumor according to treatment arm. Curves in blue color: FOLFOX4; curves in yellow color: FOLFOX4 + cetuximab.

tumors. Moreover, this is the first study evaluating the prognostic value of CIMP$^+$ status on a homogenous large population of patients with stage III colon cancer included in a phase III trial with oxaliplatin-based adjuvant chemotherapy. Cohen and colleagues (16) evaluated CIMP phenotype in a small phase III trial ($n = 293$) with oxaliplatin-based adjuvant chemotherapy including patients with stage II or III colon cancer. Likely due to a lack of power, this study failed to demonstrate a prognostic value of CIMP$^+$ status (in univariate analysis, HR for OS was 1.27; 95% CI, 0.58–2.80; $P = 0.55$). Shiovitz and colleagues (17) evaluated the CIMP status on a larger trial ($n = 615$) that compared 5-FU and leucovorin associated or not with irinotecan in patients with stage III colon cancer, showing that patients with CIMP$^+$ tumor had a shorter OS in a univariate analysis, but failed to show any significant difference in their multivariate model. However, a significant interaction with treatment arms was observed: patients with CIMP$^+$ and pMMR tumors benefitted from the addition of irinotecan to 5-FU and leucovorin therapy.

The PETACC-8 study showed no difference in OS and DFS between the group treated by FOLFOX4 and the group treated by FOLFOX4 associated with cetuximab in the adjuvant setting for stage III colon cancer, even when excluding *KRAS*-mutant patients (21). When selecting patients wild-type for *KRAS*, *NRAS* and *BRAF*, an HR of 0.76 was observed but still not significant ($P = 0.11$; ref. 28). Here, we observed a HR greater than 1 for DFS (HR, 1.35; 95% CI, 0.87–2.10) and OS (HR, 1.49; 95% CI, 0.92–2.41), suggesting a potential deleterious effect of cetuximab in CIMP$^+$ patients, even if the difference remains not significant. This analysis was not restricted to the *RAS* and *BRAF* wild-type subgroup due to the small number of patients subsequent to the overlapping between *BRAF*-mutant and CIMP$^+$ status. Furthermore, we showed a nonsignificant trend for interaction in the subgroup of *RAS* and *BRAF* wild-type and pMMR patients in disfavor of cetuximab suggesting a possible deleterious effect of the association of FOLFOX4 plus cetuximab in CIMP$^+$ patients. These findings could be explained by the association between

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$CIMP^+$ phenotype and the downregulation of two EGFR ligands gene expression, *EREG* and *AREG* (29, 30). As anti-EGFR mAbs are currently used in *RAS* wild-type patients in the metastatic setting, to assess the predictive value of CIMP status for cetuximab efficacy in the treatment of patients with metastatic colon cancer may be interesting.

We found significantly shorter SAR and OS in patients with CIMP+ tumors, unlike DFS, showing that CIMP+ status has a prognostic value only for patients with recurrence, which could be explained by the prognostic value of CIMP+ status in the metastatic setting.

Validation of our results in the NCCTG N0147 trial (31), comparing mFOLFOX6 with mFOLFOX6 plus cetuximab in stage III colon cancer, would be interesting.

In conclusion, in a large clinically and molecularly well-defined stage III colon cancer population treated with standard adjuvant therapy, the methylator phenotype accounts for about 15% of patients and is associated with shorter OS and SAR. However, no impact of CIMP status on DFS was observed. These results suggest that the $CIMP^+$ phenotype confers a more aggressive phenotype at the metastatic stage of the disease or a resistance to some of the drugs used in this setting.

Disclosure of Potential Conflicts of Interest

J. Tabernero is a consultant/advisory board member for Amgen, Boehringer Ingelheim, Celgene, Chugai, Imclone Systems, Lilly, Merck, Millenium, Novartis, Roche/Genentech, Sanofi, and Taiho. G. Folprecht reports remuneration from Merck Serono; Roche/Genentech; Bayer; Lilly; Servier; Mundipharma; Bristol, Myers, Squibb; MSD; and Shire. C. Lepage reports receiving speakers bureau honoraria from Amgen, Novartis, and Bayer, and is a consultant/advisory board member for Novartis and Halio-DX. P. Laurent-Puig is a consultant/advisory board member for Merck Serono; Amgen; Boerhinger Ingelheim; Biocartis; Roche; Bristol, Myers, Squibb; and MSD. No potential conflicts of interest were disclosed by the other authors.

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