Research Article

Cancer Epidemiology, Biomarkers & Prevention

Carcinoembryonic Antigen Levels and Survival in Stage III Colon Cancer: *Post hoc* Analysis of the MOSAIC and PETACC-8 Trials

Edouard Auclin[1,2,3], Julien Taieb[1], Come Lepage[4], Thomas Aparicio[5], Roger Faroux[6], Enrico Mini[7], Gunnar Folprecht[8], Ramon Salazar[9], Magdalena Benetkiewicz[10], Maria Banzi[11], Christophe Louvet[12], Jean-Luc Van Laethem[13], Josep Tabernero[14], Tamas Hickish[15], Aimery de Gramont[16], Thierry André[17], and Dewi Vernerey[2,3,10]

Abstract

Background: We explored and validated the association of postoperative carcinoembryonic antigen (CEA) with disease-free survival (DFS) and overall survival (OS) in stage III colon cancer.

Methods: Patients with stage III colon cancer from the MOSAIC and PETACC-8 trials were enrolled. The relation between CEA and outcomes was continuously modeled with the restricted cubic splines (RCS) method. Association of CEA with outcomes was assessed by the Kaplan–Meier method, with two risk groups among patients with a CEA level $\leq$5 ng/mL. Multivariate Cox proportional hazard models were constructed.

Results: The CEA level was available in 1,292 (96%) and 2,477 (97%) patients in the discovery and validation cohorts. The RCS analysis confirmed that patients with a CEA level >5 ng/mL were at highest risk of recurrence or death and those with a CEA level $\leq$5 ng/mL presented a heterogeneous risk population. In the discovery cohort, the 3-year DFS rate was 75%, 65%, and 45% in a group of patients with CEA level of 0–1.30 ng/mL ($n = 630$), 1.30–5 ng/mL ($n = 613$), and >5 ng/mL ($n = 49$), respectively ($P < 0.001$). CEA was independently associated with endpoints. All findings were confirmed in the validation cohort.

Conclusions: Postoperative CEA level was highly and independently associated with DFS and OS, especially in patients with a CEA level of $\leq$5 ng/mL, suggesting that this cutoff is not optimal.

Impact: CEA levels should be applied more accurately in future trials and clinical practice.

[1]Sorbonne Paris-Cité, Paris Descartes University, Hepato-Gastroenterology and Gastrointestinal Oncology Department, Hôpital Européen Georges Pompidou, Paris, France. [2]Methodology and Quality of Life Unit in Oncology, University Hospital of Besançon, Besançon, France. [3]University Bourgogne Franche-Comté, INSERM, EFS BFC, UMR1098, Interactions Hôte-Greffon-Tumeur/Ingénierie Cellulaire et Génique, Besançon, France. [4]Department of Gastroenterology, CHU Le Bocage, INSERM U1231, Dijon, France. [5]Department of Gastroenterology and Digestive Oncology, Hôpital Saint Louis, Sorbonne Paris-Cité, Paris Diderot University, Paris, France. [6]Department of Gastroenterology, Centre Hospitalier Départemental Les Oudairies, La Roche-Sur-Yon, France. [7]Department of Health Sciences, Section of Clinical Pharmacology and Oncology, University of Florence, Florence, Italy. [8]Medical Department I, University Hospital Carl Gustav Carus, Dresden, Germany. [9]Institut Catala d'Oncologia, Oncobell Program, IDIBELL, CIBERONC, University of Barcelona. L'Hospitalet de Llobregat, Barcelona, Spain. [10]Oncology Multidisciplinary Research Group (GERCOR), Paris, France. [11]Unit of Medical Oncology, Clinical Cancer Center, AUSL-IRCCS Reggio Emilia, Reggio Emilia, Italy. [12]Unit of Medical Oncology, Institut Mutualiste Montsouris, Paris, France. [13]Gastroenterology and Digestive Department, Hopital Erasme, Université Libre de Bruxelles, Belgium. [14]Vall d'Hebron University Hospital and Institute of Oncology (VHIO), CIBERONC, University of Barcelona, Barcelona, Spain. [15]Department of Oncology, Royal Bournemouth Hospital and Bournemouth University, Bournemouth, England. [16]Department of Oncology, Institut Hospitalier Franco-Britannique, Levallois-Perret, France. [17]Sorbonne University and Department of Medical Oncology, Hospital Saint Antoine, Paris, France.

Corresponding Author: Dewi Vernerey, Besancon University Hospital, INSERM UMR1098, 3 Boulevard Alexandre Fleming, Besancon 25030, France. Phone: 338-121-8776; Fax: 381-218-775; E-mail: dvernerey@chu-besancon.fr

Cancer Epidemiol Biomarkers Prev 2019;28:1153–61

doi: 10.1158/1055-9965.EPI-18-0867

Introduction

Colon cancer is the third most common cancer in men and women (1, 2). When diagnosed at a localized stage, curative management is proposed to patients including surgery and adjuvant chemotherapy if lymph node involvement is observed (stage III colon cancer). In stage III disease, fluoropyrimidine-based adjuvant treatment improved patient outcomes (3). The addition of oxaliplatin to a fluoropyrimidine chemotherapy backbone has produced additional benefit by increasing the 3-year disease-free survival (DFS) rate (4–6), and the oxaliplatin-containing combination became the worldwide standard adjuvant treatment in the setting of colon cancer. Recently, the findings of the International Duration Evaluation of Adjuvant Therapy collaboration have provided useful information in helping oncologists discuss the duration of adjuvant therapy (7–10). To optimize the value of this treatment, better tumor markers or better awareness of tumor markers to improve the recurrence risk detection rate are needed.

Carcinoembryonic antigen (CEA) is a well-known, low-cost biological tumor marker for colon cancer (11, 12), overexpressed in 90% of colorectal cancer cases (13). The European Society of Medical Oncology (ESMO) guidelines recommend CEA determination in a postoperative follow-up period (14). Previous large studies have shown that elevated preoperative CEA levels are associated with worse prognosis in all stage colon cancer patients (15) and in stage I and II colon cancer patients who did not receive adjuvant chemotherapy (16).

The results by Konishi and colleagues showed that the preoperative CEA level is not a relevant marker of recurrence in localized colon cancer if CEA is normalized after surgery (17), thus underling the importance of postoperative CEA. Another study by Lu and colleagues identified high postoperative CEA level of $\geq$5 ng/mL as an independent prognostic predictor of early relapse (18). The CEA upper limit of normal (ULN) levels vary between different institutions, ranging from 3.0 to 5.0 ng/mL, with a cutoff of 5.0 ng/mL used by most centers even though this value remains debated (15, 16, 19). Recently, Margalit and colleagues defined preoperative CEA of 2.35 ng/mL as an optimal cut-off point for predicting survival of stage I and II colon cancer (16). Likewise, Kim and colleagues suggested preoperative CEA of 3 ng/mL as the best cut-off value to identify stage III colon cancer patients with low and high risk of death or recurrence (19).

The aims of this *post hoc* analysis were (i) to assess and precisely validate the postoperative CEA prognostic value for DFS and OS in patients with stage III colon cancer receiving standard adjuvant treatments; (ii) to challenge the standard 5 ng/mL as threshold value to discriminate patients with low and high risk of recurrence or death; and (iii) to determine the additional value of postoperative CEA for the TNM classification to better stratify patients for risk of death and of recurrence.

Materials and Methods

Population

Stage III colon cancer patients for a discovery cohort were selected from the MOSAIC phase III trial (NCT00275210; refs. 4, 20). The MOSAIC study showed a survival benefit with the addition of oxaliplatin to adjuvant 5-fluorouracil (5-FU) and leucovorin (LV; the FOLFOX4 regimen) in patients with colon cancer. External validation cohort was obtained with the PETACC-8 phase III trial population (NCT00265811; ref. 21). The PETACC-8 study failed to show a survival benefit for adjuvant combination of FOLFOX and cetuximab in patients with stage III colon cancer. Both trials were performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and approved by the appropriate Ethics Committees.

Follow-up

In both study cohorts, the radiologic evaluation was made at randomization and every 6 months during the first 5 years of follow-up.

The MOSAIC database was locked on April 25, 2014, and the PETACC-8 database on October 27, 2016.

Data extraction

Demographics, cancer history, pathologic, clinical, biological parameters, and efficacy outcomes (DFS and OS) were prospectively collected at the time of randomization.

Postoperative CEA serum measurements

For both study cohorts, a postoperative CEA value (MOSAIC: <10 ng/mL; PETACC-8: $\leq$1.5$\times$ ULN) was an inclusion criterion. The serum CEA measurements were not centralized. In the PETACC-8 study, the ULN for CEA was available for all patients; the median CEA value was 5 ng/mL [interquartile range (IQR), 3.8–5].

Statistical analysis

Median (IQR) values and proportions (percentage) were used for continuous and categorical variables, respectively. Median and proportions were compared using the Wilcoxon–Mann–Whitney test and the χ^2-test (or the Fisher exact test, if appropriate), respectively.

DFS was defined as the time between randomization and local/distant relapse, second colorectal/rectal occurrence, or death, whichever occurred first. Alive patients without relapse and second colorectal cancer were censored at the date of their last follow-up. OS was defined as the time between randomization and death from any cause. Patients known to be alive were censored at the date of their last follow-up.

DFS and OS were estimated using Kaplan–Meier method and described using median or rate at specific time points with their 95% confidence intervals (95% CI). Follow-up was calculated using the reverse Kaplan–Meier method (22).

When used continuously, the association between CEA and survival was investigated using the fractional polynomials method (23–25) and was validated by the restricted cubic splines method with graphical evaluation. Different cutoffs of CEA level in the discovery cohort were explored to investigate an association between CEA and survival (categorical). These were based on the percentiles (median and tercile) and optimal cut-off point (log-rank maximization) methods (26).

The association of demographic, clinical, biological, and molecular factors with survival was assessed with univariate Cox proportional hazards model. Variables with *P* values of less than 0.10 and/or clinically relevant variables were entered into an intermediate multivariable Cox regression model. Final multivariable model was constructed with statistically significant variables of the intermediate model. The correlation between variables was verified before construction of the multivariate models, in order to deal with potential colinearity. All multivariate models were stratified on the basis of adjuvant treatment received. Hazard proportionality was assessed for each categorical variable using the Schoenfeld residual-based test and a graphical method.

The predictive value and the discrimination ability of the final model were assessed with the Harrell's concordance index (C-index; ref. 27). The robustness of the statistical models was assessed by multiple imputation procedure (assessment of potential bias arising from missing data for parameters involved in the multivariable model; ref. 28).

The differential treatment effects on DFS and OS among CEA risk groups were evaluated with an interaction term in the Cox proportional hazards model.

All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC) and R software version 2.15.2 (R Development Core Team; http://www.r-project.org). *P* values of less than 0.05 were considered statistically significant, and all tests were two-sided.

Results

Characteristics of patients

In the discovery cohort, 1,347 patients with stage III colon cancer from the MOSAIC study were enrolled. Postoperative CEA was available in 1,292 (95.9%) patients; 1,243 (96.2%) had a CEA level below 5 ng/mL. The median time between surgery and CEA measurement was 4.3 weeks and the median follow-up was 9.6 years (Table 1).

Table 1. Characteristics of the discovery ($n = 1,347$) and external validation ($n = 2,552$) populations

		Discovery cohort	External validation cohort	
		***N* = 1,347**	***N* = 2,552**	***P***
Age (years)[a]	<70	1157 (85.9%)	2283 (89.4%)	0.001
	≥70	190 (14.1%)	269 (10.5%)	
Gender[a]	Female	621 (46.1%)	1091 (42.7%)	0.04
	Male	726 (53.9%)	1461 (57.2%)	
Body mass index	Normal	661 (49.2%)	1127 (44.8%)	<0.0001
	Underweight	49 (3.7%)	51 (2.03%)	
	Overweight	461 (34.3%)	926 (36.8%)	
	Obese	172 (12.8%)	411 (16.3%)	
	Missing	4	37	
Performance status	0–1	1153 (85.6%)	2430 (99.7%)	<0.0001
	≥2	194 (14.4%)	6 (0.25%)	
	Missing	0	116	
Treatment arm[a]	FOLFOX	672 (49.9%)	1277 (50.0%)	
	LV5FU2	675 (50.1%)	-	
	FOLFOX-cetuximab	-	1275 (50.0%)	
Tumor location	Left colon	895 (66.4%)	1549 (61.0%)	<0.0001
	Right colon	451 (33.5%)	964 (38.0%)	
	Both	1 (0.1%)	26 (1.0%)	
	Missing	0	13	
T stage	T1 – T2	119 (8.8%)	265 (10.4%)	0.08
	T3	977 (72.6%)	1765 (69.3%)	
	T4	250 (18.6%)	518 (20.3%)	
	Missing	1	4	
N stage	N1	882 (65.7%)	1595 (62.5%)	0.05
	N2	460 (34.3%)	957 (37.5%)	
	Missing	5		
Histoprognostic grade	G1/2	1085 (84.4%)	2051 (81.4%)	0.02
	G3/4	201 (15.6%)	469 (18.6%)	
	Missing	61	32	
Intestinal perforation[a]	Yes	75 (5.6%)	121 (4.7%)	0.26
Intestinal obstruction[a]	Yes	260 (19.3%)	416 (16.3%)	<0.0001
CEA (ng/mL)	Median (IQR)	1.4 (1–2.3)	1.91 (0.9–2.2)	
CEA (ng/mL)	≤5	1243 (96.2%)	2378 (95.89%)	0.63
	>5	49 (3.8%)	102 (4.11%)	
	Missing	55	72	
CEA[b] (ng/mL)	0 to 1.30[a]	630 (48.8%)	1172 (47.2%)	0.64
	1.30 to 5	613 (47.4%)	1205 (48.6%)	
	>5	49 (3.8%)	103 (4.1%)	
	Missing	55	72	
CEA[c] (ng/mL)	0 to 1	492 (38.1%)	888 (35.8%)	0.36
	1 to 2	429 (33.2%)	887 (35.8%)	
	2 to 5	322 (24.9%)	602 (24.3%)	
	>5	49 (3.8%)	103 (4.1%)	
	Missing	55	72	
Time between surgery and CEA measurement (week)	Median (IQR)	4.3 (3.3–5.4)	5.1 (4.0–6.3)	<0.0001
	Missing	56	70	
BRAF and MMR status	$BRAF^{WT}$/dMMR	28 (4.8%)	103 (6.4%)	0.003
	$BRAF^{mut}$/pMMR	51 (8.8%)	126 (7.9%)	
	$BRAF^{mut}$/dMMR	12 (2.1%)	64 (4.0%)	
	$BRAF^{WT}$/pMMR	488 (84.3%)	1308 (81.7%)	
	Missing	768	951	
Median follow-up time (95% CI), years[a]		9.6 (9.5–9.8)	5.85 (5.71–5.91)	

NOTE: Variables are described as *n* (%) except for continuous variables, which are described as median (IQR). *P* values refer to the differences between the discovery and validation cohorts.

Abbreviations: dMMR, deficient mismatch repair; LV5FU2, 5-fluorouracil chemotherapy; mut, mutated; N stage,, nodal stage according to the TNM classification; pMMR, proficient mismatch repair; T stage, tumor stage according to the TNM classification; WT, wild type.

[a]No missing data.

[b]Median approach was used to split the population of patients with a level of CEA <5 ng/mL.

[c]Tertile approach was used to split the population of patients with a level of CEA <5 ng/mL.

In the external validation cohort, 2,552 patients with stage III colon cancer from the PETACC-8 study were enrolled. The median time between surgery and CEA measurement was 5.1 weeks and the median follow-up was 5.85 years (Table 1).

Association of postoperative CEA in its continuous form with survival

In the discovery cohort, the restricted cubic splines approach showed that patients with a CEA level of >5 ng/mL were at highest risk of recurrence or death. Patients with a CEA level

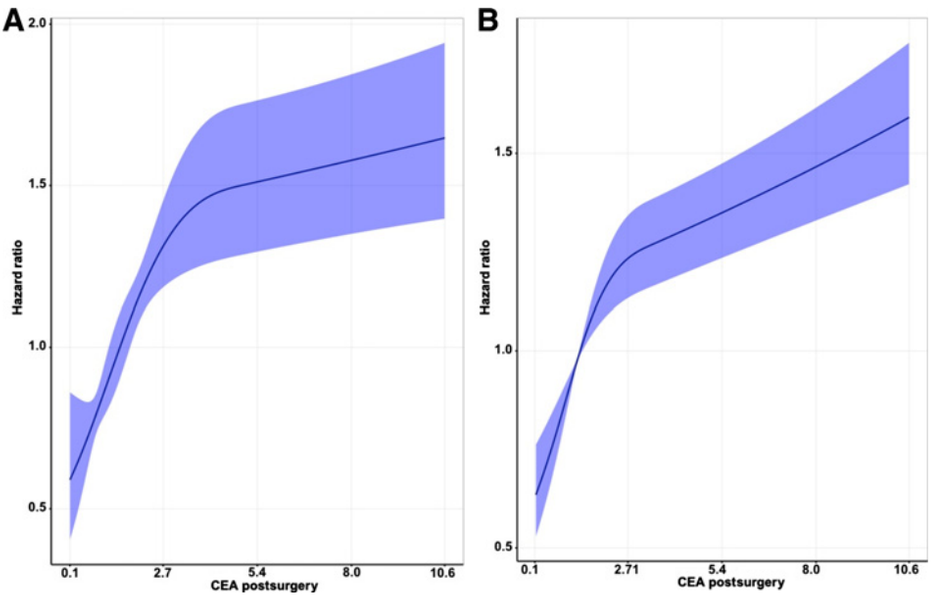

Figure 1.
Restricted cubic splines modeling of hazard ratio (HR) for DFS as a function of postoperative CEA level. HR for DFS in stage III colon cancer as a function of postoperative CEA level in the discovery cohort (**A**) and in the validation cohort (**B**). Association between CEA and DFS was modeled using restricted cubic splines, showing a square root relation between those variables. Estimates were derived from Cox model. The purple area around the blue line represents the 95% CI.

of ≤5 ng/mL did not present a homogeneous risk population. A gradual risk suggested a square root relation between CEA and DFS (Fig. 1A). The square root transformation was the best to model the relation between CEA and survival endpoints with the fractional polynomials method. Similar observations were made for the external validation cohort (Fig. 1B) and for OS endpoint (Supplementary Fig. S1). These results allowed to identify different risk populations among patients with a level of CEA lower than 5 ng/mL.

Survival according to postoperative categorical CEA

The 5 ng/mL cut-off value of CEA allowed to identified two populations with distinct DFS risk profiles (CEA ≤ 5 ng/mL population: the 3-year DFS rate of 70%; and CEA > 5 ng/mL population: the 3-year DFS rate of 45%; Fig. 2A).

Considering the important number of patients with CEA ≤ 5 ng/mL, the median and tertile approach were used to determine 2 and 3 populations in these patients (Table 1). Figure 2B shows a distinct DFS risk profile in the two median based populations identified in patients with CEA ≤ 5 ng/mL. The 3-year DFS rate was 75%, 65%, and 45% for CEA < 1.30 ng/mL, CEA 1.30 to 5 ng/mL, and CEA > 5 ng/mL groups, respectively ($P < 0.0001$). The optimal cut-off value determined by the Hothorn and Lausen method (Supplementary Fig. S2) was equal to 1.30 ng/mL for patients with a CEA level of ≤ 5 ng/mL, reinforcing the choice of CEA 1.30 to determine different risk populations in these patients. These results were fully confirmed in the external validation cohort (Fig. 2C and D) and were also validated for OS in both cohorts (Supplementary Fig. S3). Similar results were observed for DFS and OS with the tertile approach for both study cohorts (Supplementary Fig. S4).

Consistent observations were found in a subgroup analysis of treatments and of times between surgery and CEA measurement for both cohorts (Supplementary Figs. S5–S8).

Patients' characteristics according to CEA level groups are presented in Supplementary Tables S1 and S2.

Postoperative CEA level as independent prognostic factor for survival

In univariate and multivariate analyses, CEA level was found to be significantly associated with DFS. The independent prognostic value of CEA was also confirmed in multivariate analysis. Patients with CEA between 1.30 and 5 mg/mL had a HR of 1.67 (95% CI, 1.28–2.19) compared to those with CEA < 1.30 ng/mL (Table 2). Similar results were obtained for OS (Table 2).

In the sensitivity analysis, multiple imputation procedure provided analogous results in the discovery cohort (Supplementary Tables S3 and S4). All results were replicated in the external validation cohort (Table 3; Supplementary Table S5).

Additional value of postoperative CEA for DFS risk stratification within TNM risk subgroups

In the discovery cohort, 729 (54.3%) and 614 (45.7%) patients had low-risk (T3N1) and high-risk (T4 or N2) stage III colon cancer, respectively. As expected, DFS differed according to these subgroups (Supplementary Fig. S9). Among the low and high-risk subgroups, the postoperative CEA level classification allowed to identify populations with different DFS and OS risk profiles (Fig. 3; Supplementary Figs. S9 and S10). Similar results were obtained in the external validation cohort for both DFS and OS (Fig. 3; Supplementary Fig. S9).

Chemotherapy regimen benefit across CEA risk groups

In the discovery cohort, the survival benefit from the addition of oxaliplatin to 5-fluorouracil was not different across different CEA groups in term of DFS and OS. No statistically significant interaction between CEA groups and treatment the arm (5-FU/LV vs. FOLFOX) was found ($P = 0.5371$ and $P = 0.6761$, respectively; Supplementary Fig. S11).

In the validation cohort, no interaction between the CEA risk groups and the treatment arm (cetuximab-FOLFOX vs. FOLFOX) in terms of DFS and OS was found ($P = 0.8629$ and $P = 0.2412$, respectively).

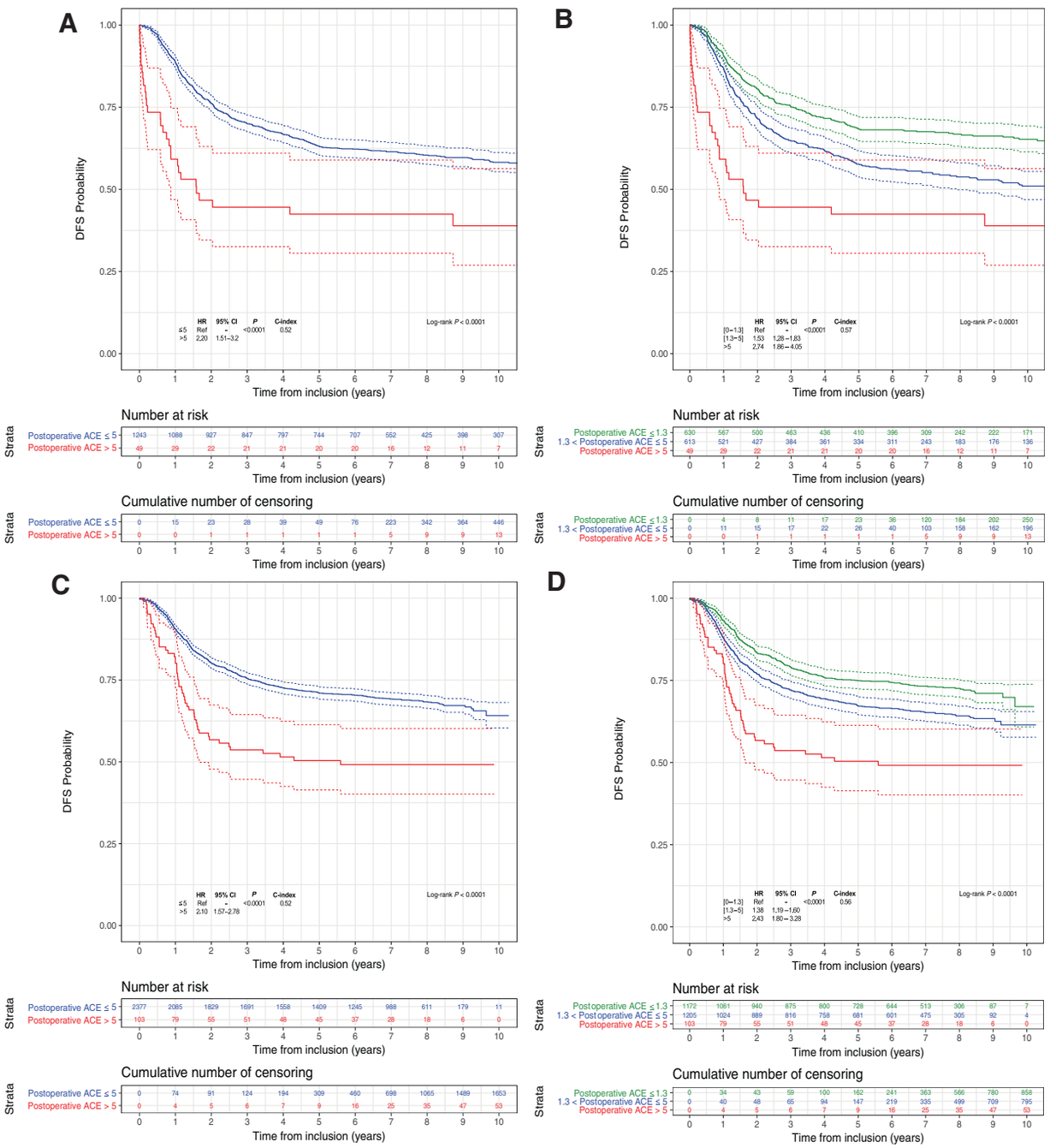

Figure 2.

Association between CEA and DFS in the discovery and validation cohorts. Kaplan–Meier survival curves for DFS of stage III colorectal cancer patients in the MOSAIC cohort according to the standard CEA levels (**A**; ≤ or >5 ng/mL), in the MOSAIC cohort according to the new CEA levels (**B**; ≤1.30 ng/mL, 1.30–5 ng/mL, >5 ng/mL), in the PETACC-8 cohort according to the standard CEA levels (**C**; ≤ or >5 ng/mL), and in the PETACC-8 cohort according to the new CEA levels (**D**; ≤1.30 ng/mL, 1.30–5 ng/mL, >5 ng/mL). *P* values were estimated using the log-rank test. The discrimination ability of the CEA level was evaluated with the C-index. HR and 95% confidence intervals were obtained with a Cox regression method.

Discussion

This study confirmed strong prognostic value of postoperative CEA level for DFS and OS in two large cohorts of patients with stage III colon cancer treated with adjuvant chemotherapy. Patient with a CEA level of >5 ng/mL had a worse risk profile for recurrence and death. Distinct risk profiles were identified in patients with CEA ≤ 5 ng/mL currently considered as a single population with normal CEA values.

Table 2A. Univariate and multivariate Cox proportional hazards model for DFS in the discovery cohort

				Univariate		Final multivariate N patients = 547	
		N pts	N evt	HR (95% CI)	*P*	HR (95% CI)	*P*
Age	>70	190	92	1.27 (1.01–1.58)	0.04	1.5 (1.09–2.07)	0.01
Gender	Male	726	294	0.98 (0.83–1.16)	0.84		
Body mass index	Underweight	49	18	0.96 (0.60–1.55)	0.14		
	Overweight	461	207	1.20 (1.00–1.44)			
	Obese	172	64	0.91 (0.69–1.20)			
Treatment arm	LV5FU2	675	293	1.26 (1.07–1.49)	0.007		
Tumor location	Right colon	452	187	1.08 (0.90–1.29)	0.40		
Histoprognostic grade	G3/4	201	90	1.27 (1.01–1.59)	0.04		
Performance status	≥ 1	970	388	0.98 (0.81–1.18)	0.81		
Intestinal perforation	Yes	75	42	1.68 (1.23–2.31)	0.001		
Intestinal obstruction	Yes	260	125	1.37 (1.12–1.68)	0.002		
CEA level	0 to 1.30 ng/mL	630	212	Ref	<0.0001	Ref	<0.0001
	1.30 to 5 ng/mL	613	286	1.53 (1.28–1.83)		1.67 (1.28–2.19)	
	>5 ng/mL	49	29	2.75 (1.86–4.05)		2.68 (1.53–4.69)	
CEA level	0 to 1 ng/mL	492	166	Ref	<0.0001		
	1 to 2 ng/mL	429	175	1.25 (1.01–1.54)			
	2 to 5 ng/mL	322	157	1.62 (1.30–2.02)			
	>5 ng/mL	49	29	2.72 (1.83–4.03)			
BRAF and MMR status	$BRAF^{WT}$/pMMR	4488	212	Ref	0.42	Ref	0.22
	$BRAF^{mut}$/pMMR	51	22	1.08 (0.70–1.68)		1.03 (0.65–1.64)	
	$BRAF^{WT}$/dMMR	28	8	0.62 (0.31–1.26)		0.65 (0.32–1.32)	
	$BRAF^{mut}$/dMMR	12	3	0.57 (0.18–1.78)		0.36 (0.11–1.14)	
T stage	T3	977	384	1.90 (1.30–2.80)	<0.0001		
	T4	250	131	2.79 (1.85–4.19)			
N stage	N2	460	237	1.89 (1.59–2.24)	<0.0001	1.93 (1.49–2.50)	<0.0001

NOTE: Univariate variables that reached statistical significance ($P < 0.10$) or that did not reach significance but are known to be strongly associated with DFS were included in the multivariable model. A final multivariable model (displayed here) was realized with the statistically significant variables of the intermediate model. Abbreviations: dMMR, deficient mismatch repair; evt, events; LV5FU2, 5-fluorouracil chemotherapy; mut, mutated; N stage, nodal stage according to the TNM classification; pMMR, proficient mismatch repair; pts, patients; T stage, tumor stage according to the TNM classification; WT, wild type.

Table 2B. Univariate and multivariate Cox proportional hazards model for OS in the discovery cohort

				Univariate		Final multivariate (N patients = 531)	
		N pts	N evt	HR (95% CI)	*P*	HR (95% CI)	*P*
Age	>70	190	82	1.375 (1.08–1.75)	0.009	1.62 (1.13–2.31)	0.008
Gender	Male	726	254	1.046 (0.87–1.26)	0.63		
Treatment arm	LV5FU2	675	250	1.255 (1.04–1.51)	0.02		
Tumor location	Right colon	452	165	1.18 (0.97–1.42)	0.09		
Histoprognostic grade	G3/4	201	85	1.52 (1.20–1.93)	<0.0001	1.91 (1.25–2.91)	0.003
Performance status	≥ 1	970	335	1.08 (0.88–1.33)	0.47		
Intestinal perforation	Yes	75	38	1.886 (1.35–2.63)	<0.0001		
Intestinal obstruction	Yes	260	113	1.494 (1.21–1.85)	<0.0001		
CEA level, ng/mL	< 1.30	630	179	Ref		Ref	0.0005
	1.30 to 5	613	240	1.50 (1.23–1.82)	<0.0001	1.73 (1.27–2.34)	
	> 5	49	26	2.58 (1.71–3.89)		2.35 (1.26–4.39)	
CEA level, ng/mL	0 to 1	492	138	Ref	<0.0001		
	1 to 2	429	146	1.24 (0.99–1.57)			
	2 to 5	322	135	1.67 (1.32–2.12)			
	> 5	49	26	2.60 (1.71–3.96)			
BRAF and MMR status	$BRAF^{WT}$/pMMR	488	170	Ref	0.46	Ref	0.03
	$BRAF^{mut}$/pMMR	51	20	1.31 (0.83–2.09)		1.37 (0.83–2.25)	
	$BRAF^{WT}$/dMMR	28	7	0.71 (0.33–1.51)		0.57 (0.26–1.26)	
	$BRAF^{mut}$/dMMR	12	3	0.73 (0.23–2.28)		0.19 (0.04–0.78)	
T stage	T3	977	320	2.232 (1.421–3.507)	<0.0001	2.18 (0.95–4.97)	0.005
	T4	250	119	3.661 (2.279–5.881)		3.30 (1 ?40–7.74)	
N stage	N2	460	207	1.96 (1.63–2.357)	<0.0001	1.97 (1.47–2.65)	<0.0001

NOTE: Univariate variables that reached statistical significance ($P < 0.10$) or that did not reach significance but are known to be strongly associated with DFS were included in the multivariable model. A final multivariable model (displayed here) was realized with the statistically significant variables of the intermediate model. Abbreviations: dMMR, deficient mismatch repair; evt, events; LV5FU2, 5-fluorouracil chemotherapy; mut, mutated; N stage, nodal stage according to the TNM classification; pMMR, proficient mismatch repair; pts, patients; T stage, tumor stage according to the TNM classification; WT, wild type.

One of the questions that arises from our results is the choice of the CEA cutoff for risk prediction. Kim and colleagues showed in their recent retrospective study of 965 patients with stage III colon cancer that the optimal cutoff for preoperative CEA was 3 ng/mL in the training and validation sets (19). Other studies evaluating preoperative CEA also showed that colon cancer patients with CEA levels inferior to 2.5 or 2.7 ng/mL had better OS and DFS (29–31). Recently, in a large study of 45,449

Table 3. Multivariate proportional hazards Cox model for DFS in the validation cohort

		HR (95% CI)	*P*
Age	>70	1.27 (0.97–1.66)	0.08
CEA level	<1.30 ng/mL	Ref	<0.0001
	1.30 to 5 ng/mL	1.40 (1.16–1.68)	
	>5 ng/mL	2.69 (1.89–3.82)	
BRAF and MMR status	$BRAF^{WT}$/pMMR	Ref	0.09
	$BRAF^{mut}$/pMMR	1.11 (0.81–1.51)	
	$BRAF^{WT}$/dMMR	0.88 (0.60–1.29)	
	$BRAF^{mut}$/dMMR	0.49 (0.27–0.89)	
N stage	N2	2.18 (1.82–2.60)	<0.0001

Abbreviations: dMMR, deficient mismatch repair; mut, mutated; N stage, nodal stage according to the TNM classification; pMMR, proficient mismatch repair; WT, wild type.

patients with stage I and II colon cancer not receiving adjuvant chemotherapy, preoperative CEA level of 2.35 ng/mL was defined as the best cutoff to predict survival (16). The results of these studies suggest that a CEA value of 5 ng/mL is not optimal. We identified considerable DFS and OS heterogeneity in the population with postoperative CEA $\leq$ 5 ng/mL, providing new data in favor of a lower CEA cutoff in the postoperative setting for stage III colon cancer. Defining a lower cutoff for postoperative CEA will allow to construct more accurate prediction models and will better stratify patients for adjuvant treatment.

Nicholson and colleagues showed that the sensitivity of a CEA level of 2.5 ng/mL is better than the standard 5 ng/mL value (82% vs. 71%), but with rather low specificity (80% vs. 88%; ref. 32) Nevertheless, in adjuvant colorectal cancer, higher sensitivity is more relevant for detecting disease recurrence. If the sensitivity is good for a lower CEA cutoff, the question about reproducibility of the measures below 5 ng/mL has to be addressed. Analytic sensitivity of most automated immunoassay systems for measuring CEA is under 1 ng/mL. For instance, analytic sensitivity of the Abbott immunoassay used in our center is 0.5 ng/mL, whereas for Beckmann immunoassays, which are also widely used, is less than 0.7 ng/mL. Thus, reliable management decisions can be made with CEA levels below 5 ng/mL.

Recently, molecular and immunologic prognostic factors in predicting colon cancer patients' prognosis have been identified (33–36). In our study, the postoperative CEA performance seems similar to these molecular/immunologic scores for DFS and OS risk stratification in patients with stage III colon cancer. These observations and the fact that CEA is a low-cost biological tumor marker reinforce its interest.

With the breakthrough of liquid biopsy, circulating tumor DNA (ctDNA) has been assessed in early-stage colon cancer (37–39). Very high prognostic value of ctDNA for OS and RFS has been showed in very small cohorts (37, 39). However, no study to date has assessed the prognostic value of ctDNA in stage III colon cancer. Currently, three studies are ongoing (NCT03416478, NCT02842203, NCT03312374). Our results suggest that prognostic value of ctDNA should absolutely be evaluated together

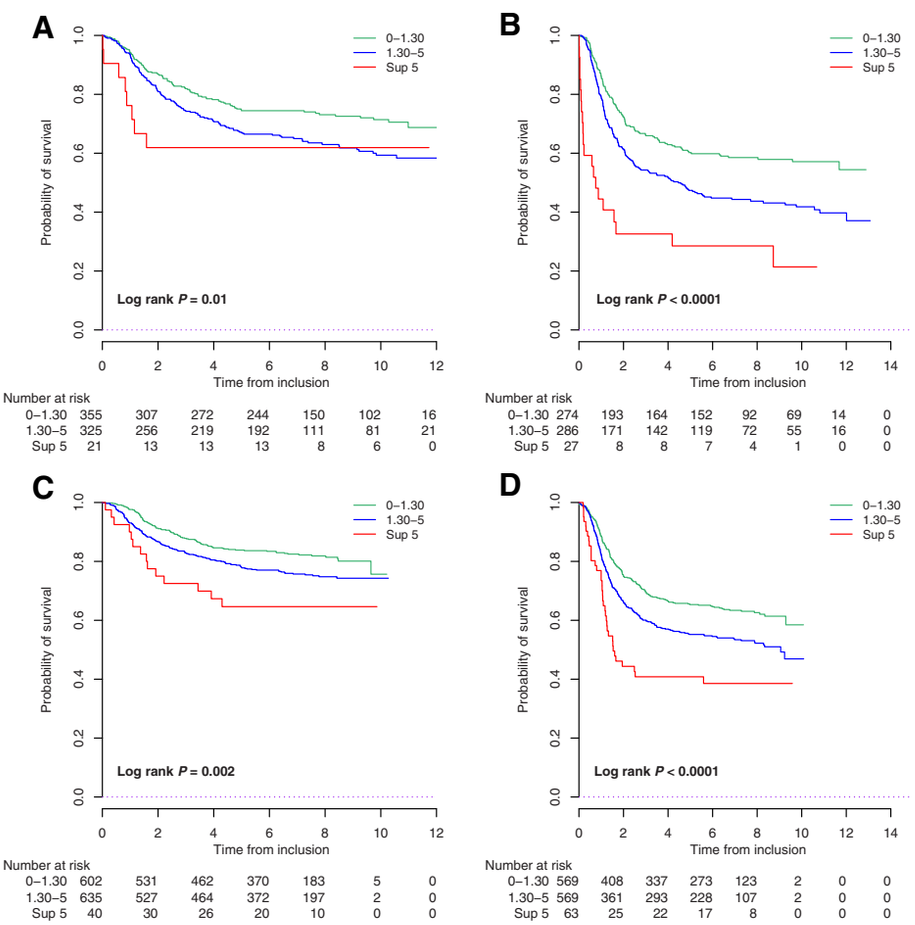

Figure 3.
Association between CEA and DFS within stage III colon cancer risk groups. Kaplan–Meier survival curves for DFS of stage III colorectal cancer patients in the MOSAIC low-risk (T3N1) group (**A**), in the MOSAIC high-risk (T4 or N2) group (**B**), in the PETACC-8 low-risk group (**C**), and in the PETACC-8 high-risk group according to the new CEA levels (**C**; $\leq$1.30 ng/mL, 1.30–5 ng/mL, >5 ng/mL). *P* values were estimated using the log-rank test.

with CEA as a covariate in multivariate analysis. Similarly, Lu and colleagues showed that combining postoperative CEA levels assessment with the presence of postoperative circulating tumor cells may be valuable tool in predicting early relapse and survival rate in patients with stage II and III colon cancer (18). Such combination was also found to be of interest for better disease prediction in the study of non–small cell lung cancer (40).

We identified the additional prognostic value of postoperative CEA for survival endpoints among conventional TNM risk classification. Recently, the results of the IDEA study suggested that shorter adjuvant chemotherapy could be administered in the subgroup of patients with T3/N1 disease (7–10). However, given that these results are still debated, postoperative CEA level might assist in clinical decision-making in this setting.

Although prediction tools already exist in localized colon cancer (41–44), these do not include postoperative CEA level. In the light of our results, a careful consideration of this measurement should be made in scoring tools to predict DFS and OS for localized colon cancer in the future.

Our study has several strengths. We evaluated postoperative CEA in adjuvant stage III colon cancer that was of more interest than preoperative CEA. This study was constructed in the framework of two large international multicenter phase III trials offering a broad spectrum of reliable parameters including molecular status at inclusion and the largest postoperative CEA assessment data in stage III colon cancer. Moreover, patients in these studies were treated with 5-fluorouracil and leucovorin in combination with oxaliplatin in majority of cases (50%). We built our analysis in a rigorous methodologic framework respecting the transparent reporting of the multivariate model TRIPOD statement (25). Showing the same results for both cohorts, we confirmed the robustness of our data.

The current study also has some limitations. Given that CEA level was an inclusion criterion in the MOSAIC and PETACC-8 trials, this analysis includes a low number of patients with high CEA. Importantly, CEA level may be affected by factors that were not available in our cohorts, mainly smoking status and other comorbidities (45). However, the robustness and the magnitude of the association observed support the notion of a cancer-derived effect on the outcomes. A second potential limitation is that in our study CEA assessment is established within different ranges, according to each manufacture, and most of the time low threshold values are considered as insufficiently sensitive. The large DFS and OS differences between subgroups of patient with CEA <5 ng/mL suggest that the findings apply across CEA values obtained from a broad range of laboratories. Despite the heterogeneity of the adjuvant regimens received and the multiples laboratories that performed the CEA measurement, postoperative CEA identified in the overall population was still relevant in all subgroups.

To conclude, in these two large cohorts, we showed that the postoperative CEA level is a highly independent prognostic factor for DFS and OS. We further confirmed that patients with CEA $\leq$5 ng/mL should not be considered as a single-risk population. These results support a routine postoperative CEA testing, suggesting its future interest for optimizing clinical trials design and for defining risk-adapted and personalized strategies for stage III colon cancer management.

Disclosure of Potential Conflicts of Interest

G. Folprecht reports receiving a commercial research grant from Merck-Serono (for a clinical study), has received speakers bureau honoraria from Merck-Serono, Roche/Genentech, Sanofi-Aventis, Lilly, Amgen, and is a consultant/advisory board member for Merck-Serono, Bayer, MSD, BMS, Shire, Roche/Genentech, Servier, Laboratoire HRA Pharma, Amgen, Mundipharma. J. Tabernero is a consultant/advisory board member for Array Biopharma, AstraZeneca, Bayer, BeiGene, Boehringer Ingelheim, Chugai, Genentech, Inc., Genmab A/S, Halozyme, Imugene Limited, Inflection Biosciences Limited, Ipsen, Kura Oncology, Lilly, MSD, Menarini, Merck Serono, Merrimack, Merus, Molecular Partners, Novartis, Peptomyc, Pfizer, Pharmacyclics, ProteoDesign SL, Rafael Pharmaceuticals, F. Hoffmann-La Roche Ltd, Sanofi, SeaGen, Seattle Genetics, Servier, Symphogen, Taiho, VCN Biosciences, Biocartis, Foundation Medicine, HalioDX SAS, and Roche Diagnostics. T. Hickish is a medical director at IQHealth Tech and is a consultant/advisory board member for Sobi International and Lilly. T. André has received speakers bureau honoraria from Sanofi and is a consultant/advisory board member for Roche. D. Vernerey is a consultant/advisory board member for OSE, HalioDX, Pfizer, and Jansen. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: E. Auclin, J. Taieb, C. Louvet, J. Tabernero, D. Vernerey
Development of methodology: E. Auclin, J. Taieb, D. Vernerey
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Lepage, T. Aparicio, R. Faroux, E. Mini, G. Folprecht, R. Salazar, M. Banzi, C. Louvet, J.-L. Van Laethem, J. Tabernero, T. Hickish, A. de Gramont, T. André
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): E. Auclin, J. Taieb, E. Mini, R. Salazar, M. Benetkiewicz, J. Tabernero, T. André, D. Vernerey
Writing, review, and/or revision of the manuscript: E. Auclin, J. Taieb, C. Lepage, T. Aparicio, R. Faroux, E. Mini, G. Folprecht, R. Salazar, M. Benetkiewicz, C. Louvet, J.-L. Van Laethem, J. Tabernero, A. de Gramont, T. André, D. Vernerey
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Tabernero, T. André
Study supervision: J. Tabernero, D. Vernerey
Other (providing final approval of the version to be submitted): M. Benetkiewicz

Acknowledgments

We thank Pr. Jean-Paul Feugeas and Dr. Laurent Bermont for their help regarding the automated immunoassay systems.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 6, 2018; revised November 16, 2018; accepted April 16, 2019; published online July 1, 2019.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018;68:7–30.
2. Malvezzi M, Bertuccio P, Levi F, La Vecchia C, Negri E. European cancer mortality predictions for the year 2014. Ann Oncol 2014;25:1650–6.
3. Efficacy of adjuvant fluorouracil and folinic acid in colon cancer. International Multicentre Pooled Analysis of Colon Cancer Trials (IMPACT) investigators. Lancet Lond Engl 1995;345:939–44.
4. André T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. N Engl J Med 2004;350:2343–51.
5. Yothers G, O'Connell MJ, Allegra CJ, Kuebler JP, Colangelo LH, Petrelli NJ, et al. Oxaliplatin as adjuvant therapy for colon cancer: updated results of NSABP C-07 trial, including survival and subset analyses. J Clin Oncol 2011;29:3768–74.

6. Haller DG, Tabernero J, Maroun J, de Braud F, Price T, Van Cutsem E, et al. Capecitabine plus oxaliplatin compared with fluorouracil and folinic acid as adjuvant therapy for stage III colon cancer. J Clin Oncol 2011;29: 1465–71.
7. Grothey A, Sobrero AF, Shields AF, Yoshino T, Paul J, Taieb J, et al. Duration of adjuvant chemotherapy for stage III colon cancer. N Engl J Med 2018; 378:1177–88.
8. André T, Vernerey D, Mineur L, Bennouna J, Desrame J, Faroux R, et al. 3 versus 6 months of oxaliplatin-based adjuvant chemotherapy for patients with stage III colon cancer: disease-free survival results from a randomized, open-label, International Duration Evaluation of Adjuvant (IDEA) France, phase III trial. J Clin Oncol 2018;36:1469–77.
9. Sobrero A, Lonardi S, Rosati G, Di Bartolomeo M, Ronzoni M, Pella N, et al. FOLFOX or CAPOX in stage II to III colon cancer: efficacy results of the Italian Three or Six Colon Adjuvant Trial. J Clin Oncol 2018;36:1478–85.
10. Iveson TJ, Kerr RS, Saunders MP, Cassidy J, Hollander NH, Tabernero J, et al. 3 versus 6 months of adjuvant oxaliplatin-fluoropyrimidine combination therapy for colorectal cancer (SCOT): an international, randomised, phase 3, non-inferiority trial. Lancet Oncol 2018;19:562–78.
11. Gold P, Freedman SO. Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. J Exp Med 1965;121:439–62.
12. Gold P, Freedman SO. Specific carcinoembryonic antigens of the human digestive system. J Exp Med 1965;122:467–81.
13. Goldstein MJ, Mitchell EP. Carcinoembryonic antigen in the staging and follow-up of patients with colorectal cancer. Cancer Invest 2005;23: 338–51.
14. Labianca R, Nordlinger B, Beretta GD, Mosconi S, Mandalà M, Cervantes A, et al. Early colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2013;24Suppl 6:vi64–vi72.
15. Thirunavukarasu P, Sukumar S, Sathaiah M, Mahan M, Pragatheeshwar KD, Pingpank JF, et al. C-stage in colon cancer: implications of carcinoembryonic antigen biomarker in staging, prognosis, and management. J Natl Cancer Inst 2011;103:689–97.
16. Margalit O, Mamtani R, Yang YX, Reiss KA, Golan T, Halpern N, et al. Assessing the prognostic value of carcinoembryonic antigen levels in stage I and II colon cancer. Eur J Cancer 2018;94:1–5.
17. Konishi T, Shimada Y, Hsu M, Tufts L, Jimenez-Rodriguez R, Cercek A, et al. Association of preoperative and postoperative serum carcinoembryonic antigen and colon cancer outcome. JAMA Oncol 2018;4:309–15.
18. Lu CY, Uen YH, Tsai HL, Chuang SC, Hou MF, Wu DC, et al. Molecular detection of persistent postoperative circulating tumour cells in stages II and III colon cancer patients via multiple blood sampling: prognostic significance of detection for early relapse. Br J Cancer 2011;104: 1178–84.
19. Kim CG, Ahn JB, Jung M, Beom SH, Heo SJ, Kim JH, et al. Preoperative serum carcinoembryonic antigen level as a prognostic factor for recurrence and survival after curative resection followed by adjuvant chemotherapy in stage III colon cancer. Ann Surg Oncol 2017;24:227–35.
20. André T, de Gramont A, Vernerey D, Chibaudel B, Bonnetain F, Tijeras-Raballand A, et al. Adjuvant fluorouracil, leucovorin, and oxaliplatin in stage II to III colon cancer: updated 10-year survival and outcomes according to BRAF mutation and mismatch repair status of the MOSAIC Study. J Clin Oncol 2015;33:4176–87.
21. Taieb J, Tabernero J, Mini E, Subtil F, Folprecht G, Van Laethem JL, et al. Oxaliplatin, fluorouracil, and leucovorin with or without cetuximab in patients with resected stage III colon cancer (PETACC-8): an open-label, randomised phase 3 trial. Lancet Oncol 2014;15:862–73.
22. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. Control Clin Trials 1996;17:343–6.
23. Royston P, Altman DG. Regression using fractional polynomials of continuous covariates: Parsimonious parametric modelling. J R Stat Soc Ser C Appl Stat 1994;43:429–67.
24. Royston P, Sauerbrei W. Multivariable model - building: a pragmatic approach to regression analysis based on fractional polynomials for modelling continuous variables. Available from: https://www.wiley.com/en-us/Multivariable+Model+Building%3A+A+Pragmatic+Approach+to+Regression+Anaylsis+based+on+Fractional+Polynomials+for+Modelling+Continuous+Variables-p-9780470028421.
25. Moons KGM, Altman DG, Reitsma JB, Ioannidis JP, Macaskill P, Steyerberg EW, et al. Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD): explanation and elaboration. Ann Intern Med 2015;162:W1–73.
26. Hothorn T, Lausen B. On the exact distribution of maximally selected rank statistics. Comput Stat Data Anal 2003;43:121–37.
27. Harrell FE, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med 1996;15:361–87.
28. Rubin D. Introduction. In: Multiple imputation for nonresponse in surveys. Hoboken, NJ: John Wiley & Sons, Inc.; 1987. p. 1–26.
29. Moreno García V, Cejas P, Blanco Codesido M, Feliu Batlle J, de Castro Carpeño J, Belda-Iniesta C, et al. Prognostic value of carcinoembryonic antigen level in rectal cancer treated with neoadjuvant chemoradiotherapy. Int J Colorectal Dis 2009;24:741–8.
30. Park YJ, Park KJ, Park JG, Lee KU, Choe KJ, Kim JP. Prognostic factors in 2230 Korean colorectal cancer patients: analysis of consecutively operated cases. World J Surg 1999;23:721–6.
31. Giessen-Jung C, Nagel D, Glas M, Spelsberg F, Lau-Werner U, Modest DP, et al. Preoperative serum markers for individual patient prognosis in stage I-III colon cancer. Tumour Biol 2015;36:7897–906.
32. Nicholson BD, Shinkins B, Mant D. Blood measurement of carcinoembryonic antigen level for detecting recurrence of colorectal cancer. JAMA 2016;316:1310–1.
33. Yothers G, O'Connell MJ, Lee M, Lopatin M, Clark-Langone KM, Millward C, et al. Validation of the 12-gene colon cancer recurrence score in NSABP C-07 as a predictor of recurrence in patients with stage II and III colon cancer treated with fluorouracil and leucovorin (FU/LV) and FU/LV plus oxaliplatin. J Clin Oncol 2013;31:4512–9.
34. Galon J, Mlecnik B, Marliot F, Ou FS, Bifulco CB, Lugli A, et al. Validation of the Immunoscore (IM) as a prognostic marker in stage I/II/III colon cancer: results of a worldwide consortium-based analysis of 1,336 patients. J Clin Oncol 2016;34(suppl 15; abstr 3500):3500.
35. Emile JF, Malicot KL, Tabernero J, Mini E, Folprecht G, Van Laethem JL, et al. Validation of the prognostic impact of lymphocyte infiltration (LI) in patients (pts) with stage III colon cancer (CC) treated with adjuvant FOLFOX+/− cetuximab: a PETACC8 translational study. J Clin Oncol 2016;34(suppl 4S; abstr 553):553.
36. Guinney J, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, et al. The consensus molecular subtypes of colorectal cancer. Nat Med 2015;21:1350–6.
37. Fan G, Zhang K, Yang X, Ding J, Wang Z, Li J. Prognostic value of circulating tumor DNA in patients with colon cancer: systematic review. PLoS One 2017;12:e0171991.
38. Lecomte T, Berger A, Zinzindohoué F, Micard S, Landi B, Blons H, et al. Detection of free-circulating tumor-associated DNA in plasma of colorectal cancer patients and its association with prognosis. Int J Cancer 2002;100: 542–8.
39. Tie J, Wang Y, Tomasetti C, Li L, Springer S, Kinde I, et al. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. Sci Transl Med 2016;8:346ra92.
40. Chen X, Wang X, He H, Liu Z, Hu JF, Li W. Combination of circulating tumor cells with serum carcinoembryonic antigen enhances clinical prediction of non-small cell lung cancer. PLoS One 2015;10:e0126276.
41. Weiser MR, Landmann RG, Kattan MW, Gonen M, Shia J, Chou J, et al. Individualized prediction of colon cancer recurrence using a nomogram. J Clin Oncol 2008;26:380–5.
42. Weiser MR, Gönen M, Chou JF, Kattan MW, Schrag D. Predicting survival after curative colectomy for cancer: individualizing colon cancer staging. J Clin Oncol 2011;29:4796–802.
43. Renfro LA, Grothey A, Xue Y, Saltz LB, André T, Twelves C, et al. ACCENT-based web calculators to predict recurrence and overall survival in stage III colon cancer. J Natl Cancer Inst 2014;106. doi: 10.1093/jnci/dju333.
44. Nagata H, Ishihara S, Oba K, Tanaka T, Hata K, Kawai K, et al. Development and validation of a prediction model for postoperative peritoneal metastasis after curative resection of colon cancer. Ann Surg Oncol 2018;25: 1366–73.
45. Alexander JC, Silverman NA, Chretien PB. Effect of age and cigarette smoking on carcinoembryonic antigen levels. JAMA 1976;235:1975–9.