

Deleted in Colon Cancer Protein Expression in Colorectal Cancer Metastases: A Major Predictor of Survival in Patients With Unresectable Metastatic Disease Receiving Palliative Fluorouracil-Based Chemotherapy

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A B S T R A C T

Purpose

To determine whether deleted in colon cancer (DCC) protein expression in colorectal cancer (CRC) metastases could predict outcome to palliative fluorouracil (FU)-based chemotherapy and to assess whether it is similar to that observed in the corresponding primary tumors.

Patients and Methods

DCC protein expression was assessed immunohistochemically on archival specimens of CRC metastases from 42 patients homogeneously treated by methotrexate-modulated bolus FU alternated to 6-S-leucovorin-modulated infused FU and was retrospectively correlated with patient characteristics and clinical outcome. In a subset analysis, DCC immunoreactivity was compared between metastatic CRC and the corresponding primary tumors and regional lymph node metastases.

Results

Positive immunoreactivity for DCC was found in 45% of patients. Eighteen (78%) of 23 patients for whom multiple samples were available displayed a similar pattern of expression in distant metastases and primary tumors. The median survival time was 14.3 months in patients without DCC expression and 21.4 months in patients with DCC-positive tumors (log-rank test, $P = .04$); the 2-year survival rates were 8.5% and 42.5%, respectively. Response rates to chemotherapy were not significantly different between the two groups. By multivariate analysis, DCC protein expression maintained its prognostic value and showed to be the single best predictor of survival, with a relative risk of 2.16.

Conclusion

Our results indicate that expression of the DCC protein in CRC metastases is similar to that observed in the corresponding primary tumors and represents a dominant predictor of survival in patients with unresectable, advanced CRC who are undergoing palliative FU-based chemotherapy.

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INTRODUCTION

Colorectal cancer (CRC) is the fourth leading cause of cancer-related death in the Western countries. Although cure may be achieved by surgery and adjuvant postoperative chemotherapy in approximately two

thirds of the patients with early stages of the disease, more than 50% of the patients actually have unresectable locally advanced or metastatic CRC at outset or develop metastases in a later phase. In this setting, palliative chemotherapy represents the main treatment option, and although recently

developed combination regimens result in a greater than 50% tumor shrinkage in approximately half of the patients, 2-year survival rates remain below 40%.¹⁻⁵ Given the limited impact on survival coupled with a substantial increase in toxicity and mortality, the use of combination chemotherapy as front-line treatment for all the patients with advanced CRC is currently the object of debate, and sequential use of fluoropyrimidines and irinotecan or oxaliplatin is being investigated as an alternative strategy.⁶⁻⁸

Therefore, improved patient selection is crucial to optimize the use of the multiple chemotherapeutic agents active in CRC that are currently available. In particular, the ability to identify patients with more aggressive disease might lead to the use of double or triple drug combinations upfront, whereas analysis of molecular markers of response to specific drugs or classes of drugs may allow the development of tailor-made treatment programs. Thymidylate synthase (TS), dihydropyrimidine dehydrogenase, thymidine phosphorylase, topoisomerase I, and the genes of the mismatch repair system are indeed actively investigated as markers of response to fluoropyrimidines, irinotecan, and oxaliplatin.^{9,10} In contrast, activation of specific oncogenes or loss of tumor-suppressor genes has been shown to be associated with tumor aggressiveness and poorer outcome in multiple malignancies.^{11,12} Alterations of genes regulating the cell death pathways down-stream of the targets directly inhibited by specific drugs may also affect chemosensitivity. This may be particularly important in CRC patients treated with fluorouracil (FU)-based chemotherapy. Although previous data from other investigators and our group have shown that patients with low TS levels in their metastases are three to 10 times more likely to respond to FU-based chemotherapy, response prediction based on TS assessment is in fact incomplete, with at least 50% of the low TS patients failing to achieve an objective response.⁹

The deleted in colon cancer (*dcc*) gene is a putative tumor suppressor gene located on a region of chromosome 18 (18q21.2) that is involved in allelic deletion in 50% to 70% of CRCs.^{13,14} This gene encodes for a transmembrane protein that can be detected by immunohistochemistry with commercially available monoclonal antibodies and is commonly expressed in most normal tissues including colonic mucosa.¹⁵ The DCC protein has considerable homology to neural cell adhesion molecules¹⁴ and may be involved in regulation of cell-to-cell or cells-to-substrate interactions with a potential functional role in the control of cell growth, cell differentiation, and development of metastases.¹⁶ 18q loss of heterozygosity is indeed observed at an increasing frequency with the progression from adenomas and intramucosal carcinomas to invasive CRC in familial adenomatous polyposis patients,¹⁷ and the *dcc* gene is considered to be the target of this loss of heterozygosity.

Lack of DCC protein expression has been shown to convey a poor prognosis to patients with radically resected

primary CRC. In particular, Shibata et al¹⁸ have demonstrated that the absence of DCC expression, as determined by immunohistochemistry, is an adverse prognostic factor in patients with stage II or III CRC. Several other studies have also shown that patients with allelic loss of 18q in their primary CRC have an increased death rate independent of stage and clinical and pathologic features.¹⁹⁻²⁴

Although DCC has never been investigated as a predictor of response to fluoropyrimidines (FPs), higher levels of DCC expression were associated with an elevated rate of spontaneous apoptosis in HT29 colonic adenocarcinoma cells,²⁵ and the DCC gene product has been shown to induce apoptosis²⁶ with a caspase-dependent mechanism.²⁷ Of note, apoptosis induced after specific inhibition of TS is also mediated via the caspases.²⁸ Thus, DCC might modulate the induction of apoptosis after TS blockade, potentially affecting chemosensitivity to FPs. In addition, significantly lower rates of loss of heterozygosity of the *dcc* gene have been reported in replication error (RER)-positive tumors.²⁹ The association with an impaired mismatch repair process is important because the RER phenotype has been shown to affect response to chemotherapy in CRC.

Because *dcc* status seems to predict clinical outcome of resected CRCs and because *dcc* also seems to have a central role in regulation of apoptosis and modulation of the cancer phenotype with a possible link with RER status, the aim of this study was to determine whether DCC protein expression in CRC metastases predicts outcome and/or objective response to palliative FU-based chemotherapy. An additional aim was to compare the pattern of DCC expression between CRC metastases and the corresponding primary tumors.

PATIENTS AND METHODS

Patients

The study population consisted of 42 patients (19 males and 23 females; median age, 62.5 years; range, 41 to 74 years; median Eastern Cooperative Oncology Group performance status [PS], 0) who were homogeneously treated with a regimen of schedule-specific biochemical modulation of FU (bolus plus methotrexate alternating to continuous infusion plus leucovorin) in two consecutive phase II trials that enrolled patients with metastatic, recurrent, or locally advanced unresectable CRC.^{30,31} These patients represent all the patients accrued in these two studies for whom tumor sections from a measurable metastatic lesion were available. The demographic and tumor characteristics of the study cohort are listed in Table 1.

The treatment consisted of two biweekly cycles of bolus FU modulated by methotrexate (24 hours earlier) alternating with (14 days later) a 3-week continuous infusion of FU modulated by leucovorin (on the first day of each week of infusion). Further treatment details have been previously published.^{30,31} Response to chemotherapy was assessed according to WHO criteria.³² Data on the outcome after chemotherapy with this regimen, along with other details, are reported elsewhere.^{30,31} Nine (21.4%) of the 42

Table 1. Patient and Tumor Characteristics of the Study Cohort According to the Level of DCC Expression (N = 42)

Characteristic	Overall		DCC Negative		DCC Positive		P
	Median	Range	No.	%	No./Total No.	%	
Age	63	41-74					
≤ 60 years			19	45	10/23	43	.80*
> 60 years			23	55	13/23	57	
Sex							
Male			19	45	11/23	48	.71*
Female			23	55	12/23	52	
PS							
0			24	57	13/23	57	.92*
1-2			18	43	10/23	43	
Primary tumor							
Right colon			14	33	7/23	30	.89*
Left colon			18	45	10/23	43	
Rectum			10	24	6/23	26	
Previous adjuvant							
Yes			5	12	2/23	9	.64†
No			37	88	21/23	91	
Timing of metastases							
Synchronous			27	64	16/23	70	.43*
Metachronous			15	36	7/23	30	
Metastatic sites							
Single			24	57	11/23	48	.18*
Multiple			18	43	12/23	52	
Liver only			21	50	11/23	48	.57*
Liver + other			12	29	8/23	35	
Other			9	21	4/23	17	
Baseline tumor area	27.4	2-1,234					
≤ 27.4 cm ²			21	50	12/23	52	.76*
> 27.4 cm ²			21	50	11/23	48	
Baseline CEA	36	1.7-6,500					
≤ 36 ng/mL			21	50	11/23	48	.62*
> 36 ng/mL			20	48	12/23	52	
Missing data			1	2			
Baseline LDH	420	190-1,500					
≤ 420 U/L			14	33	7/13	54	.84*
> 420 U/L			13	31	6/13	46	
Missing data			15	36			
Baseline ALP	222.5	60-1,054					
≤ 222.5 U/L			15	36	8/15	53	.71*
> 222.5 U/L			15	36	7/15	47	
Missing data			12	28			
Baseline WBC	7,400	2,400-13,890					
≤ 7,400 cells/μL			22	52	13/23	57	.55*
> 7,400 cells/μL			20	48	10/23	43	
TS expression							
Low			22	53	12/22	55	.90*
High			19	45	10/22	45	
Missing data			1	2			

Abbreviations: DCC, deleted in colon cancer; PS, performance status; CEA, carcinoembryonic antigen; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; TS, thymidylate synthase.
* χ^2 test.
†Fisher's exact test.

patients received second-line chemotherapy after the first regimen failed. This consisted of irinotecan in one patient, and various combinations of FU, mitomycin, methotrexate, and interferon alfa were used for the other eight patients. One patient also received irinotecan as third-line treatment, whereas none of the patients received oxalipatin.

Immunohistochemistry

Intensity and tissue localization of DCC protein expression were evaluated by immunohistochemistry using a monoclonal antibody clone (G97-449; Pharmingen, San Diego, CA) that recognizes an intracellular domain of the DCC protein. Archival, formalin-fixed, paraffin-embedded tissue samples from primary

CRC (n = 23), lymph node metastases (n = 18), and distant metastases (n = 42) were used for this study.

Tissue sections (2- μ m thick) were cut, deparaffinized in xylene, rehydrated with graded ethanol, and immersed in triethanolamine-buffered saline (TBS). Endogenous peroxidase activity was quenched with 3% hydrogen peroxide in distilled water for 15 minutes. The slides were heated in a microwave oven at 300 W for 10 minutes, cooled, and stored in TBS at pH 7.6. To block nonspecific binding of the primary antibody, a 10% normal rabbit serum (DAKO X901; Dako, Carpinteria, CA) dilution in TBS was used for 20 minutes. After removing the blocking solution, the monoclonal antibody G97-449 was applied at a dilution of 1:300 for 60 minutes in a modified chamber at room temperature. Negative control studies were performed without applying the primary antibody. Incubation with the secondary horseradish peroxidase-conjugated rabbit antimouse immunoglobulins (Dakopatts Co, Glostrup, Denmark) was performed at a 20-fold dilution in phosphate-buffered saline, including 35% immunoglobulin-free fetal calf serum, for 45 minutes at room temperature. Specifically bound antibody was then visualized, as described previously,³³ by peroxidase-catalyzed substrate conversion of 3-amino-9-ethylcarbazole with 0.03% hydrogen peroxide and, subsequently, counterstained with hematoxylin for 1 minute, dehydrated in a series of ethanols, cleared in xylene, and mounted with glass coverslips using permount. Sections known to stain positively were included in each run as positive controls. Forty-one of these samples had been assessed for TS immunoreactivity in a previous study.³⁴

Slides were then examined under a light microscope and scored by one of the authors (D.D.) blinded to both the clinical and pathologic data. DCC expression was classified on the basis of the percentage of stained cancer cells regardless of the intensity of staining. Only tumor cells with cytoplasmic immunoreactivity were counted as positive. Immunostaining was scored as +++ when more than 50% of the cells were stained positively, as ++ when staining reactivity was between 10% and 50%, as + when less than 10% of the cells were stained positively, and as - when staining was undetectable in all cells. For the purpose of correlation with clinical data, cases in which immunostaining was undetectable in all cells or less than 10% of the tumor cells displayed a cytoplasmic staining were considered negative (immunostaining patterns of - and +, respectively), whereas samples with 10% to 50% or more than 50% of the cells showing immunoreactivity were considered positive (staining patterns of ++ and +++, respectively).

Statistics

The association of DCC expression with each demographic, disease, and treatment variable was examined using the χ^2 test. When the number of expected observations within any of the four cells of a 2×2 table was equal to or less than five, the Fisher's exact test was used. Distant metastases were defined as synchronous or metachronous when detected within or after 90 days from the date of surgery for the primary tumor. For all the patients with metachronous metastases, the interval from resection of the primary tumor to the date of detection of metastatic disease was computed, and the medians were compared between DCC-positive and DCC-negative patients with the Mann-Whitney test. Survival and progression-free survival were computed from the onset of chemotherapy until death or progression, respectively; curves were then constructed according to the Kaplan-Meier method, and differences were assessed by the log-rank test. All *P* values cited are

two-sided, and *P* < .05 was considered statistically significant. To assess the relative influence of different prognostic factors on overall survival, a multivariate analysis using the Cox proportional hazards model was performed. Because of a limited sample size and missing values for some of the variables, only a limited model could be run. However, this model included all the variables that represent well-known prognostic factors in advanced CRC and/or were shown to predict for overall survival by univariate analysis in this study (response to chemotherapy, baseline TS levels, and initial PS).

RESULTS

Nineteen (45%) of 42 metastatic colorectal adenocarcinomas analyzed were DCC positive on immunohistochemistry, with a percentage of stained tumor cells greater than 50% in nine patients and between 10% and 50% in 10 patients. A representative example of positive DCC immunostaining is presented in Figure 1. Among the 23 tumors defined as DCC negative, 16 had a positive immunostaining in less than 10% of the tumor cells, whereas seven exhibited a completely negative immunostaining. All the samples of normal colonic mucosa used as internal controls uniformly showed a positive immunostaining in greater than 10% of the cells.

Tissue from the primary tumor was available in 23 patients, of whom 18 also had lymph node metastases. Eighteen (78%) of 23 of these patients showed an identical pattern of staining in the primary tumor and corresponding lymph node and distant metastases (11 DCC-positive and seven DCC-negative patients). A representative example of positive DCC immunostaining in a CRC metastasis and in the corresponding primary tumor from the same patient is illustrated in Figure 2. In four of five discordant patients,



Fig 1. Immunohistochemical analysis of the deleted in colon cancer (DCC) protein by monoclonal antibody G97-440 in a primary colorectal adenocarcinoma. DCC is uniformly expressed throughout the tumor cells with a high level of cytoplasmic immunoreactivity.

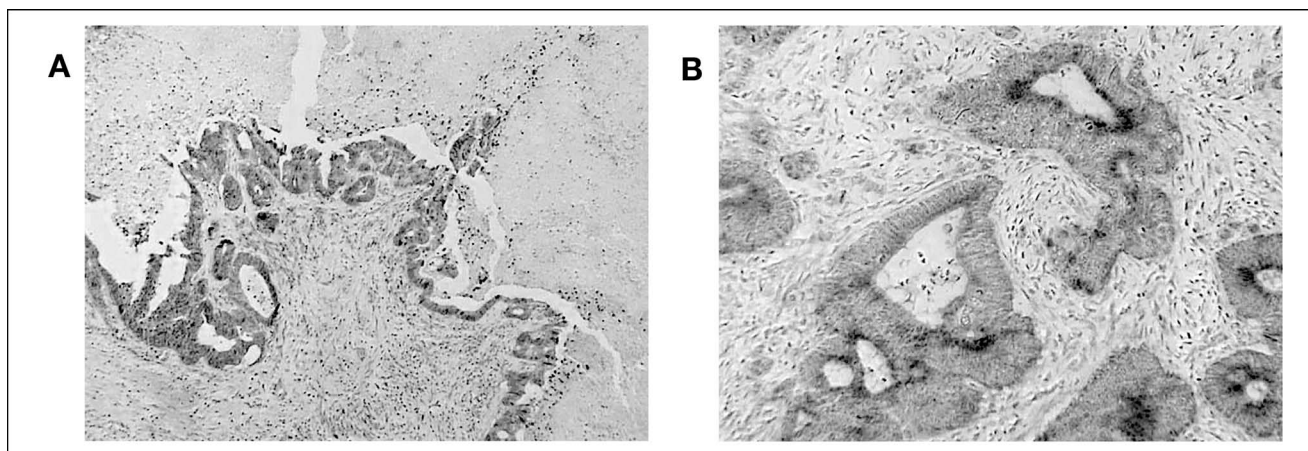


Fig 2. Immunohistochemical analysis of the deleted in colon cancer (DCC) protein by monoclonal antibody G97-440 in a liver metastasis (A) and the corresponding primary colorectal adenocarcinoma (B) from the same patient. A moderate cytoplasmic immunoreactivity in malignant cells is observed in both samples.

positive DCC immunostaining was detected in the primary tumor but not in the corresponding distant metastases. Only one patient had a DCC-negative primary tumor with DCC-positive distant metastases.

No significant differences in the distribution of patient and tumor characteristics were observed between DCC-positive and DCC-negative tumors (Table 1). The percentage of tumors displaying high levels of TS immunoreactivity was also identical between the DCC-positive and -negative groups (45% v 47%, respectively; $P = .90$). The proportion of synchronous metastases was similar in DCC-positive and -negative patients (58% v 70%, respectively; $P = .90$), whereas the time to detection of metachronous metastases seemed to be longer in the DCC-positive group (median, 31.1 months; range, 7.8 to 79.9 months v median, 16.2 months; range, 9.6 to 30.0 months, respectively; $P = .06$, Mann-Whitney test).

After a median follow-up of 3.6 years, 40 patients died, with a median survival time of 16.2 months. Kaplan-Meier survival curves (Fig 3) demonstrate that patients with DCC-positive tumors survived longer. The median survival time was 21 months in patients with DCC-positive tumors versus 14 months in patients with tumors lacking DCC expression (log-rank test, $P = .04$); the corresponding 2-year survival rates were 42.5% (95% CI, 19.9% to 64.3%) and 8.5% (95% CI, 0% to 21.1%), respectively.

Although DCC-negative patients had a worse survival, there seemed to be no statistical relationship between DCC status and either administration of or response to palliative FU-based chemotherapy. In patients with and without DCC expression, the median number of chemotherapy cycles was three versus four, the mean percentage of dose reduction was 14% versus 18%, and the median dose-intensity of bolus and continuous-infusion FU was 305 versus 300 mg/m²/wk and 853 versus 848 mg/m²/wk, respectively (difference not significant).

Ten of 19 DCC-positive patients achieved an objective response compared with eight of 23 DCC-negative patients (53% v 35%, respectively; $P = .73$); two complete responses were observed in each group (11% and 9%, respectively). Minor responses, disease stabilization, and progressions were 16% versus 30%, 16% versus 22%, and 16% versus 13% in DCC-positive and -negative patients, respectively. Expression of the DCC protein was associated with an only marginal improvement in time to progression that failed to reach statistical significance (median, 8.3 versus 7.2 months in patients with and without DCC expression, respectively; $P = .06$, log-rank test).

In this cohort, the only other variables that had a significant impact on survival by univariate analysis were response to chemotherapy and the level of TS expression (Table 2). When these factors were analyzed in a Cox proportional hazards model that also included initial PS (Table

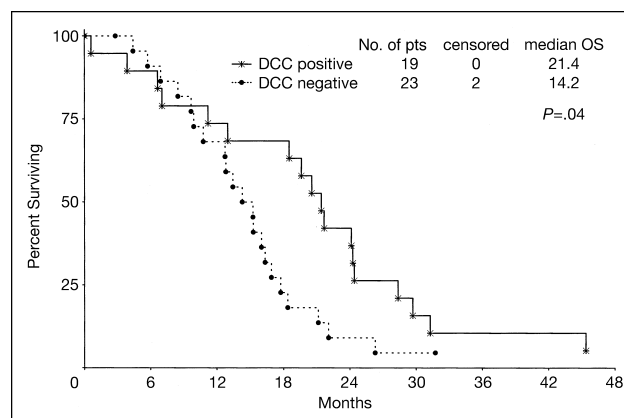


Fig 3. Kaplan-Meier survival curves of 42 patients (pts) with unresectable, metastatic colon cancer treated by bolus fluorouracil plus methotrexate alternated to continuous-infusion fluorouracil plus leucovorin. The advantage in survival time is significant for patients with tumors expressing the deleted in colon cancer (DCC) protein ($P = .04$, log-rank test). OS, overall survival.

Table 2. Survival According to Patient and Tumor Characteristics

	No. of Patients	Median Survival (months)	P
Age			
≤ 60 years	19	15.3	.93
> 60 years	23	16.9	
Sex			
Male	19	16.0	.91
Female	23	16.3	
PS			
0	24	15.8	.74
1-2	18	16.5	
Primary tumor			
Colon	32	13.9	.56
Rectum	10	20.1	
Metastatic sites			
Liver only	21	15.2	.44
Liver + other	12	16.5	
Other	9	17.7	
Baseline tumor area			
≤ 27.4 cm ²	21	16.0	.06
> 27.4 cm ²	20	17.3	
Baseline CEA			
≤ 36 ng/mL	21	16.9	.55
> 36 ng/mL	20	15.6	
Baseline LDH			
≤ 420 U/L	14	14.8	.38
> 420 U/L	13	16.0	
Baseline ALP			
≤ 222.5 U/L	15	15.3	.27
> 222.5 U/L	15	18.4	
Baseline WBC			
≤ 7,400 cells/ μ L	22	16.6	.49
> 7,400 cells/ μ L	20	14.1	
Timing of metastases			
Synchronous	27	16.9	.79
Metachronous	15	12.8	
DCC expression			
Positive	19	21.4	.04
Negative	23	14.2	
TS expression			
Low	22	18.1	.04
High	19	14.3	
Response to chemotherapy			
Yes	18	20.4	.007
No	24	13.5	

Abbreviations: PS, performance status; CEA, carcinoembryonic antigen; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; DCC, deleted in colon cancer protein; TS, thymidylate synthase.

3), DCC status in CRC metastases maintained its prognostic value and was the single best predictor of survival (hazard ratio, 2.16).

DISCUSSION

Our data indicate that immunohistochemical assessment of DCC protein expression in patients with unresectable met-

Table 3. Cox Regression Analyses of Variables Predicting Survival

Variable	Exponential Coefficient	95% CI	P
Age, ≤ 60 v > 60 years	1.22	0.63 to 2.40	.55
PS, 0 v 1-2	1.25	0.63 to 2.48	.52
Response to chemotherapy, yes v no	2.02	0.96 to 4.28	.06
TS expression, low v high	1.75	0.83 to 3.68	.14
DCC protein status, expressed v not expressed	2.16	1.04 to 4.49	.04

Abbreviations: PS, performance status; TS, thymidylate synthase; DCC, deleted in colon cancer.

astatic CRC undergoing palliative FU-based chemotherapy may help to identify specific subsets with distinctly different prognoses. Patients with tumors displaying a positive DCC immunoreactivity had, in fact, a 50% longer median survival with a four-fold increase in 2-year survival rates compared with patients with negative DCC immunostaining in their metastatic lesions.

These results are consistent with those obtained by other investigators assessing DCC protein expression in radically resected early-stage CRC. Even in that setting, DCC expression was associated with a 1.5- to 2-fold increase in overall survival.^{18,19} The longer time interval that was observed to elapse from resection of the primary tumor to development of DCC-positive compared with DCC-negative metastatic lesions in the present study underscores the overall prognostic value of this putative tumor suppressor gene in CRC.

The frequency of defective DCC expression on CRC metastases observed in our study (55%) is similar to that previously observed on samples of primary CRC by Shibata et al.¹⁸ In addition, in a subset analysis, we have investigated DCC protein expression in metastatic lesions and primary tumors from the same patients and found a similar pattern of expression with an overall concordance approaching 80%. Taken together, these results indicate that DCC expression is not related to tumor stage and argue against the notion that *dcc* inactivation represents a necessary step for the development of metastases.³⁵ The wide range of DCC protein levels observed in specimens of both primary CRC and colonic metastases to the liver by Gotley et al,³⁶ without a significant correlation between diminished DCC protein expression and development of metastases, lends further support to the contention that DCC is not associated with colorectal tumor stage. Consistently, in the study by Shibata et al,¹⁸ an identical rate of DCC-negative tumors was found in patients with and without lymph node metastases. Similarly, Sun et al¹⁹ found no difference in DCC immunostaining between matched samples of primary CRCs and regional lymph node metastases. The similar pattern of DCC protein expression observed in primary CRC and the corresponding lymph node and liver metastases suggests

that *dcc* inactivation, which originates in the primary tumor, is maintained in subsequent metastatic sites. Whether the exact genetic alterations are conserved could not be addressed in the present study because several distinctly different genetic alterations can lead to *dcc* inactivation and decreased immunoreactivity. Molecular studies on primary and metastatic sites are needed to address this issue further.

Other factors have been shown to affect overall survival in this disease, including response to chemotherapy, the number of metastatic sites, initial PS, baseline alkaline phosphatase and WBC count, and intratumor TS levels.^{34,37,38} Response to chemotherapy and intratumor TS levels were found to predict for overall survival almost to the same degree as DCC expression, even in the current study. However, the association between TS levels and survival could depend on the higher rates of objective responses obtained in patients with low TS levels, whereas DCC expression was neither significantly associated with response to chemotherapy nor linked to TS levels or p53 status (data not shown). More importantly, the prognostic role of DCC was maintained after adjusting for response to treatment and TS levels in our multivariate analysis model. Therefore, DCC may provide unique clinical information, in that groups of patients could be identified with more aggressive disease and a dismal prognosis after palliative fluoropyrimidine-based chemotherapy, regardless of the achievement of an objective response.

Because patients in this study were treated at the beginning of the 1990s, the treatment regimen did not include irinotecan or oxaliplatin. Therefore, our data cannot provide any information on the prognostic impact of DCC expression in CRC patients treated with combination chemotherapy. Studies investigating DCC expression among patients treated with chemotherapy regimens containing irinotecan or oxaliplatin are required to address this issue specifically. However, these data might provide a basis for the selection of patients who may be candidates to receive initial FU monotherapy (followed by irinotecan and/or oxaliplatin on disease progression) and patients who may be candidates to receive a standard combination of FU and irinotecan or oxaliplatin up-front. The median survival time of almost 2 years that was observed in the group with positive DCC protein expression in this study is, in fact, almost twice as long as that generally obtained with FU-based palliative chemotherapy in advanced CRC¹ and compares well even with the survival time achieved with more aggressive modern combination regimens including either irinotecan or oxaliplatin.²⁻⁵

Despite being a major predictor of survival, we could not find a significant correlation between response to FU-based chemotherapy and DCC protein expression. Although the rate of objective responses seemed to be higher

in patients with DCC-positive tumors compared with the DCC-negative group, statistical significance was not reached. In addition, the overall pattern of response was similar in DCC-positive and -negative patients; complete responses and progressions were equally distributed between the two groups, and the minor response rate in the DCC-negative group was almost twice the rate observed in DCC-positive patients, completely compensating for the difference in partial responses. Because no difference was observed in the number of cycles administered or total dose of FU delivered between DCC-positive and -negative patients, *dcc* loss seems to be a marker of biologic aggressiveness and a determinant of the natural history of advanced CRC, influencing overall survival rather than response to chemotherapy.

Given the limited sample size of our study, caution is necessary in the interpretation of these results. In particular, the lack of a significant relationship between DCC status and response to chemotherapy could be a result of the limited number of patients analyzed in this study. The limited sample size of our study may have also resulted in an over- or underestimation of the survival difference between DCC-positive and -negative patients. Further studies on larger cohorts may confirm these findings and estimate the magnitude of the observed difference more precisely.

In summary, this study is the first to demonstrate that DCC protein expression on CRC metastases predicts for overall survival in patients with unresectable advanced disease undergoing palliative FU-based chemotherapy. Although retrospective and based on a limited sample size, these results are particularly promising considering that the observed survival difference was large and, potentially, clinically relevant. In addition, all the patients were homogeneously treated in a series of phase II clinical trials with well-defined inclusion criteria and a homogenous chemotherapy treatment. On the basis of multivariate analysis, DCC immunoreactivity seems to be an independent prognostic factor in advanced CRC patients receiving FU-based chemotherapy. The possibility to assess DCC status on currently available archival specimens with a convenient, low-cost, immunohistochemical technique could enhance the relevance of this finding. Further studies on larger and prospective series are warranted to confirm these results and to test the prognostic value of DCC expression also in patients treated with irinotecan- and/or oxaliplatin-based combination regimens.

Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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