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Adjuvant Chemotherapy With FOLFOX for Primary Colorectal Cancer Is Associated With Increased Somatic Gene Mutations and Inferior Survival in Patients Undergoing Hepatectomy for Metachronous Liver Metastases

Andreas Andreou, MD[*], **Scott Kopetz, MD**[†], **Dipen M. Maru, MD**[‡], **Su S. Chen, MD**[§], **Giuseppe Zimmitti, MD**[*], **Antoine Brouquet, MD**[*], **Junichi Shindoh, MD**[*], **Steven A. Curley, MD**[*], **Christopher Garrett, MD**[†], **Michael J. Overman, MD**[†], **Thomas A. Aloia, MD**[*], and **Jean-Nicolas Vauthey, MD**[*]

[*]Department of Surgical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX

[†]Department of Gastrointestinal Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX

[‡]Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX

[§]Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX

Abstract

Objective—We hypothesized that metachronous colorectal liver metastases (CLM) have different biology after failure of oxaliplatin (FOLFOX) compared to 5-fluorouracil (5-FU) or no chemotherapy for adjuvant treatment of colorectal cancer (CRC).

Background—It is unclear whether patients treated with liver resection for metachronous CLM after adjuvant FOLFOX for CRC have worse outcomes than those who received 5-FU or no chemotherapy.

Methods—We identified 341 patients who underwent hepatectomy for metachronous CLM (disease-free interval ≥12 months, 1993–2010). Mass-spectroscopy genotyping for somatic gene mutations in CLM was performed in a subset of 129 patients.

Results—Adjuvant treatment for primary CRC was FOLFOX in 77 patients, 5-FU in 169 patients, and no chemotherapy in 95 patients. Node-positive primary was comparable between FOLFOX and 5-FU but lower in the no-chemotherapy group ($P < 0.0001$). Median metastasis size was smaller in the FOLFOX group (2.5 cm) than in the 5-FU (3.0 cm) or no-chemotherapy (3.5 cm) groups, ($P = 0.008$) although prehepatectomy chemotherapy utilization, metastases number, and carcinoembryonic antigen levels were similar. Disease-free survival (DFS) and overall survival (OS) rates after hepatectomy were worse in patients treated with adjuvant FOLFOX [DFS at 3 years: 14% vs 38% (5-FU) vs 45% (no-chemo), OS at 3 years: 58% vs 70% (5-FU) vs 84% (no-chemo)]. On multivariate analysis, adjuvant FOLFOX was associated with worse DFS ($P <$

Reprints: Jean-Nicolas Vauthey, MD, Department of Surgical Oncology, University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 1484, Houston, TX 77030. jvauthey@mdanderson.org.

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0.0001) and OS ($P < 0.0001$). Mutation analysis revealed ≥1 mutations in 57% of patients (27/47) after FOLFOX, 29% (12/41) after 5-FU, and 32% (13/41) after no chemotherapy ($P = 0.011$).

Conclusions—Adjuvant FOLFOX for primary CRC is associated with a high rate of somatic mutations in liver metastases and inferior outcomes after hepatectomy for metachronous CLM.

Keywords

colorectal cancer; metachronous colorectal liver metastases; somatic gene mutations

Colorectal cancer (CRC) is one of the most common malignancies worldwide and the second most common cause of cancer death in Western countries.[1] CRC resection with regional lymphadenectomy is the primary treatment of choice. The postoperative survival in these patients is significantly associated with tumor stage on the basis of the TNM classification system, which takes into consideration the depth of tumor penetration in the bowel wall and the extent of lymph node involvement.[2] Patients with positive lymph node metastases have a higher risk of local recurrence and metastasis, especially in the liver. Therefore, systemic therapy after resection of node-positive CRC has routinely been used to reduce the incidence of relapse.[3]

Adjuvant chemotherapy with fluorouracil (5-FU) and leucovorin (FL) was established in the 1990s for stage III CRC to reduce recurrence and prolong survival.[4–6] Since 2004, oxaliplatin, in combination with 5-FU and FL (FOLFOX), has been used to treat stage II or stage III CRC after surgery. The MOSAIC randomized trial demonstrated that patients treated with this modern chemotherapy regimen have higher disease-free (DFS) and overall survival (OS) rates than those treated with 5-FU and FL alone.[7,8]

Despite adjuvant chemotherapy, approximately 20% of patients develop metachronous colorectal liver metastases (CLM) within 3 years.[9] Modern chemotherapy with FOLFOX has increased the recurrence-free survival rate after resection of the primary CRC; however, metastatic liver disease has not been completely eliminated. One fourth of patients with CLM are candidates for liver resection; curative hepatectomy, combined with perioperative systemic therapy, leads to 5-year OS rates as high as 58%.[10] However, in this era of modern chemotherapy, it is unclear whether patients treated with liver resection for metachronous CLM after adjuvant FOLFOX therapy for the primary CRC have poorer outcomes than those who received 5-FU or no chemotherapy.

In this retrospective study, we hypothesized that metachronous CLM have different biologic characteristics after failure of oxaliplatin compared with after 5-FU or no chemotherapy for adjuvant treatment of CRC. To investigate this hypothesis, we performed a survival analysis on 3 groups of patients who had undergone liver resection for metachronous CLM (diagnosis ≥ 1 year after resection of the CRC) and had received FOLFOX, 5-FU, or no chemotherapy for the primary tumor. The biologic characteristics of the CLM were evaluated on the basis of the presence of somatic gene mutations that are known to be associated with unfavorable outcome in metastatic CRC.

PATIENTS AND METHODS

Study Inclusion Criteria

We queried the prospectively maintained hepatobiliary surgical database at The University of Texas MD Anderson Cancer Center (Houston, TX) to identify consecutive patients who had undergone surgery for CLM between January 1993 and December 2010. Clinicopathologic data (described in detail in the Statistical Analysis section) were extracted from the patients' medical records. Patients who had been treated with radiofrequency

ablation (RFA) only or concomitant hepatectomy and RFA were not included in this analysis. All patients with disease-free interval between resection of the primary CRC and diagnosis of the CLM less than 12 months were considered to have synchronous CLM and were excluded. Patients who had undergone hepatectomy for metachronous CLM and had received adjuvant chemotherapy for the primary CRC other than 5-FU or FOLFOX did not fulfill the inclusion criteria. Institutional review board approval (Protocol PA11–0607) was obtained before data retrieval and analysis.

Preoperative CLM Assessment

Preoperative assessment included a medical history, physical examination, laboratory evaluation, and imaging studies. Helical computed tomography of chest, abdomen, and pelvis with liver protocol was used to define the extent and location of CLM. Fluorodeoxyglucose positron emission tomography was used in selected patients to rule out extensive extrahepatic disease and confirm the metastatic nature of atypical lesions.[11] Treatment plans were based on the location and extent of CLM, the presence of extrahepatic disease, and radiographic response to preoperative chemotherapy. The decision to administer preoperative chemotherapy was made by the treating physicians. Hepatectomy was considered in patients in whom computed tomographic volumetry data indicated that all CLM could be safely resected with preservation of a sufficient future liver remnant. In patients with an anticipated insufficient future liver remnant, preoperative portal vein embolization was used to induce hypertrophy.[12]

Surgical Procedure

During laparotomy, the peritoneal cavity was inspected to identify previously unrecognized extrahepatic disease. Intraoperative sonography of the liver was performed to confirm and to better define the location of CLM and their relation to portal pedicles and hepatic veins. Parenchymal transection was performed under total or selective hepatic inflow vascular exclusion using the Cavitron ultrasonic surgical aspirator (CUSA, Valleylab, Boulder, CO), and hemostasis was achieved using saline-linked cautery (dissecting sealer DS 3.0, Tissuelink Medical, Inc, Dover, NH).[13]

Postoperative Evaluation

Postoperative mortality was defined as any death within 90 days after liver resection, and *postoperative morbidity* was defined as any complication within the same time period. Postoperative complications were graded according to a standard classification.[14] Major complications were classified as complications requiring surgical, endoscopic, or radiologic intervention (grade III); life-threatening complications requiring intensive care management (grade IV); and death (grade V). *Postoperative liver insufficiency* was defined as a postoperative peak serum bilirubin level higher than 7 mg/dL.[15]

All specimens were subjected to histologic evaluation to confirm the diagnosis of metastatic CRC, the degree of pathologic response of CLM to preoperative chemotherapy,[16] and the width of the tumor-free surgical margin.[17]

Somatic Gene Mutation Profiling

To assess the tumor biologic characteristics in patients who received adjuvant FOLFOX, 5-FU, or no chemotherapy for the primary CRC, mass-spectroscopy genotyping for somatic gene mutations was performed. DNA extracted from formalin-fixed paraffin-embedded resected CLM was analyzed with Sequenom MassArray technology (Sequenom, Inc, San Diego, CA) using the protocol developed in one of our institutional core facilities.[18] A total of 159 point mutations in 33 genes commonly involved in solid tumors including *KRAS*,

BRAF, *NRAS*, *PIK3CA*, *FBWX7*, and *CTNNB1* were tested. Sequenom's MassARRAY system utilizes polymerase chain reaction amplification and single-base primer extension for mutation detection.[19–21] The analytical sensitivity of the assay [limit of detection (LOD) 5%–10% of mutant DNA in total DNA] is higher than conventional Sanger sequencing (LOD: 10%–20%) and similar to pyrosequencing (LOD: 5%–10%).[22,23] The advantages offered by the MassARRAY system include high-throughput screening for many hot-spot mutations in parallel, use of minimal DNA (10–50 ng) isolated from formalin-fixed paraffin-embedded tissues, ability to detect coexisting multiple mutations, and cost and time effectiveness.

Statistical Analysis

Quantitative and qualitative variables were expressed as medians (range) and frequencies. Comparisons between groups were analyzed with the chi-square or Fisher exact tests for proportions and the Mann-Whitney *U* test or Kruskal-Wallis *H* test for continuous variables, as appropriate. Patients were stratified by type of adjuvant chemotherapy for the CRC and the clinicopathologic characteristics of patients who received adjuvant FOLFOX were compared with those of patients who received 5-FU or no adjuvant chemotherapy. Somatic gene mutation rates were also compared between the 3 patient groups. OS and DFS rates were calculated from the date of liver resection to the date of last follow-up or recurrence, respectively, using the Kaplan-Meier method and were compared using log-rank tests.

To identify factors associated with OS and DFS in the entire study cohort (N = 341), we evaluated the following clinicopathologic variables in a univariate analysis: sex (male vs female), age (> 65 vs ≤ 65 years), primary tumor location (rectum vs colon), regional lymph nodes status of the primary tumor (positive vs negative), number of CLM (multiple vs solitary), adjuvant chemotherapy for CRC (FOLFOX vs 5-FU vs no chemotherapy), diameter of the largest CLM (>3 vs ≤3 cm), preoperative carcinoembryonic antigen (CEA) level (>5 ng/mL vs ≤5 ng/mL), preoperative chemotherapy for CLM (administered vs not), portal vein embolization (performed vs not), blood transfusion required (yes vs no), liver resection margins status on microscopic analysis (R1 vs R0), pathologic response to preoperative chemotherapy (major vs minor), postoperative chemotherapy for CLM (administered vs not), and postoperative complications (yes vs no).

All variables associated with OS or DFS with $P < 0.05$ in the univariate proportional hazards models were entered into a Cox multivariate regression model with backward elimination. $P < 0.05$ were considered statistically significant. Statistical analyses were performed using the software IBM SPSS Statistics, version 19 (IBM, Armonk, NY).

RESULTS

Patients and Treatment

Among 1250 consecutive patients with CLM treated at MD Anderson during the study period, 98 patients had been treated with RFA only and were excluded from the study. Patients with synchronous CLM (N = 587) (disease-free interval < 12 months) were also excluded. Concomitant hepatectomy and RFA had been performed in 77 of the remaining 565 patients with metachronous CLM; these patients were excluded from the analysis. Of the remaining 488 patients treated with curative hepatectomy for metachronous CLM, 147 had undergone adjuvant chemotherapy for the primary tumor with agents other than 5-FU or FOLFOX and were thus not included in the analysis. The final study cohort consisted of 341 patients who underwent curative liver resection for metachronous CLM and had received FOLFOX (N = 77), 5-FU (N = 169), or no adjuvant systemic therapy (N = 95) after the resection of the primary CRC (Fig. 1).

Patient Characteristics by Adjuvant Chemotherapy Type for CRC

Patients' characteristics, listed by adjuvant chemotherapy type for CRC, are summarized in Table 1. Patients who received no adjuvant chemotherapy for CRC have been treated in our center from 1993 to the end of the study period (2010). Patients who received adjuvant 5-FU have been treated in the same time period (1993–2010). FOLFOX has been used for the adjuvant therapy of CRC since 2005. Patients' median age in the FOLFOX group was significantly lower than that in the 5-FU and no chemotherapy groups ($P = 0.035$). The number of node-positive primary tumors was similar between FOLFOX and 5-FU but lower in the no-chemotherapy group ($P < 0.0001$). The median metastasis size was smaller in the adjuvant FOL-FOX group (2.5 cm) than in the 5-FU (3.0 cm) and no-chemotherapy (3.5 cm) groups ($P = 0.008$). Postoperative complications were more common in the FOLFOX group ($P = 0.047$), but there was no difference in the major complication rates among the 3 groups ($P = 0.204$). The remaining patient characteristics in the 3 groups were similar, including prehepatectomy chemotherapy utilization ($P = 0.110$), CLM number ($P = 0.579$), and preoperative CEA serum level ($P = 0.239$).

Postoperative Mortality and Morbidity

The postoperative 90-day mortality rate was 2% (6 patients died). Three patients died as a result of postoperative liver insufficiency after an extended hepatectomy following prolonged preoperative chemotherapy. Two deaths were related to pulmonary infection, and 1 patient died of thromboembolic complications (pulmonary embolism). The postoperative 90-day morbidity rate was 27% (93 of 341 patients). Thirteen percent of patients experienced a major complication that necessitated operative, endoscopic, or radiologic intervention.

Long-Term Survival

At a median follow-up duration of 53 months (1–196 months), the 3- and 5-year DFS rates of the entire cohort were 36% and 33%, respectively. The 3- and 5-year OS rates were 72% and 55%, respectively. The DFS rates after resection of CLM were significantly lower in patients treated with adjuvant FOLFOX than in patients treated with 5-FU or no chemotherapy after resection of the primary CRC (DFS at 3 years: 14% vs 38% vs 45%, respectively, $P < 0.0001$) (Fig. 2). Likewise, OS rates were lower in FOLFOX patients than in 5-FU and no chemotherapy patients (OS at 3-years: 58% vs 70% vs 84%, respectively, $P = 0.002$) (Fig. 3).

Predictors of Outcome

The results of univariate and multivariate analyses of factors associated with DFS are summarized in Table 2. On univariate analysis, positive lymph node metastases for the primary tumor ($P = 0.023$), adjuvant FOLFOX therapy for the primary CRC ($P < 0.0001$), pre-operative chemotherapy for CLM ($P = 0.028$), and positive surgical margins at CLM resection ($P = 0.012$) were associated with poor DFS. On multivariate analysis, only the adjuvant FOLFOX therapy for the primary CRC [hazard ratio (HR) = 1.52, 95% confidence interval (CI): 1.23–1.89, $P < 0.0001$] was independently associated with worse DFS.

The results of the analysis of OS predictors are shown in Table 3. On univariate analysis, positive lymph node metastases for the primary tumor ($P = 0.022$), multiple CLM ($P = 0.009$), adjuvant FOLFOX therapy for the primary CRC ($P = 0.002$), largest CLM larger than 3 cm ($P = 0.002$), and positive surgical margins at the CLM resection ($P = 0.003$) were predictive of poor OS. On multivariate analysis, multiple CLM (HR = 1.52, 95% CI: 1.07–2.17, $P = 0.021$), adjuvant FOLFOX therapy for the primary CRC (HR = 1.86, 95% CI: 1.36–2.53, $P < 0.0001$), largest CLM larger than 3 cm (HR = 1.89, 95% CI: 1.31–2.73, $P =$

0.001), and positive surgical margins at CLM resection (HR = 1.82, 95% CI: 1.13–2.93, *P* = 0.014) remained significant predictors of OS.

Somatic Gene Mutation Profiling

Among 341 patients in this series, a total of 210 patients operated in the most recent years (FOLFOX = 70, 5-FU = 70, and no chemotherapy = 70) were selected for specimen analysis. Paraffin blocks and sufficient tissue for somatic gene mutation analysis using Sequenom MassArray technology were available in 129 patients (FOLFOX = 47, 5-FU = 41, no chemotherapy = 41). The tumor biologic characteristics of patients treated with adjuvant FOLFOX, 5-FU, or no chemotherapy for the primary CRC were assessed according to the proportions of somatic gene mutations found in each group. One or more mutations were found in 57% of patients (27/47) after FOLFOX, 29% of patients (12/41) after 5-FU, and 32% of patients (13/41) after no chemotherapy (*P* = 0.011). The mutations included the genes *KRAS*, *BRAF*, *NRAS*, *CTNNB1*, *FBWX7*, and *PIK3CA*. The differences in mutation rates among the groups were related to the proportions of *KRAS* mutations in each group (*P* = 0.008). Other mutations were similarly distributed among the 3 groups (Table 4).

DISCUSSION

Patients with primary CRC and lymph node metastases and those at high risk for metachronous CLM (stage II/III) have been treated with adjuvant chemotherapy, including oxaliplatin in recent years.[3,7,8] Nevertheless, some of these patients will develop CLM, which can be successfully treated if they can be completely resected with histologically negative margins.[24,25] In this study, we analyzed DFS and OS rates after resection of metachronous CLM according to adjuvant chemotherapy type for the primary tumor. After controlling for primary and metastatic disease stage, the primary risk factor associated with poor outcome was treatment with adjuvant FOLFOX after resection of CRC. These findings suggest that the type of adjuvant therapy given after colon resection impacts the tumor biology of the subsequent metastases. To validate this hypothesis, we analyzed somatic gene mutations in CLM. We found a higher rate of mutations in FOLFOX-treated patients, with *KRAS* mutational status being entirely responsible for this difference.

Prior to the oxaliplatin-era, published series reporting on patients who developed metachronous CLM indicated 46% to 62% 3-year DFS and 64% to 75% 3-year OS rates.[9,26,27] Our retrospective analysis of prospectively collected CLM patient data demonstrated that the natural history of the subset of metachronous CLM from stage III CRC may have changed after the introduction of oxaliplatin-based chemotherapy. Patients with metachronous CLM treated with 5-FU experienced longer survivals (3-year DFS 38% and OS 70%) than those treated with FOLFOX (3-year DFS 18% and OS 58%). Clinical trials indicate that the use of FOLFOX after primary resection prevents or delays CLM in a larger number of patients than does 5-FU alone,[7,8] but it may at the same time contribute to the selection of patients with a more aggressive form of metastatic disease—that is, resistant to oxaliplatin and responsible for a poorer OS and DFS after resection of metachronous CLM.

Two prospective randomized studies on adjuvant chemotherapy in stage II and III colon cancer patients have demonstrated that patients who recur after adjuvant FOLFOX have shorter OS than patients who recur after randomization to adjuvant 5-FU.[8,28] In the MOSAIC trial, the median time from relapse to death was 21 months for the FOLFOX group and 24 months for the 5-FU group.[8] This has been previously attributed to the lower efficacy of oxaliplatin regimens upon retreatment of previously FOLFOX-treated patients. According to this hypothesis, these patients have fewer effective chemotherapy regimens and a resulting lower OS. However, our data supports the alternate suggestion that

FOLFOX-resistant colon cancer has a different biology than 5-FU–resistant tumors. Preclinical studies suggest that oxaliplatin resistant cell lines develop epithelial-to-mesenchymal transition, characterized by a migratory and proinva-sive phenotype.[29,30] As DFS after hepatectomy is dictated by unrecognized microscopic disease outside of the visible metastases, such migratory behavior may contribute to the higher recurrence rates after FOLFOX.

KRAS mutation in primary tumors represents a modest prognostic marker for metastatic CRC patients in some, but not all, clinical series. However, it is clearly associated with resistance to epidermal growth factor receptor inhibitors.[31–34] *KRAS* mutation in CLM has also been shown to be associated with lower survival and accelerated disease progression in patients with resected CLM in an era predating FOLFOX chemotherapy.[35] The same study reported a low rate of *KRAS* mutations (16%), similar to our study, after resection of metachronous CLM. *KRAS* mutation analysis was additionally used in 2 previous studies to assess the minimum surgical margins in resected CLM.[36,37] *KRAS* mutation has recently been associated with higher rates of lung metastases, a common location of recurrence for patients with resected CLM.[32] The current study is the first to characterize *KRAS* mutation in patients undergoing curative liver resection for metachronous CLM in the era of adjuvant FOLFOX chemotherapy for CRC; the higher rate of *KRAS* mutation in patients treated with FOLFOX underscores the association between modern chemotherapy and the long-term selection of worse tumor biology.

This study is limited by its retrospective nature. The selection of adjuvant chemotherapy regimens in routine patient care is based on many clinical and pathologic factors not fully captured by multivariate analysis; however, the degree of magnitude of the observed effect and the inclusion of multiple prognostic variables argues against this. A prospective study to confirm our findings may not be feasible because chemotherapy with FOLFOX for lymph-node positive CRC is currently the standard of care on the basis of randomized trials.[3,7,8] Efforts to replicate this finding from completed randomized adjuvant studies are ongoing. Our study is also limited by the fact that somatic gene mutation profiling using Sequenom MassArray technology could only be performed in a subset of 129 patients. However, this high-throughput technology enabled the testing of 159 different mutations on 33 different genes, allowing evaluation of genes and pathway interactions that could not be evaluated with a single gene study. Another possible limitation of this study is the absence of *KRAS* status evaluation of the primary tumor. Thus, it was not possible to determine whether a discordance in mutation rates existed between patients who did and did not receive oxaliplatin after resection of the primary. However, numerous studies of *KRAS* mutational status in primary and metastatic disease sites have shown high concordance rates, ranging from 84% to 100%[38–44], whereas only one study, in 21 patients, reported a low concordance rate of 52%.[45]

In conclusion, this study suggests that oxaliplatin-based adjuvant therapy may provide a selection pressure favoring a chemotherapy-resistant subset enriched for *KRAS* mutations while on balance preventing liver recurrences in patients with *KRAS* wild-type tumors. This change may be responsible for the early recurrence and lower OS observed after resection of metachronous CLM. The selection of patients with chemotherapy-resistant CLM and predestined worse prognosis represents a new challenge for hepatobiliary surgeons in an era that is characterized by multimodal therapy of CLM and the increasing use of perioperative chemotherapy with molecular profiling.[16,46,47]

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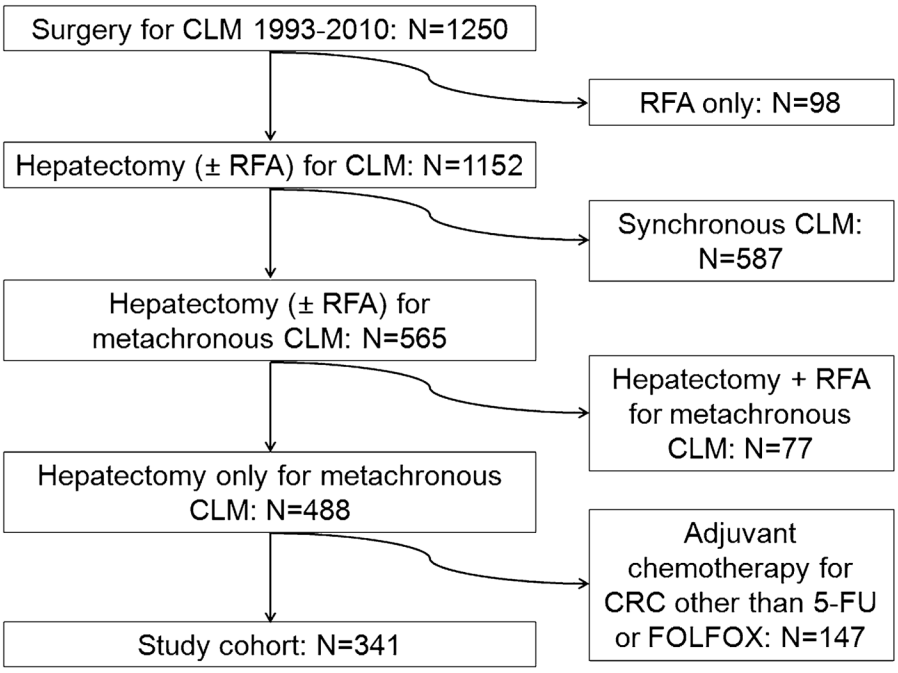

FIGURE 1.
Study inclusion criteria.

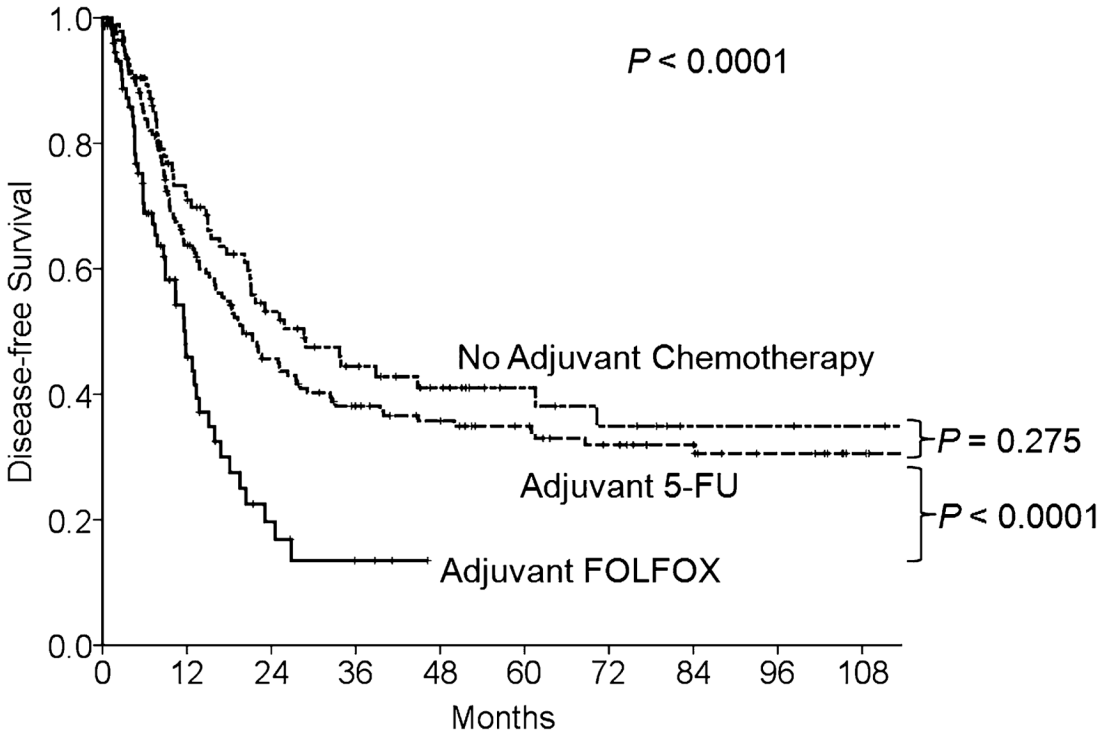

FIGURE 2.
DFS by adjuvant chemotherapy type for primary CRC in 341 patients who underwent hepatectomy for metachronous CLM.

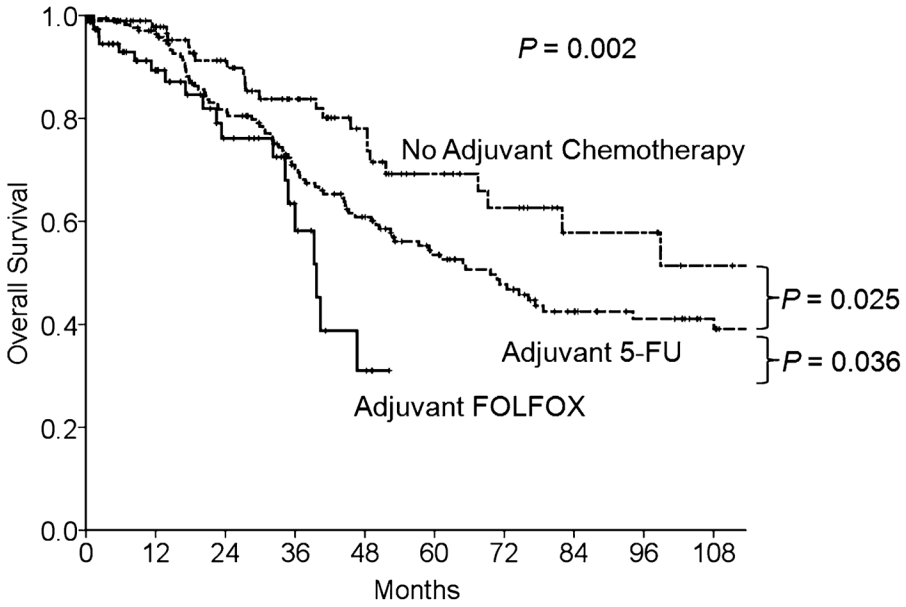

FIGURE 3.

OS by adjuvant chemotherapy type for primary CRC in 341 patients who underwent hepatectomy for metachronous CLM.

TABLE 1

Characteristics of Patients who Underwent Hepatectomy for Metachronous CLM by Adjuvant Chemotherapy Type for CRC

Variable	No Adjuvant Chemotherapy (N = 95)	5-FU (N = 169)	FOLFOX (N = 77)	P^*	$P^\dagger$
Male sex, %	68	57	58	0.166	0.810
Median age, (range), y	60 (37–84)	62 (23–82)	57 (32–87)	0.035	0.015
Age > 65 y, %	40	38	25	0.074	0.042
Median DFS after CRC resection (range), mo	20 (12–142)	20 (12–204)	19 (12–48)	0.187	0.177
Rectal primary tumor (%)	31	29	30	0.952	0.857
Positive nodes for primary tumor, %	8	68	79	<0.0001	0.059
Median number of CLM (range)	1 (1–10)	1 (1–7)	1 (1–9)	0.579	0.420
Multiple CLM (%)	43	37	38	0.619	0.954
Median size of CLM, (range), cm	3.5 (3–15)	3 (0.5–17)	2.5 (0.4–8.5)	0.008	0.036
Size > 3 cm, %	52	50	36	0.092	0.052
Median CEA (range), ng/mL	4 (1–2477)	7 (1–387)	5 (1–210)	0.239	0.092
CEA > 5 ng/mL	47	56	46	0.275	0.168
Preoperative chemotherapy for CLM, %	61	50	61	0.110	0.098
Portal vein embolization, %	8	4	13	0.09	0.027
Median estimated blood loss, (range), mL	250 (0–2000)	300 (0–3000)	250 (0–2000)	0.770	0.507
Transfusions, %	13	9	9	0.604	0.965
Postoperative complications, %	31	21	35	0.047	0.023
Major postoperative complications, %	15	10	17	0.204	0.094
Positive surgical margins, %	13	11	10	0.884	0.831
Resection for recurrence, %	17	15	10	0.449	0.298
Major pathologic response to preoperative chemotherapy for CLM, %	60	38	43	0.06	0.545
Postoperative chemotherapy for CLM, %	66	67	54	0.137	0.054

*Comparison of patients with FOLFOX vs 5-FU vs no chemotherapy for adjuvant treatment of CRC.

†Comparison of patients with FOLFOX vs 5-FU for adjuvant treatment of CRC.

TABLE 2

Univariate and Multivariate Analysis of Clinicopathologic Variables Associated With DFS in 341 Patients Who Underwent Hepatectomy for Metachronous CLM

		Univariate Analysis			Multivariate Analysis*		
Variable		**N = 341 (%)**	**Median DFS, mo**	***P***	**HR**	**95% CI**	***P***
Sex	Male	60	19	0.340			
	Female	40	20				
Age, y	>65	35	19	0.268			
	≤65	65	20				
Primary tumor	Rectal	29	20	0.820			
	Colon	71	18				
Lymph nodes for primary	Positive	54	16	0.023			NS
	Negative	46	25				
Number CLM	Multiple	61	15	0.065			
	Solitary	39	22				
Adjuvant therapy for CRC	None	28	29	< 0.0001	1.52	1.23–1.89	< 0.0001
	5-FU	49	20				
	FOLFOX	23	12				
Size, cm	> 3	47	20	0.434			
	≤3	53	18				
CEA, ng/mL	> 5	51	17	0.924			
	≤5	49	16				
Preoperative chemotherapy for CLM	Yes	55	15	0.028			NS
	No	44	25				
Portal vein embolization	Yes	7	11	0.174			
	No	93	20				
Transfusions	Yes	10	16	0.602			
	No	90	20				
Positive surgical margins at CLM resection	Yes	12	13	0.012			NS
	No	88	20				
Pathologic response to preoperative chemotherapy for CLM (n = 189)	Major	48	16	0.089			
	Minor	52	12				

		Univariate Analysis			Multivariate Analysis*		
Variable		**N = 341 (%)**	**Median DFS, mo**	***P***	**HR**	**95% CI**	***P***
Postoperative chemotherapy for CLM	Yes	64	18	0.113			
	No	36	23				
Complications	Yes	27	19	0.687			
	No	73	20				

*Cox regression multivariate analysis included all variables with $P < 0.05$ in univariate analysis.

NS indicates not significant.

TABLE 3

Univariate and Multivariate Analysis of Clinicopathologic Variables Associated With OS in 341 Patients Who Underwent Hepatectomy for Metachronous CLM

		Univariate Analysis			Multivariate Analysis*		
Variable		**N = 341 (%)**	**Median OS, mo**	***P***	**HR**	**95% CI**	***P***
Sex	Male	60	61	0.087			
	Female	40	94				
Age, y	> 65	35	59	0.103			
	≤65	65	94				
Primary	Rectal	29	94	0.265			
	Colon	71	65				
Lymph nodes for primary	Positive	54	59	0.022			NS
	Negative	46	94				
Number CLM	Multiple	61	53	0.009	1.52	1.07–2.17	0.021
	Solitary	39	108				
Adjuvant therapy for CRC	None	28	NR	0.002	1.86	1.36–2.53	< 0.0001
	5-FU	49	70				
	FOLFOX	23	40				
Size, cm	>3	47	50	0.002	1.89	1.31–2.73	0.001
	≤3	53	NR				
CEA, ng/mL	>5	51	57	0.209			
	≤5	49	71				
Preoperative chemotherapy for CLM	Yes	55	76	0.978			
	No	44	72				
Portal vein embolization	Yes	7	45	0.534			
	No	93	71				
Transfusions	Yes	10	41	0.227			
	No	90	71				
Positive surgical margins at CLM resection	Yes	12	40	0.003	1.82	1.13–2.93	0.014
	No	88	77				
Pathologic response to preoperative chemotherapy for CLM (n = 189)	Major	48	NR	0.264			

Variable		Univariate Analysis			Multivariate Analysis*		
		N = 341 (%)	Median OS, mo	*P*	HR	95% CI	*P*
	Minor	52	114				
Postoperative chemotherapy for CLM	Yes	64	67	0.417			
	No	36	82				
Complications	Yes	27	57	0.129			
	No	43	75				

*Cox regression multivariate analysis included all variables with $P < 0.05$ in univariate analysis.

NR indicates not reached; NS, not significant.

TABLE 4

Somatic Gene Mutation Rates in 129 Patients who Underwent Hepatectomy for Metachronous CLM by Adjuvant Chemotherapy Type for the CRC

Mutation	No Adjuvant Chemotherapy (N = 41)	5-FU (N = 41)	FOLFOX (N = 47)	P^*	$P^\dagger$
Patients with somatic gene mutations	13 (32%)	12 (29%)	27 (57%)	0.011	0.008
KRAS	8 (20%)	9 (22%)	22 (47%)	0.008	0.015
BRAF	1 (2%)	2 (5%)	0	0.317	0.126
NRAS	1 (2%)	0	3 (6%)	0.217	0.100
CTNNB1	0	1 (2%)	0	0.339	0.282
FBWX7	2 (5%)	1 (2%)	0	0.317	0.282
PIK3CA	4 (10%)	5 (12%)	3 (6%)	0.640	0.344

*Comparison of patients with FOLFOX vs 5-FU vs no chemotherapy for adjuvant treatment of CRC.

†Comparison of patients with FOLFOX vs 5-FU for adjuvant treatment of CRC.