Adjuvant 5-Fluorouracil and Leucovorin With or Without Interferon Alfa-2a in Colon Carcinoma: National Surgical Adjuvant Breast and Bowel Project Protocol C-05

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Background: **National Surgical Adjuvant Breast and Bowel Project (NSABP) protocol C-03 showed a benefit from leucovorin (LV)-modulated 5-fluorouracil (5-FU) adjuvant therapy (5-FU + LV) in patients with Dukes' stage B or C carcinoma of the colon. Preclinical and clinical phase I/II data suggested that interferon alfa-2a (IFN) enhanced the efficacy of 5-FU therapy. Accordingly, in NSABP protocol C-05, the addition of recombinant IFN to 5-FU + LV adjuvant therapy was evaluated.** ***Methods:*** **Data are presented for 2176 patients with Dukes' stage B or C cancer entered onto protocol C-05 during the period from October 1991 through February 1994. Individuals with an Eastern Cooperative Oncology Group performance status of 0–2 (ranges from fully active to ambulatory and capable of self-care but unable to work), a life expectancy of at least 10 years, and curative resection were stratified by sex, disease stage, and number of involved lymph nodes and were randomly assigned to receive either 5-FU + LV or 5-FU + LV + IFN; the mean time on the study as of June 30, 1997, was 54 months. All statistical tests were two-sided.** ***Results:*** **There was no statistically significant difference in either disease-free survival (5-FU + LV, 69%; 5-FU + LV + IFN, 70%) or overall survival (5-FU + LV, 80%; 5-FU + LV + IFN, 81%) at 4 years of follow-up. Toxic effects of grade 3 or higher were observed in 61.8% of subjects in the group treated with 5-FU + LV and in 72.1% of subjects in the group treated with 5-FU + LV + IFN; fewer patients in the latter group completed protocol-mandated 5-FU + LV therapy than in the former group (77.1% versus 88.5%).** ***Conclusion:*** **The addition of IFN to 5-FU + LV adjuvant therapy confers no statistically significant benefit, but it does increase toxicity. [J Natl Cancer Inst 1998;90:1810–6]**

The rationale for the National Surgical Adjuvant Breast and Bowel Project (NSABP) protocol C-05 was based on the early results from NSABP protocol C-03 in which a benefit for leucovorin (LV)-modulated 5-fluorouracil (5-FU) (i.e., 5-FU + LV) was demonstrated in patients with Dukes' stage B or C carcinoma of the colon *(1,2),* as well as the preclinical and phase I/II data that were emerging relative to interferon alfa-2a (IFN). The potential for further modulation of LV and 5-FU with IFN was considered pharmacologically and clinically compelling. IFN had been shown to enhance the cytotoxic activity of 5-FU in human cancer cell lines *(3).* The mechanism of this enhancement is likely multifactorial, and several mechanisms have been implicated, including the abrogation of 5-FU-induced augmentation of thymidylate synthase protein levels and enhanced metabolism of 5-FU to the active nucleotide forms, via increased levels of thymidine phosphorylase *(4–6).* Furthermore, studies performed on cultured colon adenocarcinoma cells showed that this enhancement could be attained at clinically achievable concentrations of IFN and LV *(7).* Studies in murine models suggested that IFN selectively protected normal tissue from the untoward effects of 5-FU, which permitted 5-FU-dose escalation and improved efficacy *(8,9).* Pharmacokinetic studies performed on patients with colon cancer indicated that coadministration of 5-FU and IFN resulted in a decrease in 5-FU clearance, a prolongation of 5-FU half-life, and a 1.5-fold increase in 5-FU exposure. Although the addition of LV appeared to abrogate the IFN-induced changes in 5-FU kinetics, this latter observation was thought to be of particular relevance and served as a major rationale for protocol C-05 *(10,11).*

Several uncontrolled clinical trials in patients with advanced gastrointestinal adenocarcinomas offered supporting evidence for the use of IFN as a 5-FU modulator. Wadler et al. *(12)* reported a 76% objective response rate with 5-FU + IFN in 17 previously untreated patients with metastatic disease. Although other phase II trials failed to duplicate this high response rate, they nevertheless seemed to confirm that 5-FU + IFN was active in gastrointestinal cancers. The Eastern Cooperative Oncology Group phase II study *(13),* EST P-Z289, registered 36 assessable chemotherapy-naive patients in whom a 42% objective response rate was demonstrated. Studies involving chemotherapy-naive patients from The University of Texas M. D. Anderson Hospital (Houston, TX) and Memorial Sloan-Kettering Cancer Center (New York, NY) demonstrated a 35% objective response rate in

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See ''Note'' following ''References.''

45 assessable patients and a 26% objective response rate in 35 assessable patients, respectively *(14,15).* The National Cancer Institute (NCI), Bethesda, MD, conducted a phase II study of 5-FU + LV + IFN in patients with metastatic colon cancer; at the conclusion of that study, an objective response rate of 54% was noted in 44 assessable patients who had received no prior 5-FU treatment *(10).*

As a consequence of these findings, the NSABP implemented protocol C-05 to evaluate the role of the addition of IFN to 5-FU and LV in patients with Dukes' B or C colon cancer. Preliminary findings from this study have been presented in abstract form *(16);* the present article reports the first full analysis of the data.

Patients and Methods

Patient Eligibility, Randomization, and Protocol Design

NSABP protocol C-05 was initiated in October 1991; accrual was completed at the end of February 1994, after 2176 patients had been entered into the trial. Eligibility criteria required that patients have a histologically confirmed Dukes' stage B or C lesion of the colon and that random assignment and the commencement of treatment occurred within 42 and 49 days of curative resection, respectively. A colon cancer was defined as any lesion of the large bowel that did not require opening of the pelvic peritoneum to define the distal extent of the tumor. Criteria for anatomic location within the colon were described previously *(17).* Dukes' classification was according to the classical criteria for carcinoma of the rectum as subsequently modified for carcinoma of the colon *(18).* Dukes' stage B lesions were characterized by extension of the tumor through the muscularis propria into the pericolic tissue without regional lymph node involvement (T3–4, N0); Dukes' stage C tumors were those having regional lymph node metastases with any depth of tumor penetration (T1–4, N1–3). Patients with tumors that extended beyond the scope of curative operative resection were ineligible for this protocol *(19).*

Patients with more than one synchronous primary colon tumor, intestinal obstruction, or direct extension of the tumor into adjacent structures were eligible, provided that the tumor could be resected *en bloc* with no residual disease. Patients were ineligible if they were pregnant, had a concomitant or previous cancer (except squamous or basal cell carcinoma of the skin or carcinoma *in situ* of the cervix), an Eastern Cooperative Oncology Group performance status worse than 2, or nonmalignant systemic disease precluding administration of the assigned therapy. Additional ineligibility criteria included a life expectancy less than 10 years or prior treatment (other than operative resection) of the colon cancer. Patients were required to have adequate renal and hepatic function as well as adequate blood cell counts.

Patients were randomly assigned by the NSABP Biostatistical Center to receive either 5-FU + LV (control arm) or 5-FU + LV + IFN (treatment arm). To ensure equal distribution of prognostic factors between treatment groups, treatment assignments were balanced by institution, sex, and lymph node status (0, 1–4, or ⩾5) by use of a biased coin minimization algorithm *(20,21).* As a consequence of random assignment, other patient characteristics were also balanced between the treatment groups (Table 1).

Thirty-nine (1.8%) of 2176 patients randomly assigned to treatment were found to be ineligible (18 on the control arm and 21 on the treatment arm). Reasons for ineligibility included the following: Dukes' stage A (two patients); Dukes' stage D (10 patients); rectal primary tumors (seven patients); either concomitant or previous cancer (four patients); tumor with free perforation (six patients); noncurative surgery, including involved adjacent structures not removed *en bloc* (five patients); involved margins (three patients); late randomization (one patient); and consent refusal (one patient). An additional eight patients were without follow-up.

Table 1. Patient entry and distribution according to selected characteristics*

Characteristic	Control arm: 5-FU + LV	Treatment arm: 5-FU + LV + IFN
No. of patients randomly assigned	1088	1088
No. ineligible	18	21
No. eligible without follow-up	1	7
No. eligible with follow-up	1069	1060
Mean time on study, mo	54	54
Age, y†		
⩽59	50.3	48.2
⩾60	49.7	51.8
Sex		
Male	55.1	55.0
Female	44.9	45.0
Race		
White	85.4	86.4
Black	8.7	7.4
Other	5.9	6.1
Unknown	0.0	0.1
Dukes' classification		
Stage B	43.8	44.4
Stage C		
1–4 positive lymph nodes	42.4	42.5
⩾5 positive lymph nodes	13.7	13.1
Unknown No. of positive lymph nodes	0.2	0.0
Location of tumor		
Left	18.8	19.2
Right	41.0	43.7
Rectosigmoid	37.5	35.0
Multiple	2.6	2.1
Unknown	0.1	0.0

*5-FU = 5-fluorouracil; LV = leucovorin; IFN = interferon alfa-2a.
†Values are percent of eligible patients with follow-up.

Chemotherapy and IFN

Patients randomly assigned to the control group received six 28-day cycles; each cycle consisted of LV (500 mg/m^2) as a 30-minute intravenous infusion daily for the first 5 days of each cycle followed by 5-FU (370 mg/m^2) given by intravenous bolus 1 hour after the completion of each daily LV infusion. This regimen was a departure from the monthly administration of 5-FU + LV used in other NSABP studies in order to comply with the NCI schedule *(13).* Patients randomly assigned to the treatment arm received the identical (5-FU + LV) therapy along with IFN (5×10^6 U/m^2) administered subcutaneously beginning 24 hours before the first dose of 5-FU + LV and then daily immediately before chemotherapy for the first 5 days of each cycle. A seventh dose of IFN was given 24 hours after the last dose of 5-FU + LV in each cycle. The sequence and intervals of the administration of IFN, LV, and 5-FU were based on previously published information *(10,22).* Recombinant IFN (Roferon-A) was supplied by Roche Laboratories, Inc., Nutley, NJ, and was distributed by the Pharmaceutical Managerial Branch of the NCI. In both the control and the treatment groups, dose reductions were initiated according to the directives of the protocol, and a new treatment cycle was not to be begun beyond 6 months, regardless of dose modifications or delays.

Follow-up and Diagnosis of Treatment Failure

Before each course of therapy, patients received a physical examination, hematologic evaluation, and renal and liver function tests. Nadir hemograms were repeated at days 15 and 21. These tests were conducted every 3 months through the 2nd year and every 6 months in the 3rd through the 5th years. The diagnosis of first treatment failure was made only when protocol-defined clinical and laboratory criteria for such an event were met. Tumor recurrence was proven by tissue examination whenever possible. Findings characterized as suspicious did not constitute sufficient criteria for treatment failure. When positive cytology or biopsy was not available for a suspected liver recurrence, any three of the following that were not associated with previously documented benign disease were taken as evidence for recurrence: recent or progressive hepatomegaly; abnormal liver contour; positive radionuclide liver scan, sonogram, or magnetic resonance or computed tomographic scan; abnormal liver function studies; and elevated carcinoembryonic antigen (CEA) level.

Radiographic evidence of lytic, blastic, or mixed lesions on plain films, with or without bone scan confirmation, was necessary for the diagnosis of skeletal metastases. A bone scan consistent with bony metastases in a patient with bone pain was regarded as positive, as were progressive bone scan changes over a 4-week period in asymptomatic patients. Histologic proof of bony metastases

was obtained whenever possible. Pulmonary metastases required either positive cytology or biopsy or the presence of multiple pulmonary nodules consistent with pulmonary metastases.

Radiologic studies were performed as required by the protocol during the first 5 years of follow-up. A chest x-ray and barium enema and/or endoscopic examination was performed every 12 months. CEA levels were determined every 6 months, and investigation of elevated CEA levels was performed at the discretion of the clinical investigator.

Follow-up forms were to be submitted for each patient on a quarterly basis for the first 2 years, on a semiannual basis for the next 3 years, and yearly thereafter. Data for this analysis are current as of June 30, 1997. The mean time on study (time from surgery to June 30, 1997) was 54 months (range, 41–70 months). Sixty-five percent of eligible patients had known 4-year disease-free survival (DFS) status; i.e., 65% either had had an event prior to 4 years or had been followed for more than 4 years; 59% of eligible patients had known 4-year survival status. These percentages were virtually identical on both treatment arms. Of the 1696 eligible patients alive at last follow-up, 90% have submitted follow-up within the past year.

Quality Control and Data Monitoring

Mechanisms were in place to monitor adjuvant therapy compliance, acute toxicity, and long-term complications of protocol therapy on an ongoing basis. Participating physicians submitted copies of the dictated surgical and pathology reports and were asked to submit blocks and slides of the surgical specimens. This information was used whenever needed to verify data provided at the time of randomization and on data entry forms. Follow-up forms were screened for consistency and, where appropriate, were reviewed by NSABP Headquarters medical review staff.

Treatment and toxicity reports were submitted after each course of therapy. An additional report evaluating toxicity was submitted 90 days after the completion of chemotherapy and thereafter only in the event of severe or unusual toxic side effects. In the event of life-threatening toxic reactions, the institution immediately notified the NSABP Biostatistical Center. Monthly toxicity reports were generated throughout the trial, and overall summaries of toxic effects and severe toxic reactions were reviewed *(23)*.

Statistical Considerations and Study Design

The study was initially designed to accrue 1545 patients. This sample size was chosen to provide a two-sided .05-level comparison of survival having power equal to 0.81 against the alternative hypothesis of a 31% reduction in mortality (85.7% versus 80.0% 4-year survival) and was based on mortality rate estimates derived from NSABP protocol C-03, an assumed accrual rate of 100 patients per month, and an assumed ineligibility rate of 3%. It was projected that a sample size of 1545 patients would result in a sufficient number of events to permit the definitive analysis 5 years after the initiation of the study. The protocol also specified semiannual interim analyses to begin after 30 deaths had been observed.

Subsequent monitoring of dose modifications and compliance to therapy raised concerns that the estimated treatment effect might be attenuated by a greater than expected dropout rate on the experimental arm. Calculations suggested that a 31% reduction in mortality rate could be attenuated to as little as a 25% reduction based on the drug delivery pattern observed up to that point in time. Accordingly, the protocol was amended on September 13, 1993, to increase the target sample size to 2108 patients. (Actual accrual was 2176 patients.) Definitive analysis was rescheduled to take place following the 388th total death in order to maintain the desired power of 81% against the attenuated mortality reduction. Results reported here are based on all data received at the NSABP Biostatistical Center as of June 30, 1997, at which point there were 433 deaths (both groups included) among eligible patients.

End-point definitions. The primary end points in the study were DFS and overall survival (OS). DFS was defined as the time from surgery to either the recurrence of colon cancer, occurrence of a second primary cancer, or death without evidence of recurrence or second primary. Deaths from all causes were considered in the analysis of OS.

Survival comparisons between treatment groups. The primary treatment comparisons regarding DFS and OS were based on the cohort of eligible patients with follow-up. Patients were analyzed as randomized, regardless of the treatment and dose actually received. Virtually identical results were obtained when ineligible patients were also included.

Curves for OS and curves for DFS were estimated by use of the Kaplan–Meier method, and statistical comparisons were made by use of the logrank test stratified by sex, stage of disease, and lymph node status. The Cox proportional hazards model was used to compute relative risks (RRs) and 95% confidence intervals (CIs), to examine the effect of prognostic variables, and to test for interactions between treatment and covariates. Treatment-by-covariate interaction terms were added one at time, and Wald tests were used to test for significance. All reported *P* values are two-sided.

Because the two treatment arms differed in terms of the percentage of patients failing to begin their assigned therapy, DFS and OS comparisons were also performed only on that cohort of eligible patients who accepted and started their assigned treatments. Similarly, because drug delivery differed as a result of increased toxicity on the treatment arm, several exploratory analyses were carried out that adjusted for differences in drug delivery between treatment and control arms in patients who accepted the assigned treatment. Cox proportional hazards models were used to compare the arms after stratification for sex, stage of disease, and number of positive lymph nodes, in which time-dependent covariates were used to account for differences in 5-FU doses. Dosages were calculated as the proportion of total dose received, relative to the total dose scheduled up to that point in time. In order to avoid bias in survival comparisons caused by the termination of protocol therapy at the time of a treatment failure, the proportion of scheduled dose received was fixed thereafter at its pre-failure level. Toxicity data are reported and summarized for all patients, regardless of eligibility status.

Results

DFS and OS Comparison

Those patients randomly assigned to receive 5-FU + LV + IFN did not have a better DFS than did those who were randomly assigned to receive 5-FU + LV (RR = 0.93; *P* = .34; 95% CI = 0.80–1.08) (Fig. 1). At 4 years of follow-up, the DFS for patients randomly assigned to receive 5-FU + LV + IFN was 70% (95% CI = 68%–73%) compared with 69% (95% CI = 66%–72%) for the group treated with 5-FU + LV. Likewise, there was no significant OS difference (RR = 0.92; *P* = .41; 95% CI = 0.76–1.11) (Fig. 2). At 4 years, OS was 81% (95% CI = 78%–83%) for patients randomly assigned to receive 5-FU + LV + IFN and 80% (95% CI = 77%–82%) for those randomly assigned to receive 5-FU + LV. Recurrence as a first event occurred in 22.4% patients on the treatment arm and in 24.0% patients on the control arm (Table 2); 19.5% of patients in the treatment group and 21.1% of those who received 5-FU +

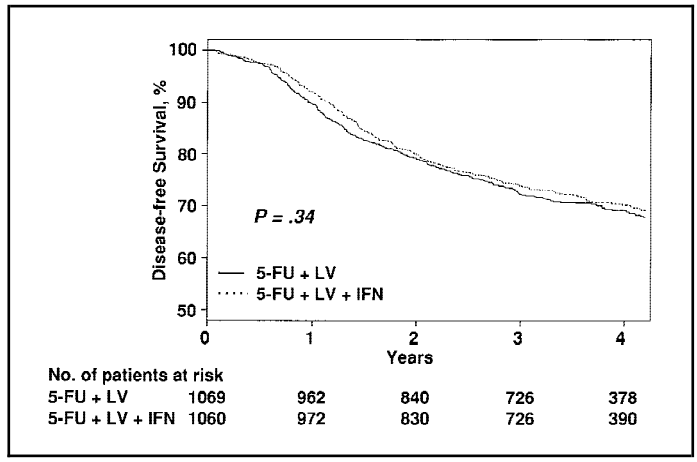

Fig. 1. Disease-free survival: 5-FU + LV versus 5-FU + LV + IFN. 5-FU = 5-fluorouracil; LV = leucovorin; IFN = interferon alfa-2a. Two-sided *P* value was determined with the use of the logrank test stratified by sex, stage of disease, and lymph node status.

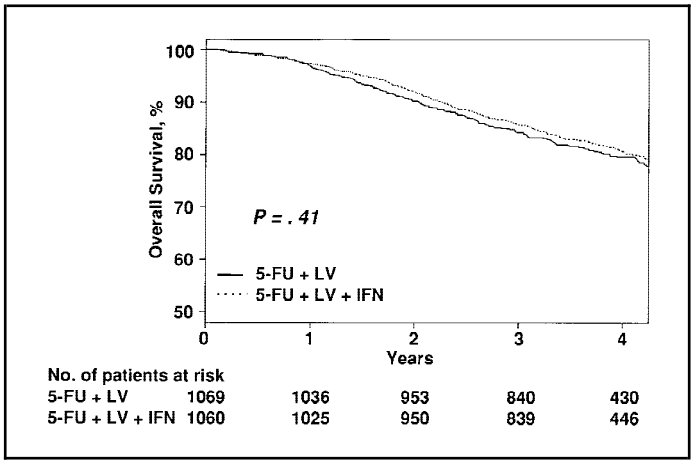

Fig. 2. Overall survival: 5-FU + LV versus 5-FU + LV + IFN. 5-FU = 5-fluorouracil; LV = leucovorin; IFN = interferon alfa-2a. Two-sided *P* value was determined with the use of the logrank test stratified by sex, stage of disease, and lymph node status.

Table 2. Summary of treatment failures and other first events for eligible patients with follow-up*

	Control arm: 5-FU + LV		Treatment arm: 5-FU + LV + IFN	
	No.	%	No.	%
Alive, no TF, or second primary tumor	730	68.3	749	70.7
TF	257	24.0	237	22.4
Alive at last follow-up	75	7.0	74	7.0
Dead	182	17.0	163	15.4
Second primary tumor	51	4.8	47	4.4
Prostate	9		12	
Colon	9		4	
Uterus	0		4	
Breast	6		6	
Lung	5		3	
Rectum	2		3	
Other site	20		15	
Alive at last follow-up	38	3.6	30	2.8
Dead	13	1.2	17	1.6
Dead, No TF, or second primary tumor	31	2.9	27	2.5
Total deaths	226	21.1	207	19.5
Eligible patients with follow-up	1069		1060	

*TF = treatment failure; 5-FU = 5-fluorouracil; LV = leucovorin; IFN = interferon alfa-2a.

LV have been reported to have died; 4.4% of patients who received 5-FU + LV + IFN developed second primary tumors as a first event, compared with 4.8% of those patients receiving 5-FU + LV alone. One patient developed leukemia in each arm of the study; there were no other blood dyscrasias reported. The distribution of secondary primary tumors as first events is shown in Table 2.

When Cox proportional hazards models were used to test for treatment-by-subset interactions, including interactions with age, sex, the number of positive lymph nodes, and tumor location, in no case did the results approach statistical significance. Cox proportional hazards models were also used to test patient and tumor characteristics in order to determine which were of prognostic significance. The number of positive lymph nodes was the most important prognostic variable for either DFS ($P<.0001$) or OS ($P<.0001$). Patients with one to four positive lymph nodes had an RR for mortality of 2.28 when compared with patients with negative lymph nodes. Patients with five or more positive lymph nodes had an RR of 6.46 when compared with patients with negative lymph nodes.

Sites of Recurrence

Two hundred fifty-seven patients randomly assigned to the control arm and 237 patients randomly assigned to the treatment arm had a recurrence as the first event (Table 3). In roughly two thirds of these patients, the recurrence was at a distant site. One hundred sixty-six patients (15.5%) randomly assigned to the control arm and 154 (14.5%) randomly assigned to the treatment arm had distant recurrence (either singly or in combination) as a first event, with the liver being the most common site. There were 6.8% of patients in the control group and 7.1% of patients in the treatment group who had treatment failure in the liver (approximately 30% of all first recurrences). As with the distant recurrences, there was no significant difference in the incidence of extrahepatic abdominal recurrences between the two treatment groups: 8.3% for 5-FU + LV and 7.6% for 5-FU + LV + IFN.

Toxicity

Information related to toxicity following treatment was obtained for 2140 patients (98%). Seventeen deaths among patients on therapy were judged to be possibly treatment related; nine of these patients were on the control arm and eight were on the treatment arm. Causes of these deaths included the following: five from sepsis, four from myocardial infarction, three from cardiac arrest, two from intracerebral hemorrhage, one from bowel obstruction, and two that were gastrointestinal related.

Major nonhematologic toxicity *(23)* occurred more frequently among patients receiving IFN. Thirty-six percent of patients in the control group had a maximum toxicity grade of 3, whereas

Table 3. Site of treatment failure among 2129 eligible patients with follow-up*

	Control arm: 5-FU + LV (1069 patients)		Treatment arm: 5-FU + LV + IFN (1060 patients)	
Site of recurrence	No.	%	No.	%
Abdominal				
Anastomotic	20	1.9	22	2.1
Peritoneal	44	4.1	34	3.2
Retroperitoneal lymph nodes	14	1.3	13	1.2
Multiple abdominal sites	11	1.0	12	1.1
Subtotal	89	8.3	81	7.6
Distant				
Liver	73	6.8	75	7.1
Lungs	33	3.1	23	2.2
Other	20	1.9	24	2.3
Multiple	7	0.7	7	0.7
Subtotal	133	12.4	129	12.2
Combination of abdominal and distant sites	33	3.1	25	2.4
Undocumented	2	0.2	2	0.2
All sites	257	24.0	237	22.4

*5-FU = 5-fluorouracil; LV = leucovorin; IFN = interferon alfa-2a.

25% had grade 4. In the treatment group, 40% had a maximum toxicity grade of 3 and 31% had grade 4. The major toxic effects are summarized in Table 4.

Gastrointestinal toxicity. Grade 3 or higher diarrhea (seven or more bowel movements per day) was experienced by 28.8% of patients randomly assigned to the control arm and by 43.2% of patients randomly assigned to the treatment arm. Grade 3 nausea (resulting in no significant oral intake) occurred in 6.3% of 5-FU + LV-treated patients as compared with 15.2% of 5-FU + LV + IFN-treated patients. Five percent of patients on the control arm and 12.2% of patients on the treatment arm had six or more episodes of vomiting over a single 24-hour period; 1.6% of patients receiving 5-FU + LV required parenteral support compared with 6.2% of 5-FU + LV + IFN-treated patients. Grade 3 or higher stomatitis occurred in 16.6% of 5-FU + LV-treated patients and 36.4% of 5-FU + LV + IFN-treated patients.

Liver toxicity. Liver function studies were obtained at day 0 of each course. The aspartate aminotransferase levels were elevated beyond 1.5 times the upper limit of normal in 7.2% of patients on the control arm compared with 12.6% of patients on the treatment arm. A similar difference was seen in the levels of alanine aminotransferase. The alkaline phosphatase level was elevated beyond 1.5 times of the upper limit of normal in about 4% of patients on either arm. The bilirubin level was greater than 2.5 times the upper limit of normal in 1% of 5-FU + LV-treated patients and in 0.6% of 5-FU + LV + IFN-treated patients. No patient was reported to have liver enzyme or serum bilirubin levels greater than 10 times the upper limit of normal.

Hematologic toxicity. Less hematologic toxicity was reported in patients on the treatment arm than on the control arm. In particular, 31.2% of control patients had grade 3 or higher nadir granulocytopenia as compared with 12.7% of the patients on the treatment arm.

Fever of any grade was reported in 14.5% of assigned patients on the control arm and in 49.8% of the patients on the treatment arm. This fever was accompanied by symptoms that were associated with flu-like syndrome (arthralgia, myalgia, abdominal pain, chills, and rigor) in 3.9% of control patients and 17.3% of patients receiving 5-FU + LV + IFN; grade 3 or higher fever was seen in 0.7% and 1.8% of patients on the control and treatment arms, respectively. Asthenia of any grade was described in 43.8% of patients on the control arm and in 54.1% of patients on the treatment arm; 4.3% of the patients on the control arm and 11.2% on the treatment arm had grade 3 asthenia. At randomization, the percentage of patients with performance status greater than or equal to 1 was similar in the two arms (control arm: 14.6%; treatment arm: 13.2%). By the beginning of the second course, 19.1% of patients on the control arm had a performance status greater than or equal to 1 as compared with 29.5% of patients on the treatment arm.

Table 4. Distribution of toxic effects (≥grade 3; greatest toxicity per patient)*

	Control arm: 5-FU + LV	Treatment arm: 5-FU + LV + IFN
No. of patients with toxicity data	1080	1060
No. of toxicity-associated deaths	9	8
Average No. of courses per patient	5.6	5.0
Type of toxicity, %		
Overall toxicity†	61.8	72.1
Granulocytopenia (nadir)	31.2	12.7
Alopecia	2.3	6.3
Nausea	6.3	15.2
Vomiting	5.0	12.2
Headache	0.5	0.8
Diarrhea	28.8	43.2
Stomatitis	16.6	36.4
Fever	0.7	1.8
Septic episode	3.5	2.5
Skin	3.2	5.7

*5-FU = 5-fluorouracil; LV = leucovorin; IFN = interferon alfa-2a.
†Overal toxicity excludes alopecia, nadir grades, and weight gain or loss.

Adequacy of Drug Delivery

Of 2176 patients, 46 did not begin all their assigned therapy. On the control arm, eight patients did not begin treatment. An additional two patients on this arm began their initial infusion of LV, but treatment was discontinued prior to receiving any 5-FU when they experienced allergic reactions. Twenty-nine patients on the treatment arm did not begin therapy. An additional four patients on the treatment arm accepted treatment with 5-FU + LV but refused IFN treatment. Three more patients on the treatment arm received their initial dose of IFN (course 1, day 0) but discontinued treatment after experiencing substantial reactions. These patients received no 5-FU + LV on protocol and no additional IFN. Of the 46 patients not beginning therapy, 35 were eligible with follow-up.

The distribution of the number of courses of 5-FU or IFN received by those patients who began their assigned therapy is shown in Table 5. More of the assigned therapy was delivered in the control arm than in the treatment arm. The proportion of patients receiving all scheduled courses of 5-FU was 88.5% in the control arm compared with 77.1% in the treatment arm; 72.9% of patients on the treatment arm received IFN up to six courses or treatment failure. In the large majority of cases, patients on the treatment arm discontinuing IFN also discontinued

Table 5. Number of courses of therapy received before discontinuation and percent of cumulative target dose received in patients beginning their assigned therapy (n = 2130)*

		Treatment arm, %	
	Control arm, %: 5-FU	5-FU	IFN
No. of courses			
1	4.0	11.4	12.8
2	1.4	5.1	6.7
3	2.1	2.9	3.8
4	1.7	2.0	2.1
5	2.3	1.4	1.7
6 or to TF	88.5	77.1	72.9
% dose received			
≤50	8.5	20.6	24.1
50.1–60.0	1.9	4.0	2.1
60.1–70.00	4.0	7.4	2.9
70.1–80.0	5.9	14.2	5.4
80.1–90.0	17.7	17.9	9.8
90.1–100	61.9	35.9	55.8
Total No. of patients	1078	1052	1052

*5-FU = 5-fluorouracil; IFN = interferon alfa-2a; TF = treatment failure.

5-FU + LV at the same time. Table 5 also shows the percentage of total 5-FU (in terms of mg/m^2) or IFN (10^6 units/m^2) received among the patients beginning their assigned therapy; 61.9% of patients on the control arm received greater than 90% of the target total of 5-FU as compared with only 35.9% of patients on the treatment arm. In addition, slightly over half (55.8%) of patients on the treatment arm received greater than 90% of the targeted total IFN dose.

On the control arm, 124 patients discontinued therapy prior to the completion of six cycles or treatment failure; 62 of the discontinuations (50.0%) were patient withdrawals due to toxic effects; 26 of the discontinuations (21.0%) were physician withdrawals due to toxic effects; 36 of the withdrawals (29.0%) were for reasons other than toxic effects. On the treatment arm, 285 patients discontinued some or all of their therapy prior to the completion of six cycles or treatment failure; 178 discontinuations (62.5%) were patient withdrawals due to toxic side effects; 67 were physician withdrawals (23.5%) due to toxic side effects; 40 of the withdrawals (14.0%) were for reasons other than toxic side effects.

Additional Analyses

Because more patients on the treatment arm refused their assigned therapy than did those on the control arm, DFS and OS comparisons were repeated after we restricted the analyses to the 2094 eligible patients with follow-up who actually began their assigned treatment, to determine whether the results of the primary analyses were influenced by this imbalance. When treatments were compared by use of the logrank test stratified for stage of disease, lymph node status, and sex, results were very similar to those that were obtained in the primary analyses: For DFS, the RR (5-FU + LV + IFN versus 5-FU + LV) was 0.93 ($P = .37$); in contrast, for OS, the RR (5-FU + LV + IFN versus 5-FU + LV) was 0.93 ($P = .43$). Inclusion of ineligible patients in these logrank tests gave almost identical results.

The difference in drug delivery between the control and treatment arms raised the possibility that the lack of differences in DFS and OS between the two arms may be due in part to the discontinuation of effective therapy in the treatment arm. To address this issue in an exploratory analysis, we compared the two arms by fitting a Cox proportional hazards model to the cohort of eligible patients with follow-up who began their assigned treatment. This model included a term representing the treatment comparison and a continuous term representing the amount of 5-FU received, expressed as a proportion of scheduled dose. To avoid bias caused by the cessation of protocol therapy following treatment failures, the 5-FU dose was modeled as a time-dependent covariate, as described in the ''Patients and Methods'' section. The model also included stratification variables representing stage of disease, lymph node status, and sex. Results were as follows: For OS, RR = 0.91 ($P = .34$); for DFS, RR = 0.94 ($P = .46$). Similar results were obtained from models in which the 5-FU dose was modeled categorically ($\leqslant$50% of scheduled dose; 51%–80%; 81%–90%; 91%–100%) and in models in which both the 5-FU and the IFN dosages were treated as continuous variables. The adjustment for drug delivery had little effect on the estimated RRs for either mortality or treatment failure.

Discussion

Our results are, to our knowledge, the first from a large, randomized clinical trial comparing 5-FU + LV and 5-FU + LV + IFN administered after surgery in patients with Dukes' B or C colon cancer. The data are consistent with the hypothesis that systemic adjuvant therapy with 5-FU + LV + IFN was not superior to treatment with 5-FU + LV alone. While these results do not preclude the possibility of some benefit, they do indicate that the benefit, if any, is likely to be small; the 95% CI for the RR of recurrence ranged from 0.80 to 1.08. Furthermore, there was little indication that IFN was of benefit in any patient subset, since there was no statistically significant interaction between the effect of treatment and age, sex, or the number of positive lymph nodes.

The outcomes seen in the control arm of this study were comparable to those reported in the 5-FU + LV arm of NSABP protocol C-04 (DFS at 4 years, 69% versus 68%; for OS, 80% versus 79%). While no firm conclusions can be drawn from these across-protocol comparisons, the demographic and disease characteristics of patients accrued to the two protocols are similar, and the results suggest that the monthly 5-FU + LV regimen used in the current study does not differ greatly in efficacy from the weekly regimen used in protocol C-04 [*(24);* Wolmark N, Rockette H, Mamounas E, Jones J, Wieand S, Wickerham L: manuscript submitted for publication].

The overall toxicity was greater in the treatment arm than in the control group. Increased frequencies of nausea, vomiting, liver dysfunction, diarrhea, stomatitis, asthenia, flu-like symptoms, and fever were seen in the treatment arm relative to the control arm. The increases in gastrointestinal toxic effects at the grade 3 and higher levels were particularly statistically significant: nausea (6.3% versus 15.2%), vomiting (5.0% versus 12.2%), diarrhea (28.8% versus 43.2%), and stomatitis (16.6% versus 36.4%). The decrease in hematologic toxicity associated with IFN remains speculative but is not inconsistent with findings from murine models in which IFN was shown to have a myeloid protective effect *(8,9).* As a consequence of overall greater toxicity, the average number of courses and cumulative doses delivered per patient were different in the two arms; the addition of IFN to the 5-FU + LV combination resulted in more frequent dosage reductions than were seen in the 5-FU + LV alone arm. Moreover, a greater proportion of patients on the treatment arm failed to begin their assigned treatment than those on the control arm (3.3% versus 0.9%). Consistent with the intention-to-treat principle, these patients were included in the primary treatment comparison, since it is generally recognized that their exclusion could significantly bias results *(25).* In order to ascertain whether this imbalance influenced conclusions, a secondary comparison of the two arms included only those patients beginning their assigned therapy. The estimated RRs derived from this comparison were nearly identical to those obtained in the primary comparison, providing assurance that the differential treatment refusal had little impact on the results.

A potential confounding influence in the interpretation of the results of this study is caused by the fact that patients accrued to the IFN arm received a lower proportion of their assigned therapy because of increased toxic effects. The question as to whether the addition of IFN to 5-FU + LV would have improved

patient OS and DFS if drug delivery rates had been equivalent on both arms is clinically and pharmacologically relevant. While additional exploratory analyses were performed to address this issue, it must be emphasized that any such method is based on assumptions that are not fully verifiable and that could lead to biased comparisons. These analyses attempted to adjust for differences in the amount of 5-FU delivered in the control and treatment arms, leading to compliance-adjusted RR estimates. The adjusted estimates turned out to be similar to the unadjusted estimates; there was little evidence of a sharp 5-FU dose–response relationship for either mortality or treatment failure rate. Similarly, models that simultaneously corrected for variations in 5-FU and IFN doses did not indicate a statistically significant benefit for the addition of IFN to the 5-FU + LV regimen. Therefore, while it remains possible that the addition of IFN to the 5-FU + LV regimen could prove effective if delivery could be accomplished with substantially less toxicity and better compliance, exploratory analyses of data from this protocol do not suggest this to be the case.

The data fail to support the hypothesis that the addition of IFN to 5-FU + LV is superior to systemic adjuvant therapy with 5-FU + LV alone. Furthermore, the addition of IFN increases overall toxicity and adversely affects patient compliance with protocol-mandated therapy.

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NOTE

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