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Combination Chemoimmunotherapy for Metastatic Colorectal Cancer Using 5-Fluorouracil, Leucovorin and Interleukin-2

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25 patients with metastatic colorectal cancer were entered into a phase II trial of combination chemoimmunotherapy using a sequential regimen of 5-fluorouracil (5-FU) and leucovorin and high-dose recombinant human interleukin-2 (rIL-2). Patients initially received three cycles of chemotherapy consisting of 500 mg/m^2 of intravenous leucovorin followed by 375 mg/m^2 of bolus 5-FU both given daily on days 1–5 of a 21 day cycle. Ten days after the last dose of chemotherapy in cycle 3, patients began high-dose rIL-2 at 720 000 IU/kg intravenously every 8 h to the maximum tolerated number of doses. After 7–10 days of recovery, this rIL-2 treatment was repeated to complete one full course of chemoimmunotherapy. There was no grade IV toxicity associated with 183 cycles of chemotherapy. Other than slight increases in the frequency of diarrhoea, stomatitis and hyperbilirubinaemia, rIL-2 toxicity was similar to that seen in patients given rIL-2 without chemotherapy. Of 23 evaluable patients, the overall response rate (partial + complete response) was 46% with 2 complete responses. Only 3 patients showed major tumour regression during the rIL-2 phase of therapy, but these 3 patients included both complete responders and the 3 most durable responses (15, 16 and 24 months). We conclude that sequential 5-FU/leucovorin and rIL-2 can be given safely without major increases in toxicity over either therapy alone, and although nearly all responses seen are largely attributable to chemotherapy, a contribution of immunotherapy to the minority of patients achieving complete or durable responses cannot be ruled out.
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INTRODUCTION

THE TREATMENT of metastatic colorectal carcinoma which is not amenable to curative resection is limited. Despite recent chemotherapy regimens which appear to have increased activity against this malignancy, none of these approaches seem capable of achieving either cures or long-term complete regressions. One current regimen which has considerable activity is a combination of 5-fluorouracil (5-FU) and leucovorin [1–3]. The addition of leucovorin to 5-FU, appears to stabilise the binding of 5-FU to its target enzyme, thymidylate synthetase and enhance efficacy without major increases in toxicity. In randomised trials, these agents have produced significantly improved response rates and in some cases, improved survival [4–6]. Despite this progress, the median survival of patients treated with this combination ranges from 9–15 months, not much different than reported in descriptions of the natural history of this disease [7].

Another modality with some activity against colorectal carcinoma is immunotherapy. Immunisation trials in the adjuvant setting have suggested that immune manipulation can impact on recurrence of colorectal cancer [8]. The utilisation of recombinant human interleukin-2 (rIL-2) in combination with other agents such as lymphokine activated killer (LAK) cells or interferon alfa (IFN-α) can lead to major regression of metastatic disease. The overall experience of the Surgery Branch, NCI with high-dose rIL-2 (with or without LAK cells) indicates that of 42 patients treated, 5 partial or complete responses were seen [9]. Although the frequency of response is too low to have a major impact on the treatment of colorectal cancer, this experience was of interest in that some of these responses were complete and of long duration. This supported the potential activity of immunotherapy in this disease, but emphasised that major improvements were necessary to develop therapies with overall survival benefit.

A possible approach to improving these results was suggested in preclinical murine models testing chemotherapy combined with rIL-2. Papa *et al.* investigated the combination of cyclophosphamide (CY) and rIL-2 in the treatment of advanced metastatic sarcoma in mice and noted that synergistic benefit resulted from this combination [10]. Although neither CY nor rIL-2 alone could cure mice of 8-day old pulmonary metastases, the combination (only when CY was given first) was able to cure the majority of mice. Investigations into the mechanism of this synergy were not conclusive [11], but one possibility is that the two differing therapeutic modalities were acting independently with complementary anti-tumour effects. Immunomodulation with chemotherapy (including elimination of suppressor cell activity) could not be demonstrated.

Following these studies, the Surgery Branch, NCI attempted to combine single-dose CY followed by rIL-2 in the treatment of malignancies known to respond to rIL-2. 2 of 16 patients with either metastatic melanoma or renal cell carcinoma (RCC) achieved responses (both partial) with this regimen [9]. A possible explanation for this low response rate was that any contribution of chemotherapy may depend on tumour destruction (rather than immune system modulation) and melanoma and RCC are not responsive to single dose CY. In order to address this possibility, a protocol was developed for the

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treatment of patients with metastatic colorectal cancer using 5-FU and leucovorin followed by high-dose rIL-2. Colorectal cancer was selected as a malignancy with some response to rIL-2 based immunotherapy and 5-FU and leucovorin was selected as an active systemic chemotherapy regimen currently available for this disease. Because the two components of this treatment have some overlapping toxicities (diarrhoea, stomatitis, hepatotoxicity, marrow suppression), it was elected to treat patients in a sequential fashion with a recovery period between chemotherapy and immunotherapy. The chemotherapy was given first based on the preclinical murine models and the hypothesis that chemotherapy might modulate a subsequent immunotherapy response.

PATIENTS AND METHODS

Patients

25 patients with measurable metastatic colorectal cancer which could not be resected with curative intent, constituted the study population. All had pathology slides reviewed and diagnoses confirmed at NCI. Patients were screened for their suitability for high-dose rIL-2 therapy and were required to have normal cardiopulmonary, hepatic, renal and haematological parameters including normal stress cardiac evaluation (for patients over 50 years old), bilirubin $\leq$ 2.0 mg/dl, SGOT (serum glutamic-oxalacetic transaminase) and SGPT (serum glutamic-pyruvic transaminase) < twice normal, normal creatinine and normal white blood cell and platelet counts. Patients with CNS metastases, requirements for glucocorticoid administration, or previous rIL-2 therapy were excluded. No patient had undergone any other therapy within 30 days of starting treatment or during the follow-up period.

Treatment

The treatment sequence is shown in Table 1. Patients started with three cycles of chemotherapy, each 21 days long with therapy given on days 1–5. Leucovorin was given intravenously each day at 500 mg/m^2, followed 1 h later with 5-FU at 375 mg/m^2. Doses were escalated (for entire cycles only) by 75 mg/m^2 for toxicity $\leq$ grade I and reduced by the same amount for toxicity $\geq$ grade III. Ten days after the last dose of chemotherapy in cycle 3, patients were admitted to the hospital to begin high dose rIL-2 (Cetus Corp., Emeryville, California) at 720 000 IU/kg given intravenously every 8 h to the maximum tolerated number of doses. After a recovery period of 7–10 days, a second cycle of rIL-2 was given in a fashion identical to the first cycle. RIL-2 treatment was discontinued for any grade IV toxicity or grade III major organ toxicity which was not correctable within 24 h.

Table 1. Treatment sequence of combination chemoimmunotherapy using 5-FU, leucovorin and RIL-2

Course 1				Course 2			
5 FU+ Leuc	5 FU+ Leuc	5 FU+ Leuc	rIL-2	5 FU+ Leuc	5 FU+ Leuc	5 FU+ Leuc	rIL-2
:	:	:	:	:	:	:	:
D_{1-5}	D_{22-26}	D_{43-47}	D_{57+}	$D_{113-117}$	$D_{134-138}$	$D_{155-159}$	D_{169+}

5 FU + Leucovorin: Leucovorin = 500 mg/m^2 intravenously once a day × 5,
5-FU = 375 mg/m^2 intravenously once a day × 5.
rIL-2: Recombinant human IL-2,
720 000 IU/kg intravenously every 8 h to maximum tolerance ($\leq$ 15 doses) with an identical repeat cycle 7–10 days later.

Patient evaluations

All patients had measurable disease. Hepatic disease was measured using magnetic resonance imaging (MRI; T1 and inversion-recovery sequences) and pulmonary disease with full lung tomograms. All patients received evaluation of their known sites of disease prior to starting treatment, immediately prior to starting the rIL-2 component of treatment and prior to restarting any subsequent chemotherapy or rIL-2 sequences in an attempt to attribute responses to the various components of this treatment combination. Disease was recorded as the sum of perpendicular diameters of all measurable lesions (unless there were many in one organ where at least the 10 most significant lesions were used as a representative sample). Standard criteria for response were used with partial responses requiring a $\geq$ 50% decrease in the sum of the product of the maximal perpendicular diameters of all lesions with no increases of $\geq$ 25% in any lesion and no new lesions. Complete responses required disappearance of all evidence of disease (including CEA elevations). Responses of less than 2 months were not included. Response durations were measured from the start of therapy. Transient progression during rIL-2 therapy was not considered relapse if further chemotherapy reattained a major response.

RESULTS

24 of 25 patients completed at least three cycles of chemotherapy and one course of rIL-2 and have a minimum of 2 months of subsequent follow-up. 1 patient withdrew without evaluation after only two cycles of chemotherapy. The characteristics of the other 24 patients are shown in Table 2. Only 1 patient had previous 5-FU (intra-peritoneal) and 13 presented with synchronous metastases. There were no treatment-related deaths in this study. 9 patients required 5-FU dose reduction within the first three cycles of chemotherapy and 3 tolerated

Table 2. Patients' characteristics

Sex	
M	13
F	11
Age	
Median	52
Range	34–63
Performance	
ECOG 0	23
ECOG 1	1
Previous therapy	
Surgery	24
Radiation	3
Chemotherapy	1
Sites of disease	
Liver	11
Lung	3
Liver, lung	5
Liver, pelvis	3
Liver, nodal	2

Table 3. Toxicity of 5-FU and leucovorin

Toxicity by cycle	Grade I	II	III	IV
Neutropenia	35	14	0	0
Stomatitis	59	23	8	0
Nausea/vomiting	21	11	2	0
Diarrhoea	38	30	2	0
Skin	7	9	0	0
Infection	2	0	0	0
Total	162	87	12	0

24 patients receiving 183 cycles of chemotherapy.

dose escalation. No grade IV toxicity was seen from chemotherapy (Table 3).

Only 1 patient required a delay in starting rIL-2 due to chemotherapy toxicity (gastrointestinal). A mean of eight doses of rIL-2 was tolerated in the first cycle and six in the second cycle (3 patients were unable to receive a second cycle). The limiting toxicities in these 45 cycles are shown in Table 4. Diarrhoea, patient refusal and elevation of bilirubin were the most common indications for stopping rIL-2. Bone marrow suppression from chemotherapy (which could have complicated rIL-2 administration) was not a major limiting toxicity during rIL-2 therapy. The first cycle of rIL-2 immediately after chemotherapy was not significantly different in toxicity from the second, more remote cycle of rIL-2. The mean number of doses for the first full course of rIL-2 (14 doses) was not significantly different from the number tolerated by patients with other malignancies concurrently undergoing their first course of identical rIL-2 therapy without chemotherapy (15 doses).

9 of these 25 patients achieved a partial response and two achieved complete responses for an overall response rate of 44%. 10 of these 11 responding patients achieved at least a partial response prior to starting immunotherapy (at the interim evaluation after three cycles of chemotherapy). Of the 11 responding patients, 3 showed significant regression during rIL-2 therapy (Fig. 1) (defined using standard criteria for partial response and compared with disease measurements taken immediately prior to starting rIL-2), one had a minor response (> 25%), 2 patients were stable and 5 showed progression (≥ 25% increase). These results for all 11 responders are graphically displayed in Fig. 2. 2 of the 3 patients showing significant tumour regression associated with rIL-2 went on to complete responses with continuing therapy. The third patient had total regression of multiple lung metastases, but retained a sub-centimetre liver lesion compatible with either tumour or haemangioma and was therefore classified as a partial responder. None of the patients responded to rIL-2 without a major response to 5-FU and leucovorin. Partial response durations were 7, 7, 8, 10, 11, 11, 12, 14 and 16 months and complete response durations were 15 and 24 months. All responding patients have now relapsed either during therapy or after discontinuing therapy. With a median potential follow-up of 17.5 months, 5 of 24 patients have died, all of their disease.

Table 4. Limiting toxicity of rIL-2

	No. of patients First cycle	Second cycle
Constitutional, pt. refusal	8	8
Hyperbilirubinaemia	4	5
Diarrhoea, emesis	6	4
Dyspnoea, pulmonary oedema	2	2
Thrombocytopenia	2	0
Confusion	1	1
Tachycardia	1	2
Gastrointestinal bleeding	1	0
Small bowel obstruction	1	0
Renal insufficiency	0	3
Hypotension	0	1
Total patients*	24	21
Both cycles combined	Median (range)	
Peak bilirubin (mg%)	3.9 (0.6–11.7)	
Nadir white blood cells ($\times 10^{-3}$/mm^3)	2.5 (0.6– 5.6)	
Nadir platelets ($\times 10^{-3}$/mm^3)	70 (37–217)	

* More than one cause may be listed per patient.

DISCUSSION

Combining agents which have different mechanisms of action has been effective in the curative therapy of several malignancies. This can result from the independent anti-tumour activity of each agent when each is effective alone and the combination is not associated with overlapping dose-limiting toxicities. In addition, some combinations can result in synergy rather than merely additive benefits. The administration of chemotherapy prior to immunotherapy in the treatment of cancer has been postulated to result in elimination of host suppressor T-cells [12], alteration of the population of tumour infiltrating immune cells [13] and redirection of circulating immune cells to sites of tumour. To study these possibilities clinically, we administered a combination of cytotoxic chemotherapy and immunotherapy in treating colorectal cancer. A sequential, alternating schedule was employed because some of the toxicities of 5-FU and rIL-2 are similar and could also prove synergistic if these agents were used simultaneously.

Pre-clinical murine studies of chemoimmunotherapy indicate that malignancies not curable by either modality alone could be cured by a combination of the two and that giving chemotherapy followed by immunotherapy was most effective. The results of this clinical trial of chemoimmunotherapy did not fulfil these expectations from murine models. Although this patient population showed a high overall response rate and survival with this treatment approach, much of this is certainly due to patient selection and good performance status. Toxicity was acceptable although some minor increases in diarrhoea, hyper-bilirubinaemia and haematological suppression may have occurred during rIL-2 due to the antecedent 5-FU. None of these patients were cured by the combination of 5-FU, leucovorin and rIL-2. Nevertheless, some observations of potential value resulted. Frequent patient evaluation showed that chemotherapy alone was responsible for the major portion of the tumour regressions seen. In view of the previous poor experience with rIL-2 therapy (without LAK cells) [9] for patients with colorectal cancer, a detrimental impact of chemotherapy on IL-2 therapy cannot be ruled out, but no augmentation of responsiveness was seen. No patient achieved a partial or complete response without a partial

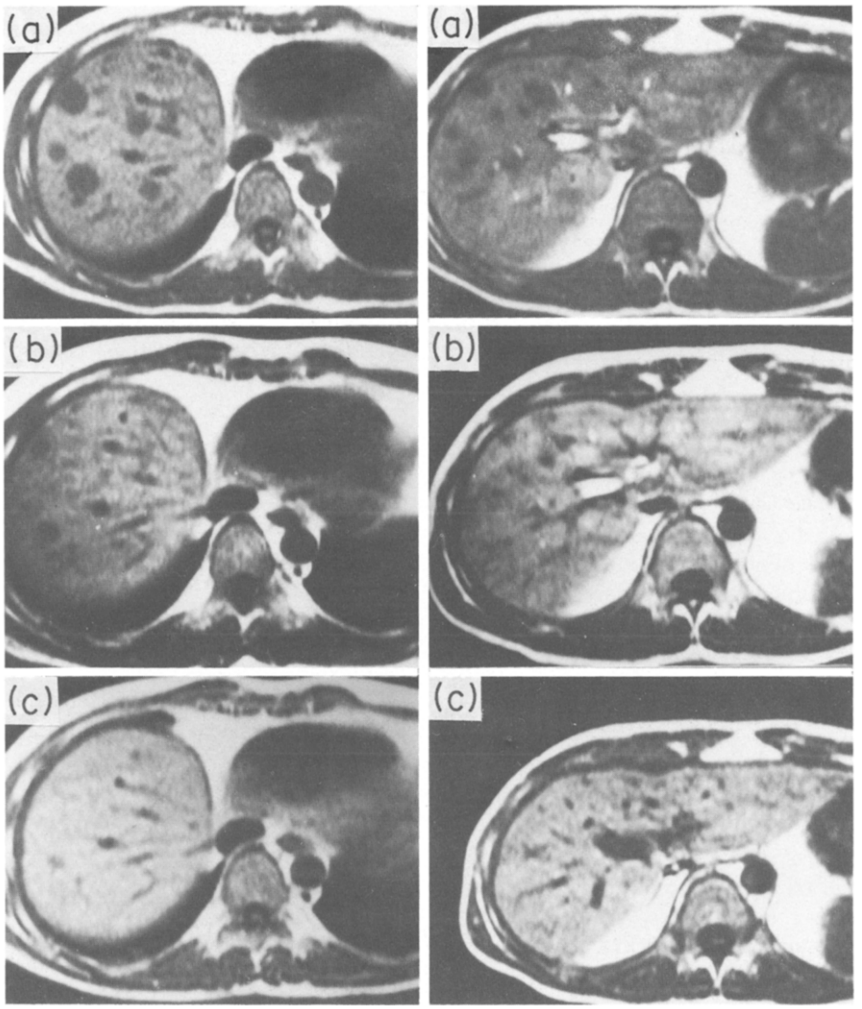

Fig. 1. MRI of liver lesions at the start of therapy (a), following 5-FU and leucovorin (b) and following rIL-2 (c). Significant regression is seen after each phase of therapy.

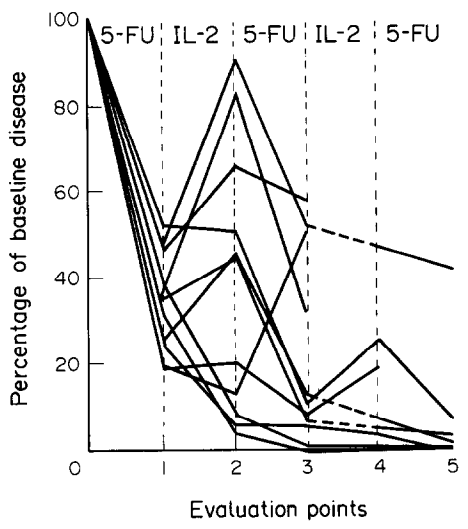

Fig. 2. Graphic representation of tumour regression in 11 responding patients. Total disease measured in cm^2 as in Patients and Methods and recorded as percentage of baseline. Phase of treatment is indicated at top of graph. Interrupted lines denote rIL-2 treatment omitted due to previous progression on rIL-2.

response to the initial chemotherapy alone. 3 responders had continued tumour regression throughout the 2 month period of immunotherapy, although the antecedent chemotherapy could potentially be responsible for these regressions seen during rIL-2 administration. Nevertheless, these 3 patients with regression during rIL-2 eventually achieved the greatest degree of overall tumour reduction and response duration (15, 16 and 24 months). If immunotherapy was contributing to the overall responses in some patients, a regimen with somewhat greater individual activity against colorectal cancer would greatly assist in confirming this contribution. In that direction, we have begun to investigate the combination of 5-FU, leucovorin, lymphokine activated killer (LAK) cells and rIL-2. In a previous experience with the rIL-2 regimen used here alone or combined with LAK cells, only the administration of rIL-2 with LAK cells resulted in major responses (17% of patients). If a favourable overall response rate is seen with this combination, and complete, durable responses achieved, then a prospective randomised evaluation of such a combination versus chemotherapy alone would be warranted in order precisely to determine the benefits of combination chemoimmunotherapy.

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Zeniplatin in Patients with Advanced Ovarian Cancer, a Phase II Study with a Third Generation Platinum Complex

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25 patients with residual or recurrent ovarian cancer were treated with the new platinum complex zeniplatin (CL 286,558) and 23 patients were evaluable for response. Responses were achieved in 4 patients, 1 complete and 3 partial remissions (16%). 7 patients had stable disease and 12 patients had tumour progression. At a median follow-up of 12 months, the median progression-free survival in responding patients was 11 months and overall survival 81%. The median overall survival of progressive patients amounted to 9 months, indicating the advanced stage of disease in most patients. Renal function was monitored by isotope clearance studies. There was no significant change in effective renal plasma flow (ERPF) or glomerular filtration rate (GFR) in 10 patients who completed six cycles of treatment. 1 patient with a marginal creatinine clearance at baseline suffered from sudden and severe renal failure during the first cycle. Zeniplatin may be active in relapsing, platinum-pretreated patients, and has no direct effects on renal function as measured by isotope clearance. Despite these findings, occasional nephrotoxicity may occur in patients with compromised kidney function, even with prophylactic hydration, and thus limit the application of this new analogue.
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INTRODUCTION

THE MAINSTAY of treatment for advanced ovarian cancer over the past 10 years has been systemic chemotherapy with cisplatin-based combinations [1–3]. Several trials have demonstrated that a two-drug schedule is equivalent in terms of response rates and survival to three or four drug combinations [4, 5]. More recently, combinations of carboplatin and cyclophosphamide have shown equivalent survival when compared with cisplatin combinations [6, 7].

The use of cisplatin-containing regimens in the treatment of advanced ovarian cancer entails significant morbidity for the patient. To prevent nefrotoxicity, prolonged prehydration and admission of the patients are required. Vigorous antiemetic regimens have to be employed to control nausea and vomiting associated with cisplatin [8]. Other problems include ototoxicity, central and peripheral neuropathy [9], hypomagnesemia and anaphylactoid reactions, which may be life threatening. The use of carboplatin carries the problem of more myelotoxicity but less neurotoxicity [6]. Clearly, a platinum analogue of equal efficacy and reduced toxicity compared with the parent com-