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Phase I clinical trial of drug–monoclonal antibody conjugates in patients with advanced colorectal carcinoma: A preliminary report

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Melphalan (MEL), an alkylating agent, has been modified to a derivative, N-acetylmelphalan (N-AcMEL), which can be conjugated to anticolon cancer monoclonal antibodies (MoAbs 30.6, I-1, and JGT) and used for immunochemotherapy. The final immunoconjugates possess potent cytotoxicity and specificity in preclinical studies. In a phase I clinical study, N-AcMEL-MoAb conjugates were administered via the hepatic artery to 10 patients, nine of whom had disseminated colorectal cancer (including the liver) and one of whom had Dukes' C colon cancer that had been resected. The selection of MoAb was based on the immunoperoxidase staining of the primary colon cancer tissue. Thus far doses of 1000 mg/m² MoAb conjugated to 20 mg/m² of N-AcMEL have been administered with no significant side effects, whereas MEL unconjugated to monoclonal antibodies would have caused myelosuppression in a proportion of patients at the same dosage. Serum antimouse antibody responses were noted in all of the patients; febrile reactions were noted with higher doses but were easily controlled with antipyretics, antihistamines and, if necessary, steroids. Serum sickness developed in one patient who was given a second course of treatment in the presence of human antimouse antibody, but the episode was self-limiting. Eight of the 10 patients had evaluable disease. Subjective improvement was noted in almost all of the patients examined, and 33%, or 3 of 9, of the treatments (nine courses of treatment in eight patients with evaluable disease; one of the patients had two courses of treatment) led to antitumor responses (minor response) by objective assessment with computed tomography of the liver. It is important to note that treatment with N-AcMEL-MoAb conjugates was safe at a dose of 20 mg/m² of N-AcMEL, whereas the efficacy of such a form of treatment remains to be determined. (SURGERY 1989;106:533-45.)

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CANCER OF THE COLON and rectum is one of the most common forms of malignancy in Western countries, with approximately 120,000 new cases reported annually in the United States.[1] Hepatic metastases are present on initial diagnosis of colorectal cancer in 25% to 30% of patients.[2] After curative resection of colorectal primary tumors, the liver is again the most frequent site of relapse in 40% to 50%.[3,4] Once hepatic metastases have developed, the prognosis is poor, with an expected median survival of 6 to 9 months,[2,5] the extent of the tumor being the most important prognostic factor.[6] Many different forms of treatment, including systemic chemotherapy, have been used for colorectal hepatic metastases, without much success.[6] The only patients who may achieve 5-year survival are the highly select group suitable for surgical resection—usually those patients with less than four hepatic metastatic lesions.[6-8] It should be recognized, however,

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Table I. Characteristics and clinical features of patients treated with N-AcMEL-MoAb conjugates

Patient	*Age (yr)*	*Sex*	*Performance status (ECOG)*	*Previous therapy*	*Dose*(μg/m²) of N-AcMEL:MoAb*
1	59	M	2	HAI of *cis*-platinum	5mg/m²:120mg/m² 10mg/m²:160mg/m²
2	61	M	2	Partial hepatectomy Adjuvant chemotherapy	10mg/m²:980mg/m²
3	57	M	2	HAI of *cis*-platinum	10mg/m²:250mg/m²
4	58	M	2		15mg/m²:340mg/m²
5	62	M	3		15mg/m²:380mg/m²
6	57	M	2		15mg/m²:500mg/m²
7	46	F	3		20mg/m²:440mg/m²
8	62	M	3		20mg/m²:1000mg/m²
9	38	F	1		20mg/m²:820mg/m²
10	64	M	3		20mg/m²:1000mg/m²

Legend: ECOG, Eastern Cooperative Oncology Group; HAI, hepatic artery infusion.
The amount (mg) of N-AcMEL conjugated to MoAb and administered was expressed as mg/m² of body surface area of the patient.

that patients suitable for resection make up a very small percentage of all patients with colorectal hepatic metastases. More recently there have been encouraging reports of response to regional perfusion with chemotherapy, especially 5-fluoro-2-deoxyuridine (FUDR); however, this is still limited by complications related to chemotherapy.[9,10]

It is with this background of unsuccessful therapeutic maneuvers that alternative therapeutic avenues with monoclonal antibodies (MoAbs) are explored. By means of the hybridoma technique,[11] murine monoclonal antibodies have been produced against almost all of the major types of human cancer.[12] However, no truly tumor-specific MoAb has been derived thus far, but in most cases the antigens recognized are present on tumors in greater concentrations than on normal tissues.[13] There are several reports of clinical response to antitumor monoclonal antibodies used alone, mostly in malignant melanoma, neuroblastoma, leukemia, and lymphoma.[14-16] However, the therapeutic effects are not dramatic, presumably because murine antibodies do not adequately incite appropriate host effector mechanisms to destroy tumors. It is therefore believed that the greatest therapeutic potential for MoAbs lies in the targeting of anticancer agents (chemotherapeutic drugs, toxins, or radioactive substances) to tumors rather than their use in unmodified form. By using a "prodrug" approach, a potent immunoconjugate was produced by covalently conjugating an inactive N-acetyl derivative of melphalan (N-AcMEL) ("prodrug") to murine MoAbs.[17] The procedure removed the ability of the melphalan to enter cells by its usual active transport via the amino acid transport systems; however, the MoAb provided the alternative route of cell entry via endocytosis, and such N-AcMEL-MoAb conjugates, on binding to tumor antigen on the tumor cell surface, exert their effects after internalization and lysosomal degradation within the target tumor cell to release melphalan.[18] The immunoconjugates have displayed in vitro and in vivo specificity and cytotoxicity and specifically inhibit the growth of human colon carcinomas xenografted in athymic mice when injected intravenously.[19]

We have described a murine MoAb 30.6 that reacted with > 90% of colon cancer tissue[20] and could preferentially localize human colorectal tumor xenograft in nude mice[21] and in primary and secondary colon carcinoma in patients.[22,23] Two additional anticarcinogenic embryonic antigen MoAbs (I-1 and JGT) had been developed, and they reacted strongly with 80% of colon carcinoma on immunoperoxidase staining.[24] Immunoconjugates of N-AcMEL to these MoAbs (30.6, I-1, JGT) have been developed by means of the same principles.[17] We now report a phase I clinical study with N-AcMEL-MoAb conjugates administered via hepatic artery infusion in 10 patients: nine with disseminated (including liver) colorectal cancer and one with resection of Dukes' C colon carcinoma.

MATERIAL AND METHODS

Patients. Ten patients with advanced colorectal carcinoma were included in this study. They were estimated to have at least a 3-month expected survival, a performance status (Eastern Cooperative Oncology Group) less than or equal to 3, and had no other cytotoxic therapy for at least 1 month before administration of immunoconjugate and during the 3-month evaluation phase of the study. Table I summarizes the characteristics and clinical features of the patients. Ages ranged from 38 years to 64 years. Nine of 10

patients had extensive hepatic metastases from colorectal carcinoma, and the remaining patient had a locally advanced colon cancer (Dukes' C) that had been resected and did not have demonstrable hepatic metastases by laparotomy or computed tomography (CT). Two of nine patients with hepatic metastases also had pulmonary metastases, and one of nine patients had a primary colon carcinoma that had not been resected because of the poor general medical condition of the patient. Two of the nine patients previously had failed intensive chemotherapy (hepatic artery infusion of *cis*-platinum), and one of these two patients had two courses of immunoconjugates separated by a 2-month interval. One of the nine patients had recurrent hepatic metastases after a previous partial hepatectomy and adjuvant 5-fluorouracil. All patients (except patient 9) had progressive metastatic disease at the time they entered the study, and the hepatic metastases were too extensive for hepatic resection. All patients were followed for at least 3 months after therapy (except patient 7 who died 4 weeks after therapy with a generalized debility and patient 10—see below); they were evaluated at weekly intervals for 6 weeks, then monthly. This phase I study was approved by the Medical Research Board of the Royal Melbourne Hospital, and written informed consent was obtained from all patients.

Monoclonal antibodies. Murine MoAbs used were IgG2b antibody 30.6, directed against an antigen present on human colon secretory epithelium but also reactive against a number of colon carcinoma cell lines,[20] and IgG1 antibodies I-1 and JGT (both anticarcinoembryonic antigen), which were produced by means of a novel immunization technique with whole serum of patients with advanced colorectal cancer; they reacted with human colon carcinoma, malignant tumors of noncolonic origin (breast, thyroid), and a number of colon carcinoma cell lines but not with normal tissue or benign lesions (24, unpublished observations). The antibodies were purified on protein A-superose (Pharmacia, Inc., Piscataway, N.J.). After elution, MoAbs were concentrated by 45% ammonium sulfate precipitation, dialyzed against phosphate-buffered saline (PBS), aliquoted, and stored at −70° C. The concentration of IgG was estimated by absorbance at 280 nm. The prepared antibodies were retested for activity after all procedures (see below), filtered through a 0.22 μm Millex-GV filter (Millipore, Befored, Ann Arbor, Mich.), and batch tested for purity by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

Preparation of N-AcMEL-IgG conjugates. The MoAbs used included 30.6 (IgG2b), I-1, and JGT (IgG1). The N-acetyl derivative of melphalan was prepared and conjugated to whole IgG.[17] Briefly, MEL was acetylated with acetic anhydride and an active ester of this N-AcMEL derivative was then coupled to the amino groups of the MoAb. Any precipitated protein was removed by centrifugation, and free N-AcMEL was removed by gel filtration chromatography with a Sephadex G-25 column (PD-10; Pharmacia). N-AcMEL incorporated in the drug-MoAb conjugates was determined by absorbance spectrophotometry at 258 nm ($E_{258} = 1 \times 10^4 M^{-1}cm^{-1}$) after subtracting the protein contribution following its estimation by the Bradford dye-binding assay.[25] The alkylating activity of the conjugate was determined by a modification of the method of Epstein et al.[26] The final preparation after drug conjugation was batch tested for pyrogens and sterility (Department of Pharmacology, University of Melbourne, and Sigma Pharmaceuticals, Clayton, Victoria, Australia).

The antibody activity of N-AcMEL-IgG conjugates was demonstrated in a rosetting assay[27] and in immunoperoxidase staining with formalin-fixed (N-AcMEL-I-1, N-AcMEL-JGT) and snap-frozen (N-AcMEL-30.6) sections of human colon cancer tissue (data not shown).

Administration of drug-MoAb conjugates. By means of the Seldinger technique, the catheter was introduced percutaneously into the left axillary or high brachial artery. The catheter was placed in the common hepatic artery, and when multiple hepatic arteries were found supplying the liver, the catheter was placed in the largest vessel. The immunoconjugate was administered via hepatic artery infusion with an oxymetric pump in 100 ml of normal saline solution for 2 hours per day for 2 days. All patients had three doses of the immunoconjugates (t = 0, t = 24 hours, t = 48 hours). Between infusions of the immunoconjugates, the patency of the catheter was accomplished with heparinized saline solution (5000 IU aqueous heparin in 1 L normal saline solution at the rate of 50 ml/hr) with the oxymetric pump. At the end of the 2-day infusion period, the indwelling catheter was removed. Patients were given dexamethasone, 8 mg intravenously, just before each infusion of immunoconjugates and oral prednisolone, 10 mg daily for 7 days after completion of infusion as prophylaxis for allergic reactions. The dose escalation protocols used (Table I) were as follows: one patient received 5 mg/m^2 and 2 months later, 10 mg/m^2; two received 10 mg/m^2; three received 15 mg/m^2; and four received 20 mg/m^2 N-AcMEL conjugated to MoAbs. The study was closed at the 20 mg/m^2 dose of N-AcMEL conjugates because of the cost incurred in producing such a large quantity of

Table II. Binding of MoAb (30.6, I-1, JGT) as detected by immunoperoxidase staining on primary colon cancer

Patient	*Colon cancer tissue*	*Staining grade**			*MoAbs used in immunoconjugates*
		30.6†	*I-1*	*JGT*	
1‡	Fixed		4	3	I-1 I-1, JGT
2	Fixed		4	4	30.6, I-1, JGT
3	Fixed		4	2	I-1
4	Fresh/fixed	3	4	4	30.6, I-1, JGT
5	Fixed		3	3	I-1
6	Fixed		3	4	I-1, JGT
7	Fixed		3	3	30.6
8	Fresh/fixed	3	4	4	30.6, I-1, JGT
9	Fresh/fixed	2	4	4	I-1, JGT
10	Fixed		3	4	I-1, JGT

*Staining score was graded based on the proportion of carcinoma cells stained: 0 = no staining; 1 = up to 25%; 2 = 26% to 50%; 3 = 51% to 75%; 4 = 76% to 100%.

†30.6 MoAb tested on fresh colon cancer tissue only.

‡Patient 1 had two courses of treatment.

antibodies and the concern that the maximum tolerated dose of such a form of treatment may not be practicably achieved (see below).

Patients were monitored clinically for changes in temperature, pulse, blood pressure, and respiratory function during and after the infusion. Blood studies were also done before, during, and weekly for 6 weeks after the therapy to assess potential hematologic (full blood examination), renal (urea and electrolytes), or hepatic toxicity (liver function test) and to detect human immune responses stimulated by murine immunoglobulin (human antimouse antibody).

Human antimouse antibody response. Human antibodies against the murine MoAbs were measured by an enzyme-linked immunosorbent assay (ELISA) modified from that previously described.[28] Ninety-six well flexible polyvinyl chloride plates (Costar, Cambridge, Mass.) were coated with 50 μl/well of administered MoAb (5 μg/ml of purified 30.6, I-1, or JGT MoAbs) in a 0.1M carbonate buffer, pH 9.6, and nonspecific binding blocked with 1% bovine serum albumin/PBS, pH 7.6. Serial dilutions of patient sera and pooled normal human serum (50 μl/well) in PBS/0.05% Tween 20 to a final dilution of 1/256 were performed and added to the coated wells (50 μl/well). Plates were then washed with PBS/0.05% Tween 20 and then reacted with 50 μl/well of phosphatase labeled affinity purified goat antihuman IgM and IgG (Kirkegaard and Parry, Gaithersburg, Md.). The color reaction was developed with alkaline phosphatase substrate and read with an ELISA plate reader (Titretek, Multiscan, MC) at a wavelength of 405 nm. Results were expressed as the absorbance value of patient serum compared with pooled normal human serum, and a positive test result was considered to be a value at least twice the control.

Immunoperoxidase staining. Immunoperoxidase staining was performed[20] on 6 μm tissue sections of colon cancer tissue from all patients with I-1 and JGT MoAbs; if possible staining was also performed with 30.6 MoAb. The 30.6 MoAb only reacts with snap-frozen but not formalin-fixed colon cancer tissue, whereas I-1 and JGT MoAbs react with both snap-frozen and formalin-fixed sections. A nonreactive control antibody was used in all cases. The sections were then assessed by light microscopy to estimate the percentage of colon carcinoma cells stained with each of the antibodies; results were expressed on a scale of 0 to 4 according to whether nil (0), up to 25% (1), 26% to 50% (2), 51% to 75% (3), or >75% (4) of carcinoma cells stained. This is a semiquantitative assay and is highly reproducible.[29] The intensity of stain, the distribution of stain in the cancer cells, and the staining of extracellular material were not taken into account. The MoAbs selected for use in drug conjugation for an individual patient had to have a staining score of 3 or 4.

Evaluation of tumor responses. Patients were evaluated clinically and biochemically (liver function test, carcinoembryonic antigen [CEA] level), and the measurable lesions were measured at 1 and 2 months after therapy by CT scans of the abdomen performed with the same technique by the same radiographers and radiologists as that used for the pretherapy evalu-

ation (performed within 2 weeks before therapy). Complete response is defined as the disappearance of all evidence of tumor. Partial response is defined as a reduction of at least 50% in the sum of the products of the two greatest diameters of measured lesions. Minor response is a reduction of more than 25% but less than 50% in the size of measurable tumors in the absence of progression or occurrence of new lesions elsewhere. Stable disease is an objective regression of measurable lesions less than that required to meet the criteria for minor or partial response or an increase of less than 25% in the size of one or more measurable lesions for at least 4 weeks. Progressive disease is the appearance of new lesions or increase in size of one or more measurable lesions by at least 25%.

RESULTS

Immunohistochemical testing on primary colon cancer. A selection of MoAbs for conjugation with N-AcMEL was made for each patient, based on the binding of the particular MoAb (30.6, I-1, JGT) to sections of the primary colon cancer by immunoperoxidase staining (Table II). In general, MoAb was selected only if it had a staining score of 3 or 4. When multiple antibodies were used for drug conjugation, the final preparation of the immunoconjugates had equal proportions of the MoAbs. A combination of at least two MoAb conjugates (N-AcMEL-30.6, N-AcMEL-I-1, N-AcMEL-JGT) was used in 7 of 11 treatments (Table II). It was considered that the use of a combination of MoAb conjugates would ensure maximal immunoreactivity and help to overcome the potential problem of tumor heterogeneity within and between tumor masses. We were unable to obtain tissue from the liver metastases itself for immunohistochemical testing before treatment.

Toxicity. Tables III and IV summarize the effects of hepatic artery infusion of N-AcMEL-MoAb conjugates. In general, the therapy was well tolerated with no disturbance in gastrointestinal, renal, or cardiac parameters, and there was no evidence of myelosuppression. There was neutrophilia during the time of treatment, which restabilized after completion of treatment. The external arterial catheter was well tolerated with no complications, and all patients maintained good mobility for the duration of therapy. Patient 1 had two courses of therapy separated by a 2-month interval, despite the presence of a high titer of human antimouse antibody (see below). During the second course of therapy he had pain in the lower back, fever (39° C), urticaria, and bronchospasm. These reactions occurred about 1 hour after immunoconjugate infusion was begun on the second day of the second course of therapy, despite prior administration of dexamethasone; treatment was required with antihistamine and an additional dose of dexamethasone. The bronchospasm, urticaria, and pain rapidly resolved with such additional measures, but the fever persisted for another 4 hours after the completion of infusion of immunoconjugates. There was, however, no reaction when further infusion of immunoconjugates was given. In four patients (patients 7, 8, 9, and 10) a temperature of 38 to 38.5° C was noted during the second and third day of antibody infusion, starting about 1 hour after the infusion was begun and continuing for 1 hour after the infusion had ended. It therefore appears that febrile reactions were more common in patients receiving higher doses of N-AcMEL-MoAb conjugates.

Table III. Toxicities

Parameters	*No. of patients*
Pain	1
Febrile >38° C	5
Allergic phenomena (urticaria, bronchospasm)	1
Hematologic	
White cell count <4000/mm^3	0
Platelets <100,000/mm^3	0
Gastrointestinal	
Nausea/vomiting/ dyspepsia	0
Diarrhea	1
Bilirubin elevation >50%	0
AST/ALT elevation >50%	2
Alkaline phosphatase elevation >25%	0
Renal	
Urea elevation >25%	0
Creatinine elevation >25%	0
Proteinuria/hematuria	0
Cardiac	
Rhythm changes	0
Rate <60 or >110/min	0
Diastolic blood pressure elevation >30%	0
Catheter-related, (thromboembolism, hemorrhage, displacement, intimal tears)	0

Legend: AST, Aspartate transaminase; ALT, alanine transaminase.

Table IV. Results of hepatic artery infusion of N-AcMEL conjugates

Patient	*Known disease sites*	*Serum CEA* level (ng/ml) relative to therapy*			*HAMA†*	*Response by CT scan of liver*	*Response duration (mo)*	*Status (time from treatment)*	*Survival from diagnosis of liver metastases (mo)*
		Before	*After (4 wk)*	*Alteration (%)*					
1	Hepatic metastases	3920 1,160	920 1,050	77 9	5.0:1.0 6.2:1.0	MR SD	3 2	Deceased (12 mo)	24
2	Hepatic metastases	200	221	10	3.2:1.0	SD	3	Alive with disease (9 mo)	17
3	Hepatic and pulmonary metastases	26	15	42	3.0:1.0	MR	12	Alive with disease (12 mo)	9
4	Hepatic and pulmonary metastases	270	50	81	2.4:1.0	SD	6	Alive with disease (10 mo)	22
5	Hepatic metastases	5,425	4,300	21	5.0:1.0	PD	—	Deceased (6 mo)	12
6	Hepatic metastases	32	12	38	4.6:1.0	MR	6	Alive with disease (6 mo)	8
7	Hepatic metastases	62	144	132	4.1:1.0	PD	—	Deceased (5 mo)	6
8	Hepatic metastases and unresected primary colon carcinoma	325	42	87	4.6:1.0	—	—	Deceased (1 mo)	3
9	Resected Dukes' C	<1	<1	0	2.0:1.0	—	—	Alive with disease (10 mo)	11
10	Hepatic metastases	1,850	2,000	8	4.0:1.0	SD	1	Alive with disease (6 wk)	4

Legend: MR, Minor response; SD, stable disease; PD, progressive disease.
*Carcinoembryonic antigen (normal <5 ng/ml).
†Human antimouse antibody (IgG) response at 4 weeks after treatment expressed as the ratio of absorbance value of patient serum compared with pooled normal human serum; the higher the ratio, the higher the HAMA response. A positive test result was considered to be a value ≥2.0:1.0.

Patient 1 also had mild diffuse arthralgia and fever that developed 11 days after completion of the second course of therapy; both slowly resolved over 2 months. Patient 8, who had unresected primary colon carcinoma and multiple hepatic metastases, died 4 weeks after receiving 20 mg/m^2 N-AcMEL conjugated to 1000 mg/m^2-MoAb of general debilitating disease. There was worsening of diarrhea that started 2 weeks after the treatment was given; however, the patient had unresected advanced colon cancer causing subacute bowel obstruction, which could have accounted for the diarrhea. Patients 7 and 10 had transient increases in the aspartate transaminase level by 70% (from 71 IU/L to 120 IU/L) and 100% (40 IU/L to 81 IU/L), respectively, during treatment, which rapidly returned to pretreatment levels within 3 days of completion of treatment.

All patients had human antimouse antibody with a raised level (absorbance value twice normal) as from the twelfth day after the first antibody exposure and was of IgM as well as IgG response. The peak response usually occurred within 30 days after therapy. The geometric means of the human antimouse antibody titer were not higher in patients receiving higher doses of MoAbs.

Antitumor effects. Table IV summarizes the tumor responses evaluated by CT scan in patients treated with N-AcMEL-MoAb conjugates. Minor antitumor responses were seen in three patients (patients 1, 3, and 6).

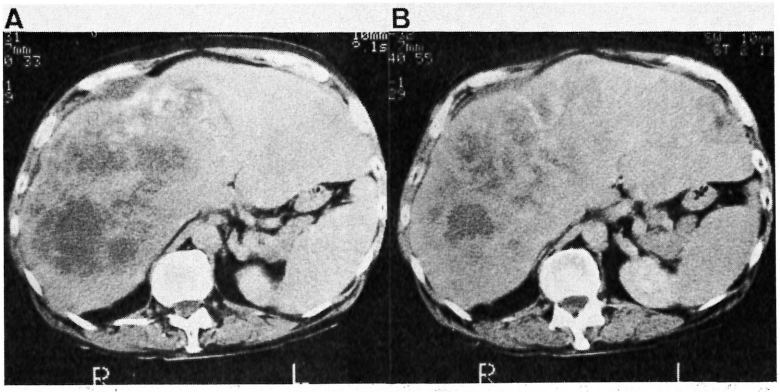

Fig. 1. CT scans of patient 1 with multiple hepatic metastases before treatment **(A)** and 1 month after treatment **(B)**.

Patient 1 had subtotal colectomy 24 months previously for synchronous carcinoma of the transverse and sigmoid colon. At laparotomy multiple large metastases in the liver were noted. The hepatic metastases were too extensive for surgical resection and were treated with two courses of hepatic artery infusion of *cis*-platinum with no response. The clinical condition continued to deteriorate over the following 10 months, with anorexia, nausea, lethargy, and severe hepatic pain. Results of liver function tests were grossly deranged with elevated transaminase (AST = 136 IU/L) and CEA (3920 ng/ml) levels. The abdominal CT scan showed multiple large metastases in both lobes occupying about 60% of the liver. Within 3 weeks after treatment with N-AcMEL-MoAB conjugates, there was a dramatic improvement in his constitution; he regained his appetite and had good relief of the hepatic pain; he felt so well that he returned to work as a headmaster. There was an improvement in liver function (AST = 60 IU/L), a decrease in the CEA level (CEA = 920 ng/ml), and an improvement in the abdominal CT scan with a reduction in the size of the hepatic metastases (25%) (Fig. 1). A repeat abdominal CT scan performed 5 months after treatment showed that the hepatic metastases had not progressed since treatment. The patient subsequently died 12 months after treatment of progression of disease.

Patient 2 had a previous left-sided hemicolectomy for carcinoma of the colon, partial hepatectomy for hepatic metastases, and adjuvant 5-fluorouracil some 18 months earlier. He subsequently had recurrent hepatic pain from recurrent hepatic metastases, which did not respond to radiotherapy, and the CEA level was rapidly rising when he entered the study. There was a subjective improvement in his general well-being with improved appetite, the CEA level remained stable after treatment, and the CT scan performed up to 3 months after treatment showed that the lesions in the liver had remained unchanged in size.

Patient 3 had hepatic and pulmonary metastases with a pretherapy CEA level of 26 ng/ml; he was lethargic and was losing weight. Within 2 weeks of treatment he became more energetic, regained his appetite and weight, and the CEA level fell to 15 ng/ml. The CT scan of the liver performed 1 month after treatment showed increased calcification in parts of the hepatic metastases as evaluated by CT scan, although the CT scan sections were not exactly comparable. Another CT scan performed 2 months after therapy showed a further increase in areas of calcification in the hepatic metastases. By that stage, although the reduction of the area of the lesions was still <50%, calcification had replaced most (50%) of the remaining hepatic metastases (Fig. 2). The pulmonary metastases remained unchanged in size for 7 months.

Patient 4 is alive with disease (hepatic and pulmonary metastases) that has remained stable since treatment (6 months), and the CEA level has been falling gradually since treatment from a pretreatment value of 270 mg/ml to 50 mg/ml 4 weeks after treatment. Patient 5 had rapidly progressive disease when entered into the study and was previously treated with *cis*-platinum via hepatic artery infusion with no response. There was a transient (6 weeks) improvement in the constitutional symptoms. Abdominal CT scan repeated 1 month after the treatment showed some insignificant regression of the extensive hepatic metastases. Both the liver function test results and CEA level remained unchanged for 3 months. Six weeks after treatment patient 5 had clinical evidence of progression of disease

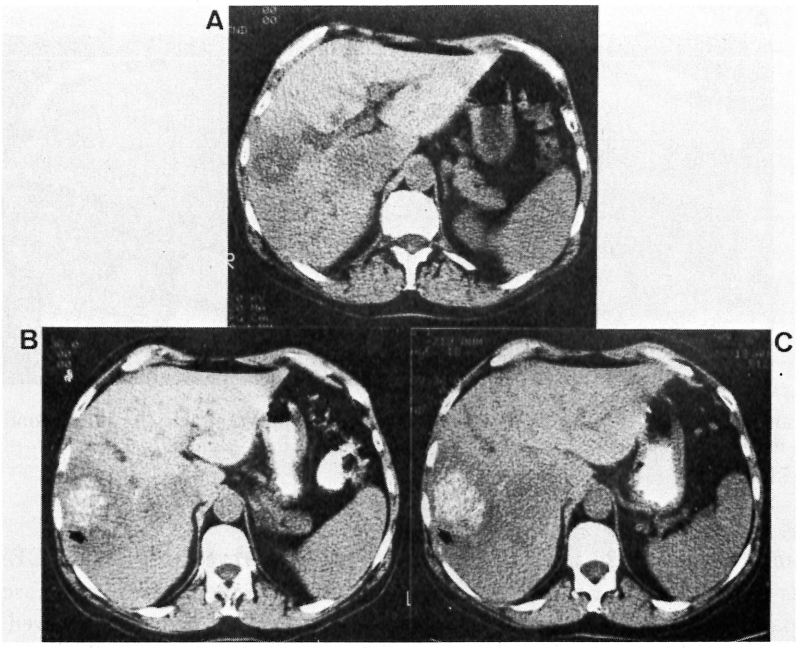

Fig. 2. CT scans of patient 3 with hepatic metastases before treatment **(A)** and 1 month **(B)** and 2 months **(C)** after treatment. Presence of increased calcification is indicated by *arrow*.

with increasing hepatomegaly and development of ascites.

Patient 6 had an anterior resection of the rectum because of rectal carcinoma and was found to have multiple bilobar hepatic metastases at laparotomy. An uneventful recovery from surgery was made, although there were complaints of frequent discomfort over the right upper quadrant of the abdomen. Within 4 weeks of treatment with the immunoconjugates, the abdominal pain disappeared and he returned to work as an engineer. The CT scan performed 1 month after treatment showed complete disappearance of two of the metastatic lesions (Fig. 3), although the overall reduction in tumor size only qualified as a minor response (>25% but <50%). However, one of the lesions (Fig. 3, *A*) was small and could have been missed in between slices of the CT scan study. There was a slight decrease in the CEA level from a pretreatment level of 32 ng/ml to 12 ng/ml 4 weeks after treatment.

Patient 7 had rapidly progressive disease (hepatic metastases) when entered into the study. There was a transient improvement in the general well-being with a notable reduction of nausea. An abdominal CT scan performed 4 weeks after treatment showed no significant change, and soon there was obvious clinical evidence of disease progression with worsening hepatic pain, increasing hepatomegaly, and a rising CEA level.

Patient 8 had widespread bilobar hepatic metastases from a locally advanced colonic carcinoma that was not resected because of the patients poor general medical condition. After treatment with immunoconjugates via hepatic artery infusion, there was a transient improvement in the general well-being and there was a significant fall in the CEA level (325 ng/ml to 42 ng/ml), but he rapidly deteriorated with fluid and electrolyte imbalance as a result of worsened diarrhea, which has already been discussed, and also from progression of generalized disease. He died 4 weeks after treatment before a repeat CT scan could be performed to evaluate the hepatic metastases. The response of the hepatic metastases to treatment was therefore not evaluable in this patient.

Patient 9 had a locally advanced colon cancer (Dukes' C) that was completely resected. She had no evidence of hepatic metastases on preoperative CT scan of the liver or at laparotomy. The CEA level had remained normal (<1 mg/ml). She was entered into the study to have the treatment in the form of adjuvant. She remained well and free of clinical recurrent disease until 7 months after treatment when three metastatic deposits in the liver were noted on CT scan. She underwent right hepatic lobectomy and the metastatic lesions were confirmed histologically. Immunoperoxidase staining of the metastatic lesions showed a poorer reactivity with the administered antibodies when com-

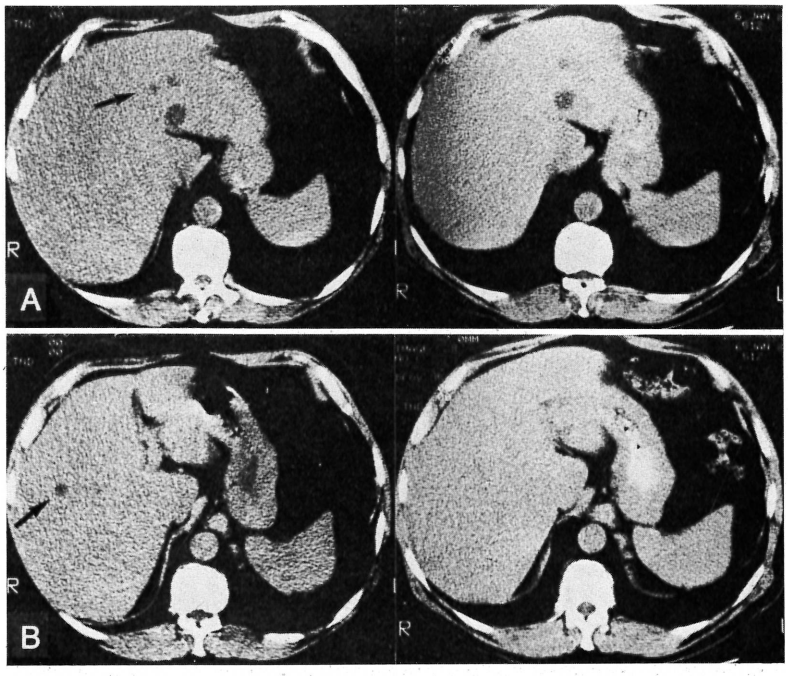

Fig. 3. Two representative CT scan sections of patient 6 with multiple hepatic metastases before treatment **(A)** and 1 month after treatment **(B).** Complete disappearance of two small metastatic deposits is indicated by *arrows.*

pared with the primary colon carcinoma tissues (Fig. 4). This illustrates the problem of tumor heterogeneity between primary tumor and its metastases.

Patient 10 had metachronous colonic carcinomas 15 years apart, and both were successfully resected. At the time the second colonic carcinoma was first seen, multiple large metastatic deposits in the liver were noted, and they increased in size rapidly over a 6-week period before treatment with monoclonal antibody conjugates. After treatment there was an improvement in the general well-being, and results of follow-up CT scan of the abdomen performed 1 month after treatment showed no further increase in the size of the metastatic deposits.

CEA levels were monitored in all of the patients before and weekly for 6 weeks after therapy. There was no significant decrease in CEA levels after therapy except in patients 1, 3, 4, 6, and 8 as discussed previously. It is of interest that despite the fact that the two MoAbs used were anti-CEA (I-1 and JGT), the CEA levels rose transiently in some patients (patients 1 and 2) at the time of treatment before stabilization after completion of therapy.

DISCUSSION

N-AcMEL-MoAb conjugates were administered via hepatic artery infusion in a phase I clinical study of 10 patients; 9 of 10 had metastatic colorectal cancer (to various sites including the liver) and 1 of 10 had a Dukes' C colon cancer that had been resected. The treatment was generally well tolerated with febrile reactions in some patients who had higher doses of the immunoconjugates. All the patients had human antimouse antibodies of both IgM and IgG classes. The immune response was still detectable at 3 months after treatments (except in patient 10 who only had the treatment 6 weeks ago), but it did not increase with the dose of MoAb received. In addition there is no obvious relationship between clinical response as evaluated by CT scan or CEA level and the levels of the human antimouse antibody response in this study. It appears that the development of human antimouse antibody response can restrict repetitive dosing as shown in patient 1 in the study who had fever, urticaria, back pain, and bronchospasm at the time of the second infusion and serum sickness 11 days after the second course of treatment.

The low incidence of toxicity with high-dose (up to 1000 mg/m^2) MoAb administration in this study is in accordance with those reported elsewhere.[14,16] In addition, there was no complication related to melphalan itself, presumably because of selective targeting of the cytotoxic agent to carcinoma cells. None of the patients had any disturbance in hematologic, gastrointestinal,

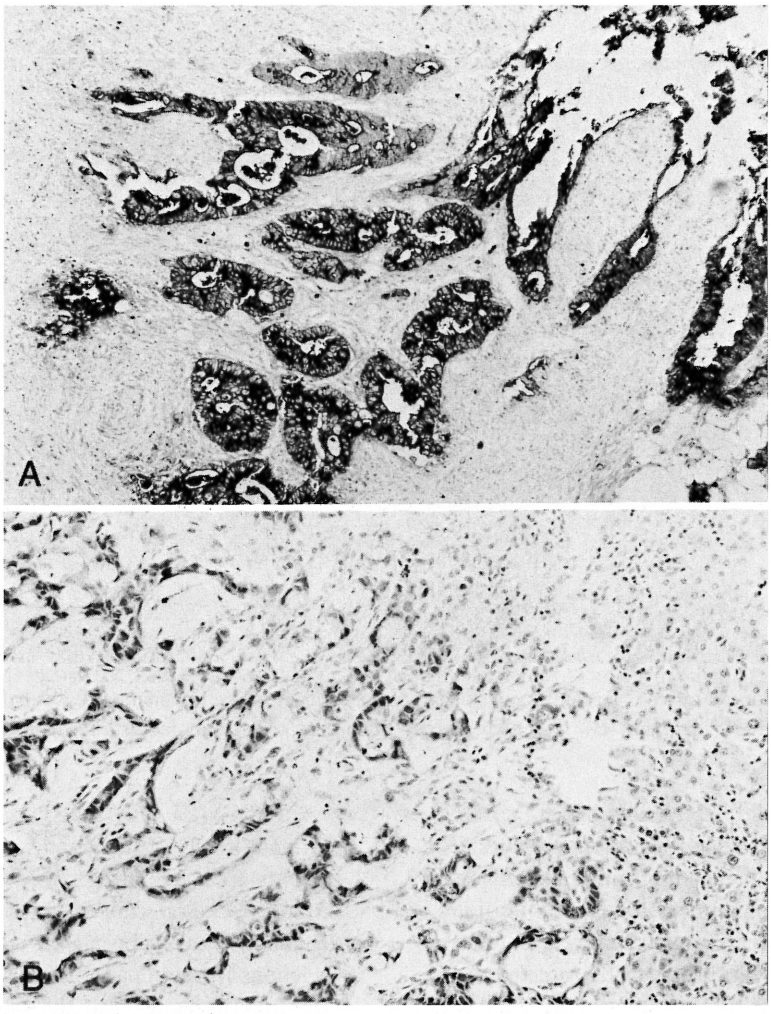

Fig. 4. Immunoperoxidase staining of primary colon cancer tissue **(A)** and hepatic metastases **(B)** with I-1 antibody. (Counterstained with hematoxylin. Original magnification ×100.) Staining pattern with JGT antibody was very similar.

renal, or cardiac parameters at a dose as high as 20 mg/m^2 of N-AcMEL, this dose being the approximate maximum tolerated dose for melphalan.[30] One of the patients had worsening of diarrhea, but it was uncertain whether this was caused by the treatment or was related to the unresected advanced colon cancer causing subacute bowel obstruction. There was also no complication related to the arterial catheter.

Of the 10 patients treated, only eight had evaluable disease status, since one patient had Dukes' C colon cancer with no hepatic metastases and one died before follow-up CT scan. Of the eight patients (nine courses of treatment), two had a minor response for 3 months and another for 7 months. All three of these patients had sustained clinical improvement with marginal decreases in circulating CEA levels and minor responses of the hepatic metastases based on CT findings. Although these findings do not meet the standard oncologic definition of an objective response,[31] they are noteworthy and are presented as evidence of a biological effect. On four occasions the treatment led to stabilization of the disease for a duration varying from 1 to 6 months. Six of the nine patients with disseminated colorectal cancer are still alive with disease, and the mean interval of survival from the time of treatment was 7.3 months, whereas the mean interval from the time of diagnosis of hepatic metastases was 11.6 months.

The response rate in this phase I study was three of nine or 33% but only if minimal response is an

acceptable grading to use; none of the responses fulfill the conventional oncologic criteria of complete or partial responses. By contrast the best response rates obtained with systemic chemotherapy used alone (5-fluorouracil, FUDR) are about 14% to 20%, although the response criteria are not strictly comparable; there is also an accompanying morbidity with chemotherapy.[32] A method for increasing the response rate with chemotherapy is to use hepatic artery infusion on the grounds that hepatic metastases derive their blood supply primarily from the hepatic artery,[33] but this is somewhat controversial.[34] Although regional perfusion with 5-fluorouracil or FUDR appears to have advantages over systemic chemotherapy, direct comparisons between studies are difficult because of variations in protocols, accrual of patients, and response criteria.[6] In addition, the complications related to chemotherapy given in regional perfusion still occur. However, the survival patterns can be compared with the accumulated knowledge of the natural history, giving some clarity as to the benefits or limitations experienced thus far.

It is possible that the pharmacologic benefit of improved hepatic extraction, as in hepatic artery infusion of chemotherapeutic agent alone, may not be applicable to immunoconjugates, the action of which depends on antigen-antibody binding. Nonetheless, the N-AcMEL-MoAb conjugates were given by hepatic artery infusion in this study to expose the hepatic metastases to a high concentration of the immunoconjugates (not applicable to patient 9). Inasmuch as two of the MoAbs (I-1 and JGT) used were anti-CEA, there was also concern that such immunoconjugates given intravenously may be bound by the circulating CEA and prevented from reaching the tumor metastases in the liver, although in this study there was a paradoxical rise in the circulating CEA level during treatment, probably related to tumor cell swelling and necrosis, but there is no direct evidence of this.

The selection of MoAbs for immunotargeting in this study was based on results of immunoperoxidase testing of the primary colon cancer tissue, and this may not be the optimum method, considering the possibility of tumor heterogeneity between the primary tumor and its metastases.[35] Attempts were made to overcome this problem of tumor antigen heterogeneity by using "cocktails" of MoAb conjugates from different MoAbs; each gave >50% tumor reactivity as assessed by the immunoperoxidase staining. In addition to using immunoperoxidase staining to assess the binding of the MoAbs to the tumor, in vivo studies such as radioimaging with radiolabeled MoAbs should be considered.[36] This was not performed in this study because of concerns of interference with subsequent therapy from antigenic modulation, competition of antigen binding and, if some time elapsed between radioimaging and therapy, development of human antimouse antibody response. Furthermore, there are also likely to be subpopulations of drug-resistant cells maintained by a constant mutation rate, which may justify the use in the future of different drugs with different modes of action in the cocktails of drug-MoAb conjugates.

The results from this phase I study indicate that N-AcMEL-MoAb conjugates are safe and well tolerated at a dose of 20 mg/m^2 of N-AcMEL and 1000 mg/m^2 of MoAbs. This is in contrast to the usual clinical experience of myelosuppression with doses of melphalan in excess of 20 mg/day in adults.[30] The study was prematurely concluded because of the difficulties in producing such large quantities of purified antibodies. Fortunately there is rapid development of large-scale production methods, and it is hoped that this will lead to comparatively low-cost MoAbs within the next few years. However, it should be noted that because of its relative specificity, the maximum tolerated dose of the immunoconjugates may be well beyond 20 mg/m^2 of N-AcMEL and 1000 mg/m^2 of MoAbs and may not be practicably achieved because of the large quantities of MoAbs required. The biological antitumor responses in patients with gross hepatic metastatic disease from selective targeting of N-AcMEL-MoAb conjugates in doses $\leq$ 20 mg/m^2 of N-AcMEL in this limited phase I study are of interest. However, it is important to recognize that the agent conjugated to the monoclonal antibody is a derivative of melphalan ("prodrug"), and although the in vivo cytotoxicity of N-ACMEL-MoAb conjugates is comparable to that of free melphalan in animal studies, the comparability of cytotoxicity between the two agents in patients is unknown.[17] The therapeutic efficacy of drug-MoAb conjugates may be enhanced by increasing the dosage of cytotoxic drug, perhaps by using an intermediate drug carrier system such as human serum albumin[37] or a combination of drugs with different modes of action, or even by conjugating more toxic drug or toxin that otherwise can never be used clinically in its free form.[38] Furthermore, the usefulness of MoAb targeted cytotoxic agent may be best realized in patients with small tumor burden and not the type of patients (except patient 9) involved in the study. As evidenced in patient 6 (Fig. 3), the smaller hepatic metastases disappeared completely with treatment, whereas the larger tumor deposits remained unchanged. Of greater importance is the potential of

extending this treatment program to patients with Dukes' C colorectal cancer because of their high risk of development of liver metastases through the course of their disease, some of whom already have occult hepatic metastases at the time they are first seen,[8, 39] or as an adjuvant for patients who have had hepatic resection for resectable hepatic metastases. However, the development of hepatic metastases with different antigenic expression as in patient 9 (Fig. 4) is an important factor that warrants further study on the selection criteria of monoclonal antibodies to be used in "cocktails" of the immunoconjugates if this problem of antigen heterogeneity is to be overcome. Nonetheless, the therapeutic value of immunoconjugates can only be truly assessed in a properly conducted phase II study with matched control subjects.

It is important to note that treatment with N-AcMEL-MoAb conjugates was safe at a dose of 20 mg/m^2 of N-AcMEL, whereas the efficacy of such a form of treatment (immunotargeting of melphalan or even more toxic drugs) remains to be evaluated.

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