PHIGENIX
Exhibit 1035

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

J. J. Tjandra, FRCS, G. A. Pietersz, PhD, J. G. Teh, BSc(Hon),
A. M. Cuthbertson, FRACS, J. R. Sullivan, FRACP, C. Penfold, FRACS, *and*
I. F. C. McKenzie, PhD, FRACP, FRCPA, *Parkville and East Melbourne, Victoria, Australia*

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.)

From the Research Centre for Cancer and Transplantation, Department of Pathology, The University of Melbourne, and the Colorectal Unit, Royal Melbourne Hospital, Parkville, and the Department of Medical Oncology, St. Andrew's Hospital, Clarendon Place, East Melbourne, Victoria, Australia

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,

Accepted for publication Nov. 22, 1988.

Reprint requests: Ian F. C. McKenzie, MD, PhD, Research Centre for Cancer and Transplantation, Department of Pathology, The University of Melbourne, Parkville, Victoria 3052, Australia.

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		10mg/m²:250mg/m²
4	58	M	2		15mg/m²:340mg/m²
5	62	M	3	HAI of *cis*-platinum	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/m² and 2 months later, 10 mg/m²; two received 10 mg/m²; three received 15 mg/m²; and four received 20 mg/m² N-AcMEL conjugated to MoAbs. The study was closed at the 20 mg/m² 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-

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.