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Adjuvant therapy of ovarian cancer with radioactive monoclonal antibody

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Summary Fifty-two patients with epithelial ovarian cancer were treated with yttrium-90-labelled monoclonal antibody HMFG1 administered intraperitoneally following conventional surgery and chemotherapy as part of an extended phase I-II trial.

The treatment was well tolerated and the only significant toxicity observed was reversible myelosuppression as previously described. Following conventional surgery and chemotherapy, 21 out of the 52 patients had no evidence of residual disease and were regarded as receiving treatment in an adjuvant setting. To date, two of these patients have died of their disease (follow-up 3-62 months, median follow-up 35 months).

This extended phase I-II study suggests that patients with advanced ovarian cancer who achieve a complete remission following conventional therapy may benefit from further treatment with intraperitoneal radioactive monoclonal antibody.

Cancer of the ovary ranks sixth as a fatal form of cancer in women (Young *et al.*, 1982). Its incidence is approximately 20 per 100,000 with 4,500 new cases and 3,700 deaths per annum in the United Kingdom (Department of Health 1987). At diagnosis most patients have tumour outside the pelvis and this probably accounts for the poor prognosis (FIGO news report 1971). Advances in cytoreductive surgery and postoperative chemotherapy in the last decade have produced response rates of 65-80% but only a small improvement in overall survival (Neijt *et al.*, 1984). Unfortunately, most patients relapse and die of their disease indicating that benefits from surgery and chemotherapy, whether these may be new drugs or new combination of old drugs have reached a plateau (Marsoni *et al.*, 1990).

More than 90% of epithelial ovarian tumours express high levels of many antigens (Bast *et al.*, 1991), including one in particular, known as polymorphic epithelial mucin (PEM) (Gendler *et al.*, 1987). PEM can be described as a 'tumour associated antigen' because although expressed extensively by many epithelial cancers it can also be found at low levels on many normal tissues (Arklie *et al.*, 1981). Several monoclonal antibodies to this antigen and its various epitopes have been made and used for *in vitro* and *in vivo* diagnosis of many cancers including ovarian cancer (Epenetos *et al.*, 1982; Pateisky *et al.*, 1985; Colcher *et al.*, 1983). Since 1983, we have been investigating the possibility of tumour targeting and therapy by the intraperitoneal administration of radiolabelled monoclonal antibodies in patients with ovarian cancer (Epenetos *et al.*, 1984).

We have previously described extensively the pharmacokinetics, biodistribution and toxicity of iodine-131 and yttrium-90-labelled monoclonal antibodies for the treatment of ovarian cancer (Epenetos *et al.*, 1987; Stewart *et al.*, 1989, 1990; Maraveyas *et al.*, 1993). In this report we present the first comprehensive survival data of patients treated in this way from October 1987 to December 1992. Based on our results we propose that this novel modality should now be considered further as a form of adjuvant in patients with cancer of the ovary.

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Patients, materials and methods

Patients

Fifty-two patients with known epithelial cancer received intraperitoneal radioimmunotherapy with yttrium-90-labelled monoclonal antibody HMFG1. Patients' ages ranged from 29-76 years. All had performance status above WHO Grade 2. All patients had previously undergone cytoreductive surgery, and all but one were subsequently treated with cisplatin or carboplatin based chemotherapy. One patient (Stage Ic) did not receive chemotherapy. Table I shows the stage and disease status at presentation of all treated patients and Table Ib shows the histology and stage of patients treated as adjuvant, as assessed at second look laparoscopy. It can be seen that there are 22 patients who had no evidence of disease at the time of laparoscopy. One (Stage 1a) was disease free following chemotherapy for relapse and the remaining 21 were regarded as receiving treatment in an adjuvant setting.

Monoclonal antibody

The monoclonal antibody used in this study was Human Milk Fat Globule 1 (HMFG1) (ICRF, London and Unipath (UK) Ltd, Bedford). HMFG1 is a mouse IgG1 monoclonal antibody that binds to the PEM molecule found on more than 90% of epithelial ovarian carcinomas (Arklie *et al.*, 1981). Patients received 25 mg of antibody.

Antibody labelling

Yttrium-90 (AERE Harwell, UK) was chelated to the antibody-DTPA, CITC-DTPA or -DOTA conjugate as previously described (Stewart *et al.*, 1990; Meares *et al.*, 1990). Free radioisotope was removed by sephadex G50 gel filtration using phosphate buffered saline as elution buffer. Specific activity of radiolabelled antibody was $\leqslant 5$ Ci mg^{-1}. The final dose of administered antibody was made up to 25 mg of total IgG by adding unlabelled HMFG1 IgG to the radiolabelled fraction. Antibody immunoreactivity was tested in an enzyme-linked immunosorbant assay (ELISA method) before and after radiolabelling and was compared with underivatised antibody using micro titre plates coated with purified antigen. No obvious reduction in immunoreactivity was seen. The administered dose of radioactivity was measured in a SIEL isotope calibration chamber that had been calibrated with an yttrium-90 source (Stewart *et al.*, 1990).

Table Ia Patient's number, FIGO stage and extent of disease at antibody treatment

Patient no.	*FIGO stage at presentation*	*Disease stage at antibody therapy*
1	Ia	No evidence of disease following relapse
2	Ia	Bulky[a] disease + ascites
3	Ic	No evidence of disease
4	Ic	No evidence of disease
5	Ic	Positive peritoneal washings
6	Ic	No evidence of disease
7	2a	Unassessable adhesions
8	2b	No evidence of disease
9	2b	No evidence of disease
10	2c	No evidence of disease
11	2c	No evidence of disease
12	III	No evidence of disease
13	III	No evidence of disease
14	III	Unassessable adhesions
15	III	Bulky disease
16	III	Bulky disease
17	III	Bulky disease
18	III	Bulky disease
19	III	Minimal[b] disease
20	III	Minimal disease
21	III	Bulky disease
22	III	Bulky disease
23	III	Bulky disease
24	III	No evidence of disease
25	III	Bulky disease
26	III	Minimal disease
27	III	Unassessable adhesions
28	III	Bulky disease
29	III	No evidence of disease
30	IV	No evidence of disease
31	IV	No evidence of disease
32	IV	Extra peritoneal disease
33	Ic	Bulky disease
34	III	No evidence of disease
35	III	Bulky disease
36	III	Minimal disease
37	III	Bulky disease
38	III	Minimal disease
39	IV	Bulky disease
40	III	Bulky disease
41	III	Minimal disease
42	IIa	No evidence of disease
43	Ic	No evidence of disease
44	IIc	No evidence of disease
45	III	No evidence of disease
46	III	Unassessable
47	Ic	No evidence of disease
48	III	Unassessable
49	IV	Bulky disease
50	III	No evidence of disease
51	III	No evidence of disease
52	III	No evidence of disease

[a]Bulky disease = >2 cm. [b]Minimal disease = <2 cm.

Treatment

A peritoneal dialysis catheter was inserted into the peritoneal cavity during laparoscopy. Open laparoscopy also allowed visual assessment of disease volume, including peritoneal lavage with normal saline for cytological assessment. Minor adhesions, particularly around the liver, were noted in some patients. A good view was obtained in all but five patients, however, all patients were treated. Following the laparoscopy, yttrium-90-labelled antibody (dose range 5.00–30.000 mCi) was infused into the peritoneal cavity with 1.5 litres normal saline or Hartman's solution and the peritoneal dialysis catheter was removed. The patient position was altered every 20 min for the first 2 h to encourage an even distribution of antibody. Patients were nursed in a radiation controlled area for 5 days, during which time blood and urine samples were counted in order to monitor the blood levels of radioactivity and urinary excretion of the radioisotope, respectively.

Table Ib FIGO stage and histology of patients treated in an adjuvant setting

No.	*Stage at presentation*	*Histology*
1	Ic	Endometrioid
2	Ic	Endometrioid
3	Ic	Serous
4	IIb	Undifferentiated
5	IIb	Serous
6	IIc	Undifferentiated
7	IIc	Endometrioid
8	III	Endometrioid
9	III	Endometrioid
10	III	Undifferentiated
11	III	Endometrioid
12	IV	Undifferentiated
13	IV	Serous
14	III	Serous cystadenocarcinoma
15	IIa	Clear cell
16	Ic	Serous cystadenocarcinoma
17	IIc	Well differentiated
18	III	Serous
19	Ic	Undifferentiated
20	III	Serous
21	III	Endometrioid

Pharmacokinetics

Pharmacokinetics, toxicity and dosimetry have been previously reported (Epenetos *et al.*, 1987; Stewart *et al.*, 1989, 1990; Maraveyas *et al.*, 1993). Approximately 30% of the intraperitoneally injected immunoconjugate was absorbed into the systemic circulation by 48 h after administration (Stewart *et al.*, 1989, 1990; Maraveyas *et al.*, 1993).

Results

Toxicity

The treatment was well tolerated by all patients. Reversible myelosuppression was observed at high doses (>15 mCi of HMFG1-DTPA-^{90}Y). This toxicity was reduced considerably by the subsequent use of more stable chelating agents known as DOTA and CITC-DTPA (Moi *et al.*, 1990). No significant myelotoxicity was observed even at higher doses of up to 20 mCi of HMFG1-DOTA-^{90}Y (Kosmas *et al.*, 1992) and 34 mCi of HMFG1-CITC-DTPA-^{90}Y. A correlation between body surface and CITC-DTPA-^{90}Y dose was found (Maraveyas *et al.*, 1993). DOTA is potentially immunogenic in patients (Kosmas *et al.*, 1992) as three out of six patients treated with HMG1-DOTA-^{90}Y conjugate developed serum sickness reactions manifested as superficial and self limiting skin rashes 10–12 days after treatment. It was also found that treated patients developed anti-DOTA (Kosmas *et al.*, 1990) and anti-CITC-DTPA antibodies. All patients developed human antimouse antibodies as previously reported (Epenetos *et al.*, 1987; Stewart *et al.*, 1989). The difference in toxicity and immunogenicity between DTPA and DOTA linkage between antibody and radionuclide as well as the HAMA levels are reported elsewhere (Kosmas *et al.*, 1992; Maraveyas *et al.*, 1993).

Survival

Figure 1 shows the survival data of the subgroup of 15 patients treated regarded as receiving adjuvant treatment and compares it with a similar group (70 patients) from the same centre (North Thames Ovarian Group). This group comprises of patients who presented with Stage 11b disease or worse and had no evidence of residual disease at laparoscopy following conventional treatment with surgery and chemotherapy. These data show a remarkable difference in survival between the group treated as adjuvant with antibody and the historical control from the North Thames Ovarian Group (Lambert *et al.*, 1993). However, this is not the result

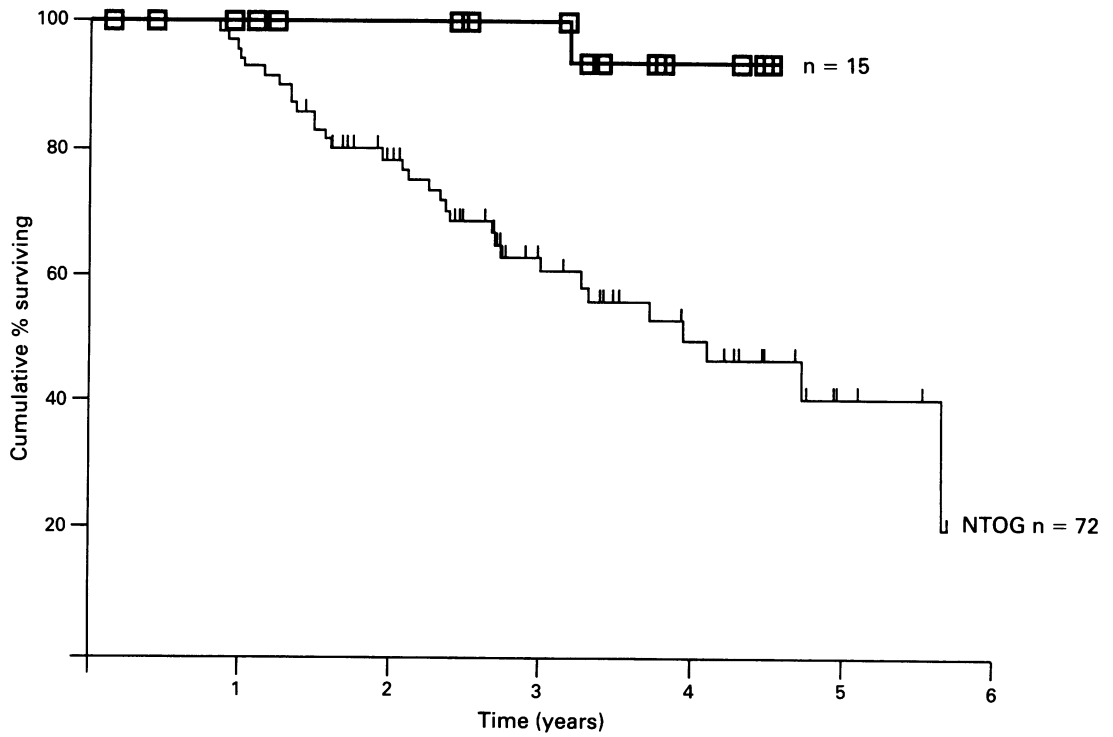

Figure 1 Actuarial survival of patients treated in an adjuvant setting with monoclonal antibodies. In this Figure a comparison is made of the 15 patients (Stage IIb or greater) adjuvant group treated with antibody and a group of 70 patients from the North Thames ovarian Group who presented with at least Stage IIb disease and who had no evidence of disease at second look laparoscopy. These patients were further randomised to receive further chemotherapy or whole abdominal radiotherapy (Lambert *et al.*, 1993). A further six patients who had stage Ic-IIa disease and were treated similarly with radiolabelled monoclonal antibodies are not shown on this graph. One patient of this group has died.

of a randomised trial, and the patient numbers are small. Survival after antibody therapy of patients with bulky disease treated with radiolabelled antibody is: median survival of 11 months (range 2–31 months), with four patients still alive.

Discussion

The application of radiolabelled antibodies as specific cytotoxic drugs against cancer has many attractions including selectivity against tumour cells, irradiation of adjacent tumour cells, lack of major side effects and simplicity of radiolabelling and administration. Although tested extensively over the last decade, radiolabelled and other immunoconjugates have had only limited success as anti-cancer agents.

For the first time, this study demonstrates that radiolabelled antibodies used in an adjuvant setting may reduce the rate of recurrence from ovarian cancer and improve the long term survival. Although survival data from this study appear superior to previously reported studies (Neijt *et al.*, 1984; Marsoni *et al.*, 1990), the patient numbers are small and need to be substantiated by larger phase III randomised studies. Furthermore, because this was a phase I–II study, our cases included a mixture of stages from Ic-IV.

The mechanisms for the action of antibody therapy are not clear from this trial. The calculated doses of radiation delivered by the radioactive antibody are thought to be insufficient for a cytotoxic effect based on calculations using conventional dosimetry tables (Snyder *et al.*, 1978) although more recent studies suggest that higher doses can be delivered (Larson *et al.*, 1991). Unfortunately, there are no comprehensive data on the therapeutic efficacy of radioactive yttrium colloid alone given intraperitoneally after chemotherapy. An alternative possibility is that HMFG1 murine monoclonal antibody when administered intraperitoneally into humans, can cause a cascade of immunological reactions leading to humoral (Courtenay-Luck *et al.*, 1988; Herlyn *et al.*, 1991) and cellular (Kosmas *et al.*, 1991) activation of the immune system with resultant antitumour effects. If this is the cause of the observed prolongation of survival in patients with ovarian cancer in this study, then, ironically, the use of murine monoclonal antibodies may be more effective than the recently described chimeric (LoBuglio *et al.*, 1989), humanised (Reichmann *et al.*, 1988) or completely human (Borrebaeck *et al.*, 1988) monoclonal antibodies.

In summary, this study provides encouragement to the concept of adjuvant therapy with monoclonal antibodies in patients with epithelial ovarian cancer who have no evidence of residual disease after initial surgery and chemotherapy.

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