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Targeting of Tumours With Murine and Reshaped Human Monoclonal Antibodies Against Placental Alkaline Phosphatase: Immunolocalisation, Pharmacokinetics and Immune Response

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Anti-tumour monoclonal murine and humanised (reshaped human) antibodies (H17E2 and Hu2PLAP, respectively) against placental alkaline phosphatase (PLAP), radioactively labelled with indium-111 (^{111}In) and iodine-123 (^{123}I), were evaluated for their ability to localise mainly testicular and ovarian tumours in sequential pilot studies of the Hammersmith Oncology Group. 33 patients with active primary and/or metastatic testicular cancer were studied with the [^{111}In]- or [^{123}I]H17E2 antibody. 8 patients with testicular cancer were studied with the same antibody after being rendered free of disease after induction chemotherapy and surgical resection of residual tumour. 3 additional patients, 2 with ovarian cancer and 1 with testicular seminoma, were studied with [^{111}In]H17E2 via a macrocyclic chelating agent (DOTA). 7 patients; 5 with ovarian cancer, 1 with breast cancer, and 1 with gastric cancer, received the reshaped human Hu2PLAP antibody [^{111}In]DOTA labelled. One of these was imaged twice, with H17E2- and Hu2PLAP-DOTA-^{111}In, respectively. In the initial 33 patients with active primary and/or metastatic testicular cancer, the presence of tumour was confirmed and correlated well with conventional radiological diagnostic methods, and in addition, the antibody scan revealed the presence of active disease in 2 patients with negative conventional imaging, but elevated serum tumour markers. In the 8 patients with complete remission (CR), imaging studies with the radiolabelled antibody did not show any localisation. The best images were obtained at 24 and 48 h after the [^{123}I]- and [^{111}In]H17E2, respectively. None of these patients developed human anti-mouse antibody responses (HAMA). Successful imaging with the reshaped human antibody, Hu2PLAP-DOTA-^{111}In, was seen in 3 patients with PLAP-positive tumours (2 ovarian and 1 gastric cancer). The 3 negative patients were 1 in complete remission, 1 with PLAP-negative tumour and 1 who cleared the Hu2PLAP antibody immediately after infusion due to the presence of anti-chelating agent (anti-DOTA) antibodies from a previous H17E2-DOTA-^{111}In scan. One patient with PLAP-negative breast carcinoma had a false-positive scan with Hu2PLAP, showing localisation to the pleural effusion. Antibody pharmacokinetics showed a mean $t_{1/2}\beta$ = 73.1 ± 30.2 h (n = 5) for Hu2PLAP versus $t_{1/2}\beta$ = 27.2 ± 5.9 h (n = 3) for H17E2 ($P < 0.05$). 2 patients receiving Hu2PLAP were excluded due to the rapid clearance of the radiolabel as a result of the presence of high HAMA and anti-chelate antibody levels, respectively. At 96 h, the mean cumulative urine excretion of ^{111}In was 11 ± 8% for Hu2PLAP versus 14 ± 5% for H17E2. HAMA developed in 1 patient undergoing sequential imaging with both antibodies, and in another who already had HAMA after intraperitoneal monoclonal antibody therapy for ovarian cancer. Antibodies to the chelating agent developed in 3 patients. In conclusion, immunolocalisation of PLAP-positive tumours is feasible with both murine and reshaped human monoclonal antibodies. The sensitivity and specificity of the method appear to be very high in this pilot study, and, in view of the absence of toxicity, the diagnostic contribution of this approach should be evaluated prospectively.

Key words: testicular cancer, seminoma, ovarian cancer, placental alkaline phosphatase, radiolabelled monoclonal antibodies, immunoscintigraphy
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INTRODUCTION

THE TREATMENT of germ cell tumours (GCT) has been revolutionised over the last decade, since the introduction of cisplatinum in 1977 [1]. The majority of patients are cured of their disease, but there is still a significant proportion, over 15–20%, who relapse after an initial complete remission, or are primarily refractory to standard induction chemotherapy and are deemed to succumb to their disease. A significant problem is often encountered in those patients who respond to induction chemotherapy, but residual masses persist in areas of previous bulky disease requiring surgical intervention. These residual masses represent, in approximately 40% of patients, reactive fibrosis/necrotic tumour, in 40% mature teratoma and in 20% active residual cancer [2]. It should, therefore, be imperative to devise

methods able to differentiate patients with active residual disease from those with fibrosis/necrotic tumour, and thus avoid unnecessary surgical intervention. In the subgroup of patients with stage I seminoma, it is common practice to administer adjuvant radiation to the clinically negative (by abdominal CT scan and/or bipedal lymphagiogram) paraaortic lymph nodes after orchidectomy for primary tumour. Since only 15–20% of these patients relapse at 2 years of follow-up without radiotherapy after diagnosis, it appears that the majority of patients (over 80%) with clinical stage I seminoma receive unnecessary radiation [3]. The patients who relapse after orchidectomy and close follow-up can be cured by radiotherapy and, therefore, do not compromise the otherwise excellent overall survival of the group, when compared to those receiving radiotherapy after the diagnosis. It would, therefore, be very helpful to identify patients with occult retroperitoneal disease by improving on the specificity of abdominal CT scan and administer post-orchidectomy radiotherapy only to these patients. The above problems could be overcome if specific and sensitive methods of *in vivo* diagnosis are employed, such as anti-tumour monoclonal antibodies (MAbs).

Radiolabelled anti-tumour MAbs hold promise in improving *in vivo* tumour diagnosis and therapy, as they have shown their ability to localise successfully to microscopic tumour deposits [4]. The largest studies in colorectal carcinoma exist in U.S.A. and European centres [5, 6]. The large multi-institutional study in Italy [5] included 284 patients with gastrointestinal adenocarcinomas imaged by $F(ab')_2$ fragments of FO23C5, an anti-CEA MAb, and demonstrated a sensitivity of 64 and 84% for lesions $\leq$2 and >2cm in diameter, respectively. Repeated administration of radiolabelled B72.3 ([^{111}In]ZCE-025) in the postoperative follow-up of colorectal carcinoma patients is safe and effective [7]. Although successful tumour localisation and sometimes therapy [8, 9] can be achieved by the use of radiolabelled murine MAbs, two major problems have been identified that limit their more widespread application. Firstly, murine antibodies when administered to humans evoke an immune response, which can lead to rapid clearance of the murine MAb with subsequent administration and hazardous type III hypersensitivity reactions from immune complex deposition [10]. These problems could be overcome by administering human or genetically engineered reshaped human MAbs ('humanised' MAbs). The latter can be produced by transplanting the genes coding for the complementarity determining regions (CDRs) of the murine MAb into a human immunoglobulin construct [11, 12]. Secondly, conjugation of MAbs with radioisotopes usually results in unstable immunoconjugates, which lose their efficacy in successful tumour targeting, as well as exposing the host to dose-limiting haematological toxicity from deposition of the free isotope, such as yttrium-90 (^{90}Y), to the bone [9]. This problem can be overcome by the use of new bifunctional macrocyclic chelating agents, such as 2-p-nitrobenzyl-1, 4, 7, 10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (benzyl-DOTA) [13], which have shown excellent stability *in vitro* [13, 14] and in preclinical *in vivo* studies [14–16]. ^{90}Y-labelled benzyl-DOTA-coupled murine MAbs have been used therapeutically in ovarian cancer [17].

Placental alkaline phosphatase (PLAP) has been shown to be expressed on the surface membrane of testicular germ cell tumours and epithelial ovarian cancers [18, 19]. PLAP has been detected in the serum of patients with testicular germ cell tumours, and only those with active seminoma have been found to have consistently elevated serum titres [20]. Immunohistochemical methods using H17E2, an anti-PLAP MAb [21], on fresh frozen tissue revealed the absence of staining of normal tissues, except full term placenta.

In the first part of our sequential studies, we studied the murine H17E2 anti-PLAP MAb for testicular tumour localisation, labelled conventionally with either ^{111}In (indium) or ^{123}I (iodine). Subsequently, we evaluated the ability of the same MAb to localise PLAP-positive tumours when labelled with [^{111}In] DOTA, in order to improve the *in vivo* stability of the radioimmunoconjugate. In the final part of our study, we combined both the advantages of improved stability with DOTA in ^{111}In chelation, as well as the potentially reduced immunogenicity of a reshaped human antibody, Hu2PLAP, and evaluated whether this novel immunoconjugate is able to localise efficiently PLAP-positive tumours, and compared its pharmacokinetics with those of its murine homologue, H17E2.

PATIENTS AND METHODS

Patients' characteristics

41 patients with germ-cell tumours, aged between 29 and 68 years (mean 47), were selected for radioimmunolocalisation with ^{111}In- or ^{123}I-labelled H17E2 MAb. Among these patients were 26 with malignant teratomas, 10 with seminomas, 4 with mixed teratomas–seminomas and 1 with embryonal carcinoma. All patients were evaluated after initial diagnosis and prestudy entry by detailed history and physical examination, full blood count, biochemical profile, serum tumour markers (β-human chorionic gonadotrophins (β-hCG) and α-fetoprotein (α-FP)) chest X-ray and chest CT, CT scan of the abdomen. 8 of these patients were in complete remission after induction chemotherapy ± adjunctive surgery to residual retroperitoneal or lung masses.

7 patients, aged between 36 and 65 years, were studied with the Hu2PLAP-DOTA-^{111}In immunoconjugate (Table 1). 5 had advanced ovarian cancer, 1 gastric cancer and 1 breast cancer with malignant pleural effusion. All patients except 2 (nos 4 and 7) had tumours expressing PLAP. One patient (no. 5) was in complete remission at the time of the MAb study. 2 patients (nos 1 and 2) had raised serum HAMA (human anti-mouse antibody) levels prior to receiving Hu2PLAP MAb. Patient no. 1 had received HMFG1-DTPA-^{90}Y intraperitoneally in an attempt to treat extensive intraperitoneal ovarian cancer and patient no. 2 had previously undergone H17E2-DOTA-^{111}In immunoscintigraphy. 3 patients, 2 with ovarian cancer and 1 with testicular seminoma, were evaluated with H17E2-DOTA-^{111}In (Table 1). Patient no. 2 was initially studied with H17E2-DOTA-^{111}In and then received Hu2PLAP-DOTA-^{111}In as indicated above.

The above studies were approved by the Institutional Review Board of the Hammersmith Hospital and London University, and informed consent was obtained from every patient before entering the study.

Monoclonal antibodies

H17E2. This is a murine IgG1k antibody directed against PLAP. This enzyme is expressed as a surface membrane antigen on many neoplasms, including 60–85% of ovarian carcinomas, as well as testicular germ-cell tumours [21].

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Table 1. Clinical/laboratory characteristics, radioimmunolocalisation results, as well as immunological responses of patients studied following i.v. administration of [^{111}In]DOTA-labelled Hu2PLAP or H17E2 MAbs

Patient	Diagnosis and disease extent	PLAP expression	MAb	HAMA (μg/ml)	Anti-DOTA (μg/ml)	RIL
1	Stage IIIc OvCa	+	Hu2PLAP	+ (4840)	−*	+
2	Stage Ia OvCa (recurrent)	+	H17E2/then Hu2PLAP	+ (407)	+ (67)	+/then −†
3	Gastric Ca	+	Hu2PLAP	− (1.7)	−	+
4	Stage III OvCa	−	Hu2PLAP	− (0.8)	−	−
5	Stage I OvCa-CR	+	Hu2PLAP	− (2.4)	N/T	−
6	Stage IIIc OvCa	+	Hu2PLAP	− (8.3)	+ (80)	+
7	Breast Ca/malignant pleural effusion	−	Hu2PLAP	− (4.3)	−	+
8	Stage III testicular seminoma-CR	+	H17E2	− (4.6)	+ (32)	−
9	Stage IIIc OvCa	+	H17E2	− (2.5)	−	+

OvCa, Ovarian cancer; CR, complete remission; N/T, not tested; RIL, radioimmunolocalisation.
*Anti-DOTA response below lower detection limit (= 0.25 μg/ml).
†Patient no. 2 had already developed both HAMA and anti-DOTA serum responses after a successful previous scanning with H17E2-DOTA-^{111}In. On subsequent imaging with Hu2PLAP, the patient cleared the Hu2PLAP-DOTA-^{111}In radioimmunoconjugate so rapidly that imaging was impossible.

Hu2PLAP. This is a reshaped human IgG1k MAb having the same specificity as the murine H17E2 MAb. It was produced by transplanting the genes of a H17E2 hybridoma cell line coding for the complementarity determining regions (CDRs) to a human IgG1 immunoglobulin construct, as previously described [12]. It has PLAP-binding capability with a lower *Ka* than its murine equivalent, H17E2. Despite this difference, the *Ka* of Hu2PLAP is not so low as to limit the application of the humanised antibody.

Radiolabelling

Labelling of H17E2 with ^{123}I (AERE, Harwell, U.K.) was performed using the iodogen method [22]. H17E2 antibody radiolabelled with ^{123}I resulted in a labelling efficiency of approximately 70–90% and a specific activity of 2–4 mCi/mg of MAb. Labelling with ^{111}In (Amersham International, Amersham, U.K.) involved conjugation with diethylenetriamine pentaacetic acid (DTPA) by means of the cyclic anhydride (Sigma Chemical Co., U.K.) [23]. Free ^{123}I or ^{111}In were separated by gel filtration using a Sephadex G-50 column.

The H17E2 and Hu2PLAP antibodies were conjugated to benzyl-DOTA [13] via a thioether linkage. This was achieved by the introduction of thiol groups into the antibody using 2-iminothiolane (Sigma Chemical Co.) followed by reaction with the bromoacetamide derivative of DOTA [24, 25]. Antibody-DOTA was subsequently radiolabelled with ^{111}In at pH 5.0 in 0.1 M ammonium acetate buffer. The radiolabelled antibody was purified on a Sephadex G-50 gel filtration column and eluted in phosphate buffered saline (PBS), pH 7.4. The protein-containing fractions were pooled and sterilised by 0.22-μm micropore filtration. Antibodies H17E2 and Hu2PLAP radiolabelled with ^{111}In resulted in a labelling efficiency of approximately 80% and a specific activity of 1–4 mCi/mg of MAb. Patients received 1–2 mCi of labelled MAb, given as an intravenous (i.v.) bolus over 1 min.

Immunoreactivity of both H17E2 and Hu2PLAP was assayed using purified PLAP (Calzyme Labs Inc., U.S.A.) as antigen coated on 96-well microtitre plates (Becton Dickinson, Mountain View, California, U.S.A.). DTPA and benzyl-DOTA conjugated antibodies were assayed for immunoreactivity by ELISA.

All DOTA-conjugated antibodies were immunoreactive as measured by ELISA. The mean chelated loading per molecule of MAb was 1.3 and 1.0 for H17E2 and Hu2PLAP, respectively. ^{111}In- and ^{123}I-labelled antibodies were immunoreactive as measured by solid-phase competitive radioimmunoassay (RIA).

The immunoreactivity of radiolabelled antibody was assayed by competitive solid-phase RIA using a 25-fold excess of underivatised specific antibody to compete. Radiolabelled non-specific antibody alone and in competition with underivatised non-specific antibody were used as negative controls. All reagents produced were tested for sterility and pyrogenicity before administration to patients by an independent pharmacy laboratory and were found to be sterile and apyrogenic. Sterile reagents were used throughout the radiolabelling and conjugation procedures and were prepared by the Pharmacy Department, Hammersmith Hospital.

Immunoperoxidase staining

Fresh frozen sections of tumour were stained by an indirect two-stage immunoperoxidase procedure [26]. The concentration of the antibody was 10 μg/ml. Sections were tested against H17E2 MAb as well as negative controls. Positive tissues were scored when 50% or more of tumour cells, seen under light microscopy, stained positive. H17E2 and Hu2PLAP MAbs showed positive staining against sections of PLAP-expressing tumours, as determined by the indirect two-stage immunoperoxidase method. Underivatised antibody and a non-specific antibody were used as controls.

Imaging studies

Imaging studies were carried out using a 40-cm field-of-view gamma camera (General Electric, Maxi-camera 400T) fitted with a medium or low energy colimator for ^{111}In or ^{123}I, respectively. Anterior and posterior whole body scans as well as planar images

Table 2. Results of immunoscintigraphy in patients with GCTs studied, using [^{111}In]DTPA and [^{123}I]H17E2

Radiolabel	Number of patients investigated	Antibody scans Positive (%)	Negative (%)
^{111}In	20	15 (75)	5 (20)
^{123}I	13	9 (69)	4 (31)
Total	33	24 (73)	9 (27)

Table 3. Comparison between CT and [^{111}In]DTPA and [^{123}I]-labelled H17E2 MAb (anti-PLAP) scanning in the detection of disease in GCTs

	Active disease CT (+)	Active disease CT (−)	Inactive disease CT (+)	Inactive disease CT (−)
PLAP scan+	22	2	0	0
PLAP scan−	9	0	0	8

were obtained. A baseline blood pool was taken 5 min following the initial injection of MAbs. The sequential scans were then carried out for up to 5 days with ^{111}In- and 3 days with ^{123}I-labelled MAb. Amounts of administered H17E2 ranged between 250 and 800 μg. Hu2PLAP-DOTA-^{111}In (220–833 μg) was administered i.v. to each patient. Gamma camera images were obtained of the whole body and abdomen (both posterior and anterior) at t = 0, 48 and 96 h postadministration. The uptake of the radiolabelled antibody by the liver was quantitated using regions of interest in whole body scans [27].

Pharmacokinetics

Blood samples were obtained at t = 0, 1 h and at the same time of subsequent scans. Serum samples were taken prior to the study and 2–3 weeks after the study. Urine was collected in 24-h aliquots from the time of injection until the time of the final scan.

Immune responses

HAMA response was determined by an ELISA method that has been previously described [10]. Anti-idiotypic response was determined by a comparative ELISA against H17E2 MAb that was administered to patients in the present study and against two other MAbs of the same isotype (IgG1k), but of irrelevant specificity (different idiotypes) as has already been described [10].

Human immune responses against the macrocycle benzyl-DOTA were determined by an ELISA method using as antigen benzyl-DOTA coupled to human serum albumin (HSA). Details of the method have been previously described [28].

Statistical analysis

Statistical analysis of the data was carried out using the Student's t-test to compare the mean and standard deviation of each group. The threshold of significance was taken as $P < 0.005$.

RESULTS

Patients

Details of patients investigated with [^{111}In]- or [^{123}I]H17E2 and H17E2 or Hu2PLAP-DOTA-^{111}In as well as imaging results are shown in Tables 1, 2 and 3, respectively. Representative results of immunoperoxidase staining are shown (Figure 1).

Imaging studies

[^{123}I] *and* [^{111}In]*DTPA-labelled H17E2.* Antibody guided localisation of testicular tumours using [^{111}In]- or [^{123}I]H17E2 was shown in most patients with active disease and results are summarised in Table 2. In 24 of 33 patients with active testicular cancer, there was a positive correlation between the conventional radiological investigations (mostly CT scans, some bipedal lymphagiogram) and the MAb scan (Figure 2). In 2 patients with active disease, as defined by elevated serum tumour markers, the antibody scan provided definite identification of the site of the disease in the presence of a negative CT scan (Figures 3, 4). Exploratory laparotomy with retroperitoneal lymph node dissection in these 2 cases revealed the presence of active

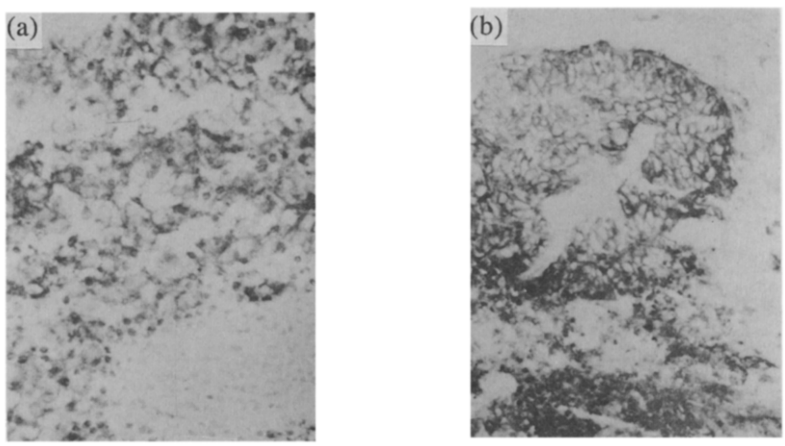


Figure 1. Immunoperoxidase staining of the testicular primary tumour with H17E2 antibody in a case of (a) seminoma and (b) malignant teratoma undifferentiated.

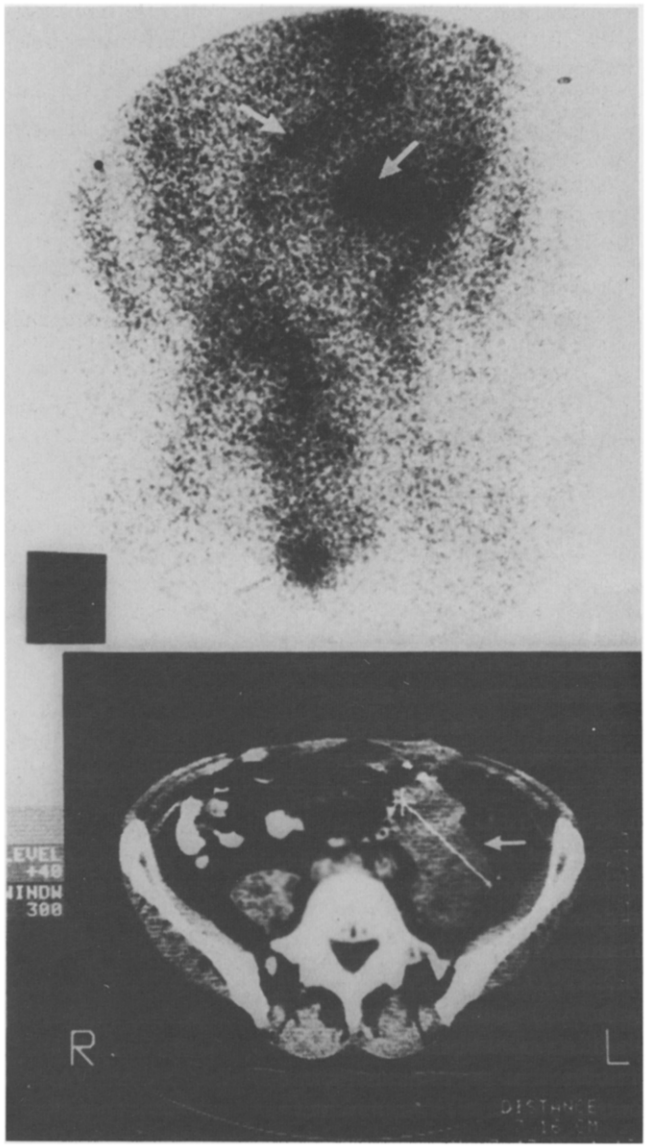


Figure 2. [^{123}I]H17E2 MAb scan 3 days after injection shows uptake in lower paraortic and left iliac lymph nodes (upper panel), which correlates with the mass in the left iliac region shown in the CT scan (lower panel).

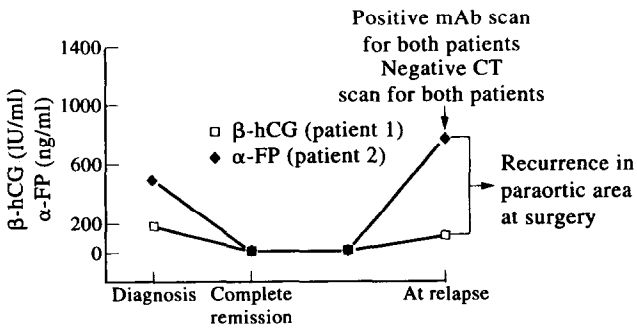
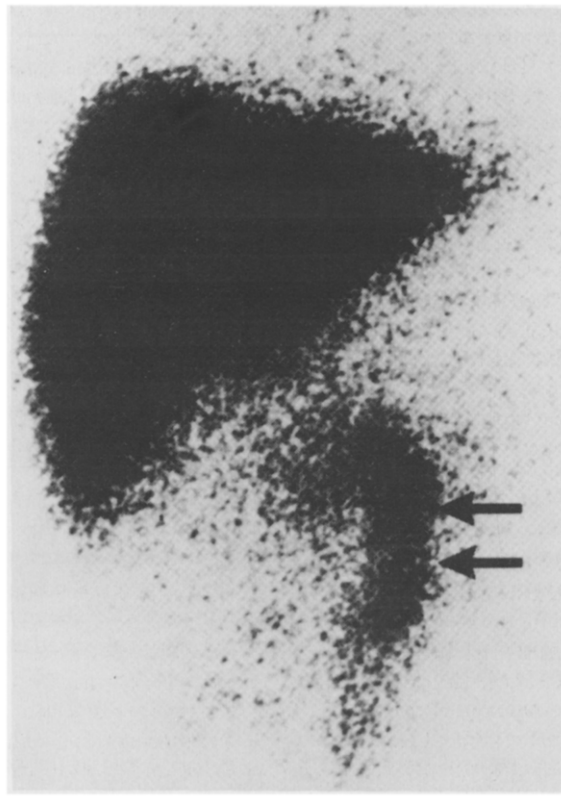


Figure 3. Results of serum marker data and conventional investigations at diagnosis, complete remission, and relapse in 2 patients; 1 with testicular seminoma and raised β-hCG (patient 1) and another with malignant teratoma undifferentiated and raised α-FP (patient 2). Both patients relapsed with rising tumour markers, had negative CT scans, but were positive after H17E2 MAb scan, indicating the presence of active tumour, which was confirmed histologically after surgical resection of the involved lymph nodes.

(a)



(b)

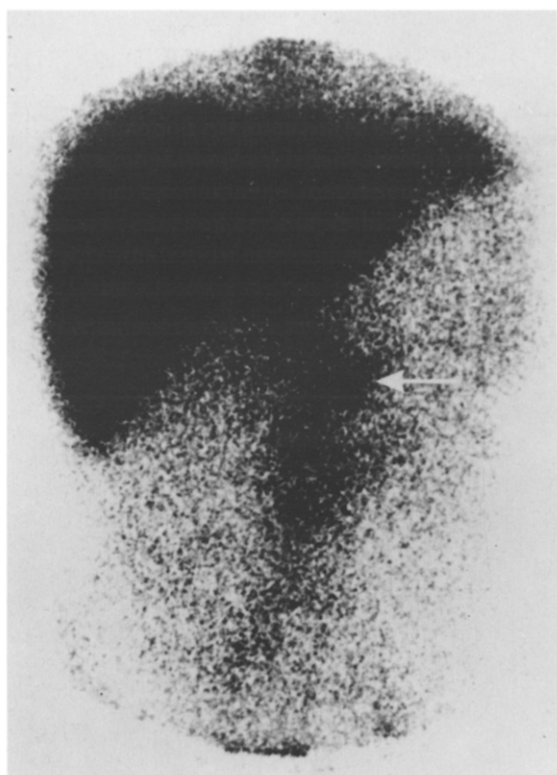


Figure 4. High uptake of [^{111}In]H17E2 in paraortic lymph nodes (arrows) in a patient with testicular seminoma (a) and a patient with malignant teratoma undifferentiated (b). Invasion of these lymph nodes by tumour was confirmed after histological examination of the retroperitoneal lymph node resection specimen obtained at laparotomy.

testicular cancer, as predicted by the antibody scan. 8 patients with GCTs in complete remission (CR), as defined by clinical, biochemical and radiological criteria, were scanned with [^{111}In]- or [^{123}I]H17E2 and did not show any uptake in new or previous sites of disease. The sensitivity of the localisation was approximately 74% and the specificity 100% (Table 3). The images obtained were of good quality without the need of computer manipulation. The best images were seen at 48 h after ^{111}In-labelled H17E2 (conventional chelation via DTPA) administration and 24 h after ^{123}I-labelled H17E2 MAb. In all patients studied with [^{111}In]H17E2, there was observable uptake of the radiolabelled MAb by the liver and spleen. The uptake of the radiolabel by the liver was quantitated and found to be 30% of the administered dose 48 h after antibody administration. Therefore, this technique is unsuitable for imaging hepatic metastases. The patients studied with [^{123}I]H17E2 had observable uptake of the radiolabel by the thyroid gland and the stomach.

[^{111}In]*DOTA-labelled H17E2 and Hu2PLAP*. Hu2PLAP radiolabelled with ^{111}In was used to image 7 patients with malignant tumours (Table 1). Successful imaging was seen in 3 patients (nos 1, 3 and 6), with PLAP-positive tumours and a representative case is shown (Figure 5). Patient no. 1 had a very high HAMA level (4.84 mg/ml) at the time of entry into the immunoscintigraphy study. The three negative scans were patient no. 5 with PLAP-positive ovarian cancer, in CR from her disease after surgery + cisplatinum based chemotherapy; patient no. 4 with an antigen-negative tumour and patient no. 2 who developed both HAMA and anti-DOTA serum responses after previous scanning with H17E2-DOTA-^{111}In, which was positive. This patient cleared the Hu2PLAP-DOTA-^{111}In radioimmunoconjugate so rapidly that imaging was impossible.

2/3 patients scanned with H17E2-DOTA-^{111}In had positive scans (Table 3). Patients nos 2 and 9 with ovarian cancer showed localisation at sites of permanent disease. The third patient, no. 8, was in CR at the time of the antibody scan after four cycles of cisplatinum + etoposide for testicular seminoma metastatic to the left supraclavicular lymph nodes.

Pharmacokinetics

Pharmacokinetics of H17E2 MAb labelled conventionally with ^{111}In or ^{123}I, as well as H17E2- or Hu2PLAP-DOTA-^{111}In, are shown in Table 4.

Patient nos 1 and 2, having high pre-existing HAMA and anti-DOTA immune responses, respectively, and very rapid clearance of the Hu2PLAP immunoconjugate, were excluded from the analysis of $t_{1/2}\beta$. Patient no. 2 had only 27% of the administered radioactivity remaining in her blood at 1 h postinjection, the remainder cleared with a $t_{1/2}\beta$ = 39 h and a cumulative urine excretion at 96 h of 31%. For patient no. 1, these values were $t_{1/2}\beta$ = 47 h and 66% cumulative urine excretion at 96 h.

Humoral immune response

None of the patients with GCT imaged with [^{111}In]- or [^{123}I]H17E2 developed HAMA within 6 months of continuous monitoring for that response, other than the pre-existing low-affinity anti-globulin reactivity. In patients undergoing repeated imaging studies, we did not observe the development of HAMA, even when a total of 800 μg of MAb was administered.

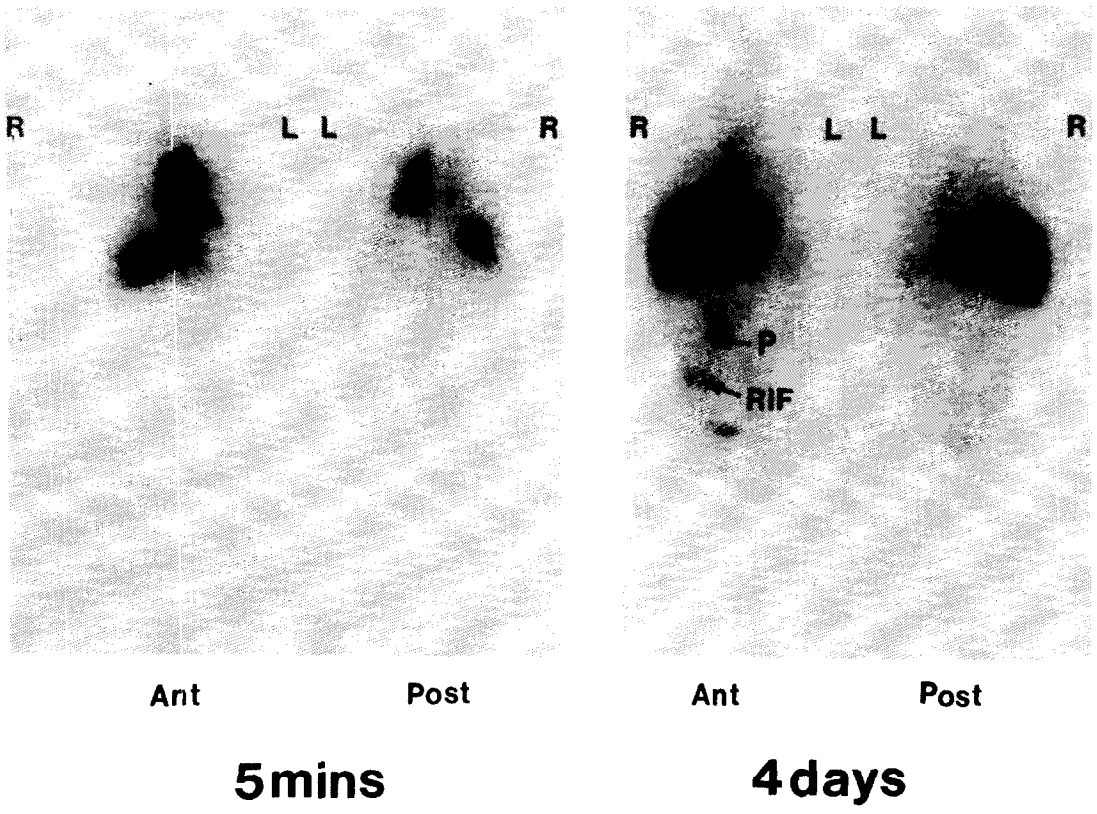


Figure 5. Gamma-camera scans taken after i.v. administration of Hu2PLAP-DOTA-^{111}In to a patient with stage IIIc ovarian cancer. Anterior (Ant) and posterior (Post) whole body images were taken at 5 min and 96 h. Positive uptake is seen at 96 h in the right iliac fossa (RIF) and the paraortic region consistent with the clinical and laparoscopic findings. R, right; L, left.

Table 4. Pharmacokinetic data of ^{123}I- or ^{111}In-labelled H17E2 and ^{111}In-DOTA-labelled H17E2 and Hu2PLAP

Parameter	[^{123}I]H17E2 (n = 8)	[^{111}In]H17E2 (n = 11)	[^{111}In]DOTA-H17E2 (n = 3)	[^{111}In]DOTA-Hu2PLAP (n = 5)
$t_{1/2}\beta$ (±S.D.) (h)	30.0 ± 6.0	36.0 ± 4.8	27.2 ± 5.9	73.1 ± 30.2
Cumulative 96-h urine excretion (%)	—	—	14 ± 5	11 ± 8

No anti-idiotypic responses against Hu2PLAP were detected following these studies, but 3 (nos 2, 6 and 8) of 9 patients tested developed anti-DOTA responses. Patient no. 8 was imaged with H17E2-DOTA-^{111}In and patient no. 2 received initially H17E2-DOTA-^{111}In and then Hu2PLAP-DOTA-^{111}In. An anti-DOTA response developed just after H17E2-DOTA-^{111}In administration. The dose of administered DOTA-immunoconjugate in μg correlated linearly with the level of anti-DOTA response, $r = 0.982$ ($P < 0.001$) (Figure 6). Patient no. 8 had a 4.84-mg/ml HAMA level from previous intraperitoneal radioimmunotherapy with ^{90}Y-labelled MAbs and patient no. 2 developed a 407-μg/ml HAMA level after the first imaging study. Patient nos 3–8 had insignificant pre-existing HAMA levels that did not rise after administration of the radioimmunoconjugate (see Table 1).

DISCUSSION

The present study has demonstrated that the presence of active disease in patients with testicular tumours can be successfully localised with an acceptable degree of accuracy using radiolabelled murine anti-PLAP MAb. Similarly, a preliminary evaluation of the reshaped human MAb Hu2PLAP labelled with ^{111}In, coupled via a new macrocyclic stable chelating agent (benzyl-DOTA), indicated that successful images could be obtained in patients with PLAP-positive tumours, mainly ovarian cancer.

The presence of active disease was consistently detected and correlated well with conventional diagnostic imaging. However, a theoretical limitation of the method is the intense non-specific liver uptake when using ^{111}In-labelled MAb, which could complicate interpretation in the presence of liver metastases and, therefore, an alternative is to use ^{123}I-labelled MAbs. This finding is in agreement with our previous experience, where ^{111}In-labelled MAbs were used for the diagnosis and treatment of liver tumours [29]. In fact, very few patients with GCTs present with liver metastases at diagnosis, representing a subgroup with poor prognosis that need to be treated more aggressively [30]. Similarly, in ovarian cancer patients, the presence of intrahepatic metastases is extremely rare, with direct extension to the serosal peritoneal surface of the liver as the usual pattern of metastases.

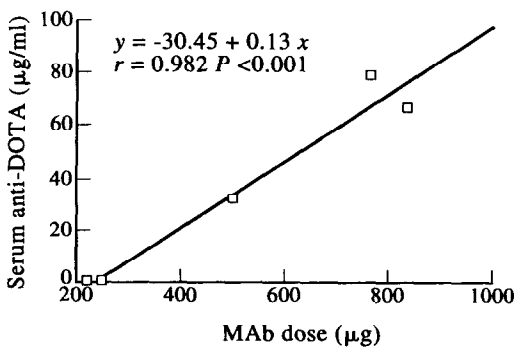


Figure 6. Correlation between the dose of the MAb (H17E2 or Hu2PLAP)-DOTA immunoconjugate and the serum level of anti-DOTA antibody immune response ($r = 0.982$, $P < 0.001$). 2 patients receiving the same dose of MAb (250 μg) did not develop an anti-DOTA response and are, therefore, represented by the same point in the graph.

The H17E2 scan revealed the presence of active disease in 2 patients; 1 with testicular undifferentiated teratoma and 1 with seminoma (Figure 4), who had no evidence of tumour by abdominal CT scan and bipedal lymphography, despite elevated β-hCG and α-FP serum tumour marker levels (Figure 3). Conversely, in 1 patient seemingly disease-free, antibody scanning was negative, thus confirming the absence of active disease, whereas the CT scan demonstrated a residual mass, which was found subsequently to be necrotic tumour after laparotomy. Therefore, this method can be valuable in helping to avoid unnecessary surgical explorations in patients with residual masses after induction chemotherapy for GCTs. Antibody scans were consistently negative in the 8 patients who were rendered disease free after induction chemotherapy ± adjunctive surgery (Table 3).

The observed successful localisation of testicular tumours with ^{111}In- or ^{123}I-labelled H17E2 MAb can be explained by the ability of this antibody to bind avidly and specifically to tumours expressing PLAP, thus resulting in a high sensitivity of the method. The observation that not all patients with active disease had positive immunolocalisation studies can be explained by the heterogeneity of PLAP expression between patients with GCTs and different tumour sites in the same patient, the latter reflecting the usually observed discordance in antigen expression between primary tumour and metastatic sites.

A further role of the monoclonal anti-PLAP MAb scan would appear to be for patients where there is uncertainty about the disease status, i.e. in patients with a raised tumour marker-PLAP, β-hCG, or α-FP but no evidence of disease on conventional imaging studies, or in patients where the results of CT scans and lymphagiograms are inconclusive, or in patients with equivocal clinical and biochemical findings.

In patients undergoing repeated imaging studies, we did not observe the development of a HAMA response and no acute hypersensitivity or type III (serum sickness) immune reaction were observed. However, repeated injections of higher doses of murine MAbs usually lead to an immune response. Therefore, this possibility has to be always monitored when murine antibodies are administered.

In order to overcome murine MAb immunogenicity, human or near-human (chimaeric or CDR-grafted) MAbs have entered therapeutic pilot clinical studies [31–33]. A CDR-grafted human MAb, Hu2PLAP, against PLAP, was constructed by incorporating the CDRs from the murine IgG1 antibody H17E2 into a human IgG1 framework. Both Hu2PLAP and H17E2 recognise human PLAP as antigen, but the humanised MAb has a lower binding affinity for PLAP compared to its murine equivalent. Before starting clinical therapy studies with Hu2PLAP, it is extremely important to show that the genetically reshaped human MAb has the ability to localise successfully PLAP-expressing tumours. In addition, improved chemistry of the new generation of metal isotope chelating agents, such as the macrocycle benzyl-DOTA, prompted us to combine all these postulated improved features into a novel radioimmunoconjugate designated Hu2PLAP-DOTA-^{111}In, and evaluate it in a pilot radioimmunolocalisation study. 3 patients with PLAP-positive tumours showed successful localisation following i.v. administration of Hu2PLAP-DOTA-^{111}In. 1 of these patients had a pre-injection HAMA response, which had developed after previous treatments with murine MAbs administered i.p., and cleared the humanised MAb with a $t_{1/2}\beta$ lower than that of HAMA-negative patients. Therefore, even in the presence of high HAMA levels, it is possible to obtain adequate tumour localisation when using a humanised MAb. This finding demonstrates that only partial cross-reactivity exists between the constant regions (Fc) of murine and human IgG1 antibodies. The 3 negative patients were 1 with PLAP-positive ovarian cancer in CR, 1 with a PLAP-negative tumour and 1 with active PLAP-positive ovarian cancer who cleared the Hu2PLAP-DOTA-^{111}In immunoconjugate with a $t_{1/2}\beta$ = 39 h, due to the development of HAMA and anti-chelate (anti-DOTA) antibodies after previous successful imaging with H17E2-DOTA-^{111}In. It can be speculated that the anti-chelate immune response was more important in clearing the Hu2PLAP antibody, although the parallel contribution of the anti-globulin response to the formation of large immune complexes (HAMA-mab-DOTA-anti-DOTA antibodies) in the systemic circulation, which shifted rapidly to the liver and spleen, cannot be excluded. One patient with a malignant pleural effusion, secondary to breast adenocarcinoma, showed a positive uptake of Hu2PLAP-DOTA-^{111}In, despite being negative for PLAP expression. This is considered as a false positive result and can be explained by the previously reported non-specific uptake of MAbs by lung tumours [27].

The *Ka* of the humanised MAb Hu2PLAP is indeed lower than the *Ka* of its murine counterpart H17E2, which is very high, but not so low as to limit its tumour targeting efficacy. We have performed animal studies in PLAP-positive tumour xenograft-bearing nude mice and found that, at 5 days post-MAb injection, there was only a 1.7-fold greater concentration of H17E2 in tumours compared to Hu2PLAP. These data may be a reflection of the higher affinity of the murine antibody, but the level of the humanised antibody in tumours was still 2.5-fold greater than that for a murine non-specific (not binding to PLAP) antibody used as a control [34].

One further limitation could be the reduced affinity of Hu2PLAP for its antigen and the high serum levels of PLAP observed in seminomas, which may, in fact, pose a problem to the successful imaging of these tumours. Unfortunately we did not have the opportunity to study any seminomas with Hu2PLAP. Therefore, additional trials evaluating the ability of Hu2PLAP to localise seminomas are needed in the future.

No anti-idiotypic immune responses were detected in any of our patients, but 2 patients developed anti-DOTA antibodies. Chimeric antibodies, constructed by genetically grafting the whole variable (V) region of the murine MAb to a human MAb constant region, has led to the development of anti-V region antibodies, which, if anti-paratopic, block MAb binding [32]. The use of humanised MAbs may modulate the HAMA response or allow administration in HAMA-positive patients, but may not protect against the immunogenicity of foreign molecules attached to it. In our case, the macrocyclic chelating agent DOTA presumably acted as an hapten on a macromolecular protein carrier, the Hu2PLAP antibody. Similar anti-chelate antibody development has been observed in patients with ovarian cancer receiving i.p. DOTA- or benzyl-DTPA-based immunoconjugates of ^{90}Y [28, 35]. Therefore, careful monitoring for such immune responses should always be undertaken in future trials.

In conclusion, this sequential study of MAb-guided imaging of PLAP expressing tumours has resulted in important information gained for the design of future immunolocalisation studies using radiolabelled MAbs. H17E2 antibody scanning should be considered in patients with testicular tumours, who come into the categories discussed above, so as to maximise the amount of information available before and/or after therapy. Since these tumours represent a potentially curable disease, antibody scanning could contribute to accurate staging, localisation of active disease after induction chemotherapy and possible avoidance of adjunctive surgery for postchemotherapy residual masses, and monitoring the presence of recurrent disease. Nevertheless, the exact role of the radiolabelled H17E2 MAb for the immunolocalisation of testicular neoplasms will only be determined by performing a prospective study in a large number of patients. Reshaped human antibodies with anti-tumour specificity may, by modulation of the HAMA response, enable repeated administration of antibodies to patients for both diagnostic imaging and therapy purposes. Targeting PLAP expressing tumours by the CDR-grafted reshaped human MAb Hu2PLAP is feasible and encouraging, and the long-term advantages of this approach will be assessed only in larger trials.

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