Table 2. Non-haematological toxicity

	WHO Grade				
Side effects	0	1	2	3	4
Nausea	9	4	5	2	1
Diarrhoea	19	2	—	—	—
Liver toxicity	18	2	—	—	1
Renal toxicity	19	2	—	—	—

after the first 4 weeks because of nausea/vomiting. All patients were considered evaluable for toxicity. There were no haematological side-effects; non-haematological side-effects are listed in Table 2. Nausea/vomiting were predominant and were present in 12 patients (57%), one of which was taken off the study because of grade 4 vomiting resistant to treatment. The other side-effects were infrequent. In 1 patient a grade 4 transaminase elevation was observed after 4 weeks, which was completely reversible after discontinuation of drug administration. The patient was taken off the study because of this toxicity. Besides these 2 patients, 1 patient was not evaluable for response because of cardiac death on day 24. Thus 18 patients were considered evaluable for response. Only 1 patient had no change for 16 weeks; all other patients progressed.

DISCUSSION

Previous studies have indicated that drugs with even moderate activity against soft tissue sarcomas can be discovered in second-line studies [1]. Miltefosine, tested in this situation, did not yield any activity. Although toxicity in the short term was manageable and apparently tolerable, the large number of patients with nausea/vomiting induced by the drug suggests that long-term treatment will be less feasible. Whether the lack of activity is related to a low bioavailability of the oral formulation in man remains to be elucidated. Miltefosine at this dose and schedule should not be tested any further in soft tissue sarcomas.

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Serum Immunoreactive and Bioactive Lactogenic Hormones in Advanced Breast Cancer Patients Treated with Bromocriptine and Octreotide

Elizabeth Anderson, Janice E. Ferguson, Helen Morten, Steven M. Shalet, E. Lawrence Robinson and Antony Howell

6 patients with advanced breast cancer who had failed first and second line endocrine therapies received bromocriptine (1.25–2.5 mg twice daily *per os*) and octreotide (Sandostatin®) via a continuous subcutaneous infusion (200–400 μg/24 h) until disease progression. Pre-treatment 24-h profiles of serum lactogenic hormones and their response to standard provocative tests were established and repeated at 2 weeks, and 3 and 6 months (or at tumour progression). Immunoreactive prolactin (ir-PRL), growth hormone (ir-GH) and insulin-like growth factor I (IGF-I) were measured by radioimmunoassay and bioactive lactogenic hormone levels (BLH) were estimated using the Nb2 rat lymphoma cell bioassay. Before treatment all patients showed episodic secretion of ir-PRL, ir-GH and BLH and provocative stimuli resulted in a peak of ir-GH and BLH maximal between 60 and 90 min after injection but no change in ir-PRL. After 2 weeks of treatment, ir-PRL levels were reduced to below the limit of detection in all 6 patients. Peaks of ir-GH and BLH were still apparent, although much reduced. Immunoreactive PRL continued to be profoundly suppressed in 3 of the 4 patients who remained on treatment for 3 to 6 months. Small pulses of ir-GH were still detectable in these patients with which BLH was, again, well correlated. After 2 weeks of treatment, serum IGF-I levels were reduced by 9–54% of the pretreatment values and generally remained suppressed throughout treatment. Clinically, 4 patients did not show disease progression for periods of up to 6 months and side-effects were minimal.
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Table 1. Clinical details of patients entered into the octreotide and bromocriptine study

Patient	Age	Sites of disease	Treatment	Response	Previous treatment
A	61	Chest wall, lymph nodes, pleural effusion, bone	Octreotide 200 μg Bromocriptine 1.25 mg twice daily	Stabilisation	Tamoxifen Megestrol
B	59	Skin, bone	Octreotide 200 μg Bromocriptine 2.5 mg twice daily	Progression	Tamoxifen Aminoglutethimide and hydrocortisone Megestrol Tamoxifen
C	70	Axilla, lungs, pleural effusions	Octreotide 200 μg Bromocriptine 2.5 mg	Progression	Tamoxifen Medroxyprogesterone acetate
D	78	Chest wall, axilla, lymphoedema	Octreotide 400 μg Bromocriptine 2.5 mg twice daily Tamoxifen 20 mg/day	Progression after 20 weeks	Tamoxifen Megestrol Tamoxifen
E	53	Lymph nodes	Octreotide 400 μg Bromocriptine 2.5 mg twice daily Megestrol	Progression after 18 weeks	Tamoxifen Megestrol
F	78	Chest wall and back, axilla	Octreotide 400 μg Bromocriptine 2.5 mg twice daily Tamoxifen 20 mg/day	Progression after 15.5 weeks	Tamoxifen Aminoglutethimide and prednisolone Tamoxifen and prednisolone

INTRODUCTION

THE ROLE of prolactin (PRL) in rodent mammary tumorigenesis is well defined and it is clear that raised levels of the hormones are associated with both the initiation and maintenance of tumours [1]. The situation in humans, however, is less clear and the roles of the lactogenic hormones, PRL and growth hormone (GH) in either the early or late stages of breast cancer are not well understood.

Early studies on advanced breast cancer patients treated by hypophysectomy suggest the involvement of a pituitary factor in the promotion of tumour growth [2]. This is supported by several reports of an association of raised serum prolactin levels with a poor prognosis and resistance to therapy [3–9]. A substantial proportion of breast tumours express cell surface receptors for PRL to which GH also binds [10, 11] and *in vitro* experiments have shown that PRL stimulates colony formation by human breast tumour cells in primary soft agar culture [12]. Additionally, stimulation of proliferation of the MCF-7 breast cancer cell line by PRL under strictly defined culture conditions has been demonstrated [13]. However, treatment of advanced breast cancer with bromocriptine, a dopamine agonist which inhibits PRL secretion, has been largely unsuccessful either alone or in combination with tamoxifen and medroxyprogesterone acetate [14–16]. This lack of effect may be due to the failure of bromocriptine to reduce circulating GH levels as well as PRL, and it may be hypothesised that either this hormone is sufficient to directly maintain tumour progression or that it is maintaining tumour growth indirectly via synthesis of insulin-like growth factor I (IGF-I) in the liver, breast and other tissues. Evidence for a direct effect of GH comes from studies showing that it is capable of binding to and stimulating the growth of human breast cancer cells in culture [17, 18]. In support of an indirect action, it is known that IGF-I is a potent mitogen for human breast cancer cell lines in culture [19] and up to 90% of human breast tumours contain receptors for this peptide [20].

The secretion of GH is inhibited by somatostatin and the development of long-acting, superpotent somatostatin analogues such as octreotide (Sandostatin®) [21] has provided a means by which the effects of GH upon human breast cancer may be studied. This has lead several groups to assess the value of octreotide alone or in conjunction with bromocriptine in the treatment of advanced breast cancer [22–24]. Most of these studies have included measurement of serum PRL and GH levels before and during therapy either in a single sample or after pituitary stimulation with insulin, arginine or L-dopa [22]. Generally, it is found that GH and PRL secretion in such situations is effectively suppressed by combined treatment with octreotide and bromocriptine. There is, however, a lack of information about the patterns of lactogen secretion throughout the day in breast cancer patients and it is not known whether octreotide and bromocriptine at the commonly used doses suppress pulsatile hormone secretion at all times. Finally, it is not known whether suppression of biological lactogenic activity accompanies suppression of the immunoreactive lactogenic hormones. In order to answer these questions we devised a protocol whereby 24-h profiles of immunoactive and bioactive lactogenic hormone secretion were determined in a small group of heavily pretreated advanced breast cancer patients before and during treatment with octreotide and bromocriptine.

PATIENTS AND METHODS

Subjects

6 patients with histologically proven breast cancer and objective evidence of disease progression after at least two endocrine agents were entered into the trial. All patients had at least one site of measurable or evaluable disease and a WHO performance

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status of 0–2. All were postmenopausal or had had previous bilateral oophorectomy and all had a life expectancy of at least 3 months. Patients were excluded if they had other current or previous malignancies with the exception of successfully treated carcinoma *in situ* of the uterine cervix and adequately treated basal cell carcinoma of the skin. Patients with meningeal or brain involvement and those with rapidly progressive lung or liver disease were also excluded. The clinical details of these patients are outlined in Table 1.

Study procedure

Pretreatment investigations included a full clinical history and examination, assessment of quality of life, full blood count, liver function tests, chest and pelvic X-rays, and bone and liver scans (where necessary). Clinical examination was repeated at 2 weeks, 3 and 6 months after the start of treatment or at tumour progression.

For determination of 24-h profiles of GH and PRL secretion, the patients were cannulated via the antecubital vein and 10-ml

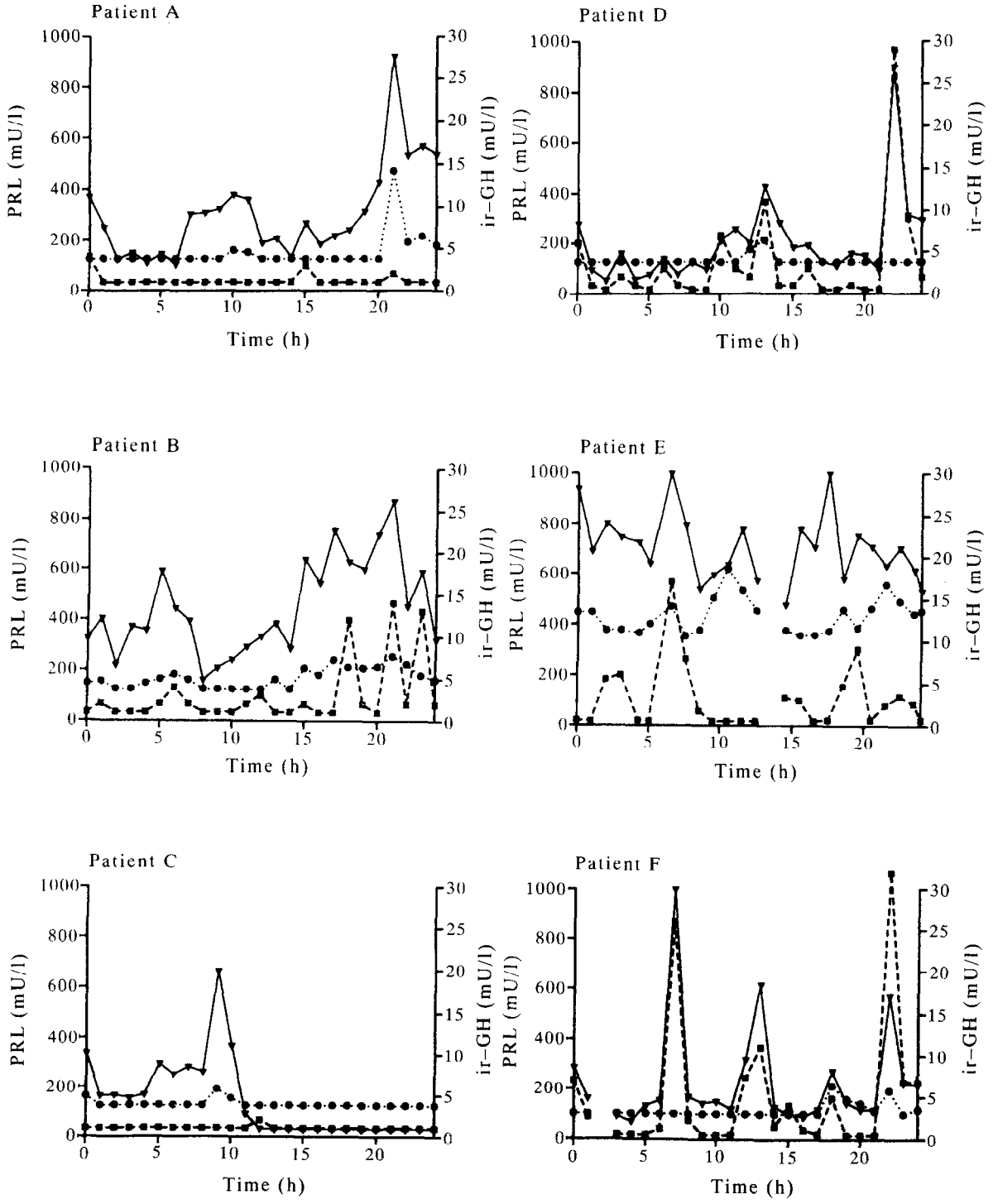

Fig. 1. Secretion of bioactive lactogenic hormone (▲), ir-PRL (●) and ir-GH (■) throughout 24 h in 6 patients with advanced breast cancer before treatment with a combination of octreotide and bromocriptine. Hourly sampling was started at 9.00 a.m. in all patients.

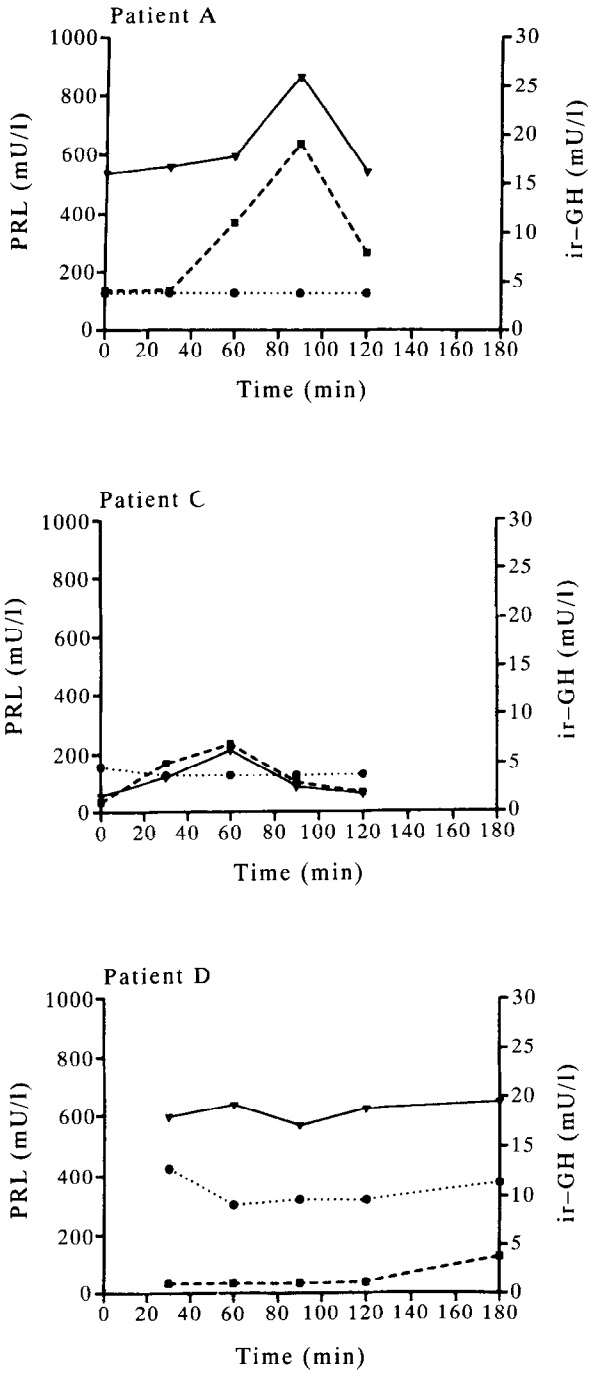

Fig. 2. Response of bioactive lactogenic hormone (▲), ir-PRL (●) and ir-GH (■) to provocative stimuli in 3 advanced breast cancer patients before treatment with a combination of octreotide and bromocriptine. The stimuli were insulin (patients A and C) and glucagon (patient D).

blood samples were taken hourly. At time 0 h, sufficient blood was collected for the estimation of baseline cortisol, follicle stimulating hormone (FSH), leuteinising hormone (LH) and oestradiol levels. At the end of the 24-h sampling period, the patients underwent an insulin tolerance test (ITT) and blood sampling was continued for a further 2 h. The ITT was not well tolerated by the patients especially when they were receiving octreotide and a glucagon stimulation test (GST) was substituted where necessary. The 24-h profiles were repeated after 2 weeks of treatment in all patients and at 3 and 6 months in those patients still receiving treatment.

Treatment

Details of treatments given to each patient are given in Table 1. Continuous infusion was chosen as the route of administration as it has previously been shown to be more effective in producing sustained GH suppression in acromegalic patients [25]. Patients who were receiving endocrine therapy (tamoxifen or megestrol) immediately prior to this study remained on this medication during treatment.

Radioimmunoassays

Immunoreactive PRL (ir-PRL) was measured by means of an in-house radioimmunoassay (RIA) that utilised [^{125}I]PRL supplied by the North East Thames Regional Immunoassay Laboratory (NETRIA, London, UK) and antiserum from the Chelsea Hospital for Women (London, UK). The assay was standardised against the 81/541 reference preparation supplied by the National Institute for Biological Standards and Control (NIBSC, South Mimms, UK) and the lower limit of detection was 125 mU/l. Immunoreactive GH (ir-GH) was measured using the NETRIA immunoradiometric assay, standardised against the 80/505 1st International Reference Preparation supplied by NIBSC. The lower limit of detection for this assay was 0.5 mU/l.

Extracted serum IGF-I levels were assayed by means of an RIA based on a rabbit polyclonal antiserum raised by Armstrong *et al.* [26] and using their method. Briefly, 100 μl of sample appropriately diluted in assay buffer (35 mmol/l NaH_2PO_4, pH 7.4, containing 9 mmol/l EDTA, 0.2 mmol/l NaN_3, 0.05% v/v Tween 20 and 0.1% RIA grade bovine serum albumin) or standard (recombinant human IGF-I supplied by Amersham International, UK) was incubated, in duplicate, with 100 μl of [^{125}I]IGF-I (approximately 30000 cpm) and 200 μl of the antiserum at a dilution of 1:4000 for 48 h at 4°C. Tubes for the estimation of non-specific binding contained 200 μl of assay buffer instead of the antiserum and non-specific binding was estimated for each serum sample. After incubation, the antibody was precipitated by adding 100 μl of donkey anti-rabbit immunoglobulin antiserum at a dilution of 1:20 in 0.5% v/v rabbit serum and incubating at room temperature for 3 h. 1 ml of 25% w/v polyethylene glycol (*M* 8000) was added to each tube and incubation was continued for 30 min. The tubes were centrifuged at 900 *g* for 30 min at 25°C after which 100 μl of a 10% w/v starch solution was added. The tubes were then recentrifuged as before. The supernatants were aspirated and the precipitates counted in a Canberra Packard gamma counter. The lower limit of detection for this was 0.4 ng/ml with an intra-assay coefficient of variation (COV) of 4.9% and an inter-assay COV of 12.7%.

Bioassay

Serum levels of total bioactive lactogenic hormones (BLH) were estimated using the Nb2 rat lymphoma cell bioassay of Tanaka *et al.* [27], modified as described in Anderson *et al.* [28]. The sensitivity of the assay was 30 mU/l, the intra-assay COV determined from the human serum control was 5.6% (mean of 14 values obtained for duplicate estimations) and the interassay COV was 10.7% (n = 15; 121 ± 12 mU/l). Although the Nb2 cells were stimulated to proliferate by both PRL and GH, no attempt was made to separately measure the contribution made by either hormone to the total lactogenic activity. The results are expressed, therefore, as total bioactive lactogenic hormone activity in mU PRL equivalent/l. Neither octreotide nor bromocriptine at therapeutic levels had any effect upon the growth of the Nb2 cells (data not shown).

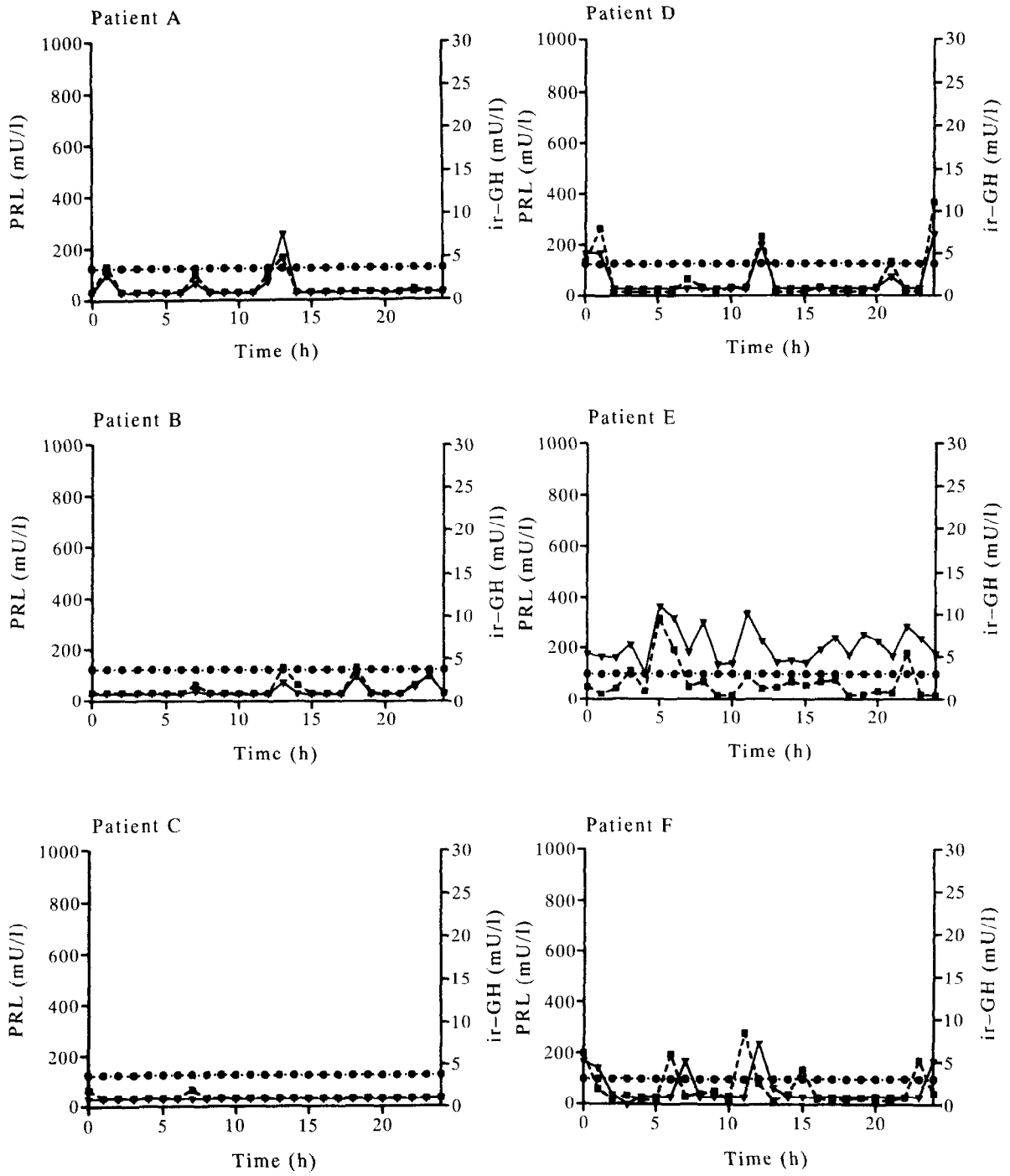

Fig. 3. Secretion of bioactive lactogenic hormone (▲), ir-PRL (●) and ir-GH (■) throughout 24 h in 6 advanced breast cancer patients after 2 weeks of treatment with combined bromocriptine and octreotide. Hourly sampling was started at 9.00 a.m. in all patients.

RESULTS

Endocrinological effects of treatment

Immediately prior to treatment, all 6 patients showed episodic secretion of ir-PRL, ir-GH and BLH (Fig. 1) and, in general, BLH levels corresponded to ir-PRL and ir-GH. Where provocative tests were performed, only ir-GH and BLH were stimulated in responding patients, with maximal responses being seen 60–90 min after injection of the stimulus (Fig. 2).

After 2 weeks of treatment, all 6 patients showed significant decreases in serum levels of ir-PRL, ir-GH and BLH, irrespective of the dose of octreotide (see Fig. 3). In all patients, ir-PRL levels were reduced to below the limit of detection of the RIA but small peaks of ir-GH were still measurable and the BLH levels correlated almost absolutely with these peaks. Serum ir-GH and BLH secretion could still be provoked by pituitary stimulation in those patients who had responded when tested before the start of treatment (data not shown).

4 patients achieved 3 months of treatment with octreotide and bromocriptine. The 24-h endocrinological profiles of these patients showed that their ir-PRL levels remained undetectable (Fig. 4) but that small peaks of ir-GH were still apparent with which BLH was well correlated. 3 of these patients had been treated with the higher dose of octreotide.

3 patients went on to receive octreotide and bromocriptine for a total of 6 months. The 24-h profiles of 2 of these patients were essentially the same as they had been at 2 weeks and 3 months after the start of treatment (Fig. 5). The third patient, however, appeared to have escaped from suppression by octreotide and bromocriptine and serum levels of ir-PRL, ir-GH and BLH during the 24-h profile and after provocative stimuli (Fig. 6) had increased to pretreatment levels or greater.

Serum IGF-I levels were measured in the baseline samples from each of the 24-h profiles. Before treatment, the serum IGF-I concentrations ranged from 322 to 840 ng/ml. Treatment with octreotide and bromocriptine resulted in a fall in circulating IGF-I levels of between 9 and 54% in all 6 patients after 2 weeks. These reduced levels were maintained throughout treatment (data not shown) with the exception of patient A whose level was raised above the pretreatment value at the 6-month profile.

Clinical effects of treatment

In 4 of the 6 patients (A, D, E and F) there was no evidence of disease progression for periods of up to 6 months. In the remaining 2 patients there was rapid progression of tumour growth and the patients ceased treatment with bromocriptine and octreotide after 2 weeks. The toxicity of the treatment was minimal, with some patients reporting gastrointestinal disturbances which were not severe enough to stop therapy, although 1 patient (A) could only tolerate a maximum bromocriptine dose of 1.25 mg twice daily because of headache and fatigue.

DISCUSSION

Studies in which secretion of both GH and PRL is inhibited by a combination of bromocriptine and octreotide indicate that this approach may have a place in breast cancer therapy [22–24]. Pituitary function in these studies was assessed either by measuring the appropriate hormones in a single blood sample or by monitoring the response to provocative stimuli and gave no information as to the importance of the variable pattern of lactogenic hormone secretion throughout the 24-h period.

Before treatment, the 6 patients presented in this study showed episodic secretion of ir-PRL, ir-GH and BLH, although their patterns of secretion throughout the day were disordered. None of the patients showed the nocturnal/early morning (12–7.00 a.m.) increase in ir-PRL normally seen in postmenopausal women [29]. These findings are not in agreement with Bartsch *et al.* [30] who have demonstrated maximal PRL secretion late at night in their group of secondary breast cancer patients, but concurs with Malarkey *et al.* [29] who have shown a similar disorder of PRL secretion in primary breast cancer patients. However, the two groups of patients investigated were not comparable. The patients in our study were a very heterogeneous group in terms of the treatment they were receiving and, in 3 patients, tamoxifen and megestrol were being administered at the same time as the bromocriptine and octreotide.

The results from this study show small pulses of ir-GH secretion with a magnitude and frequency similar to those usually demonstrated in postmenopausal women [31].

It appears that ir-GH and ir-PRL both contribute to the lactogenic bioactivity as measured by the Nb2 rat lymphoma cell bioassay. It has been postulated that a species of bioactive but not immunoreactive prolactin is associated with the development of breast cancer [32]. It cannot be determined from the data presented here whether there are additional species of

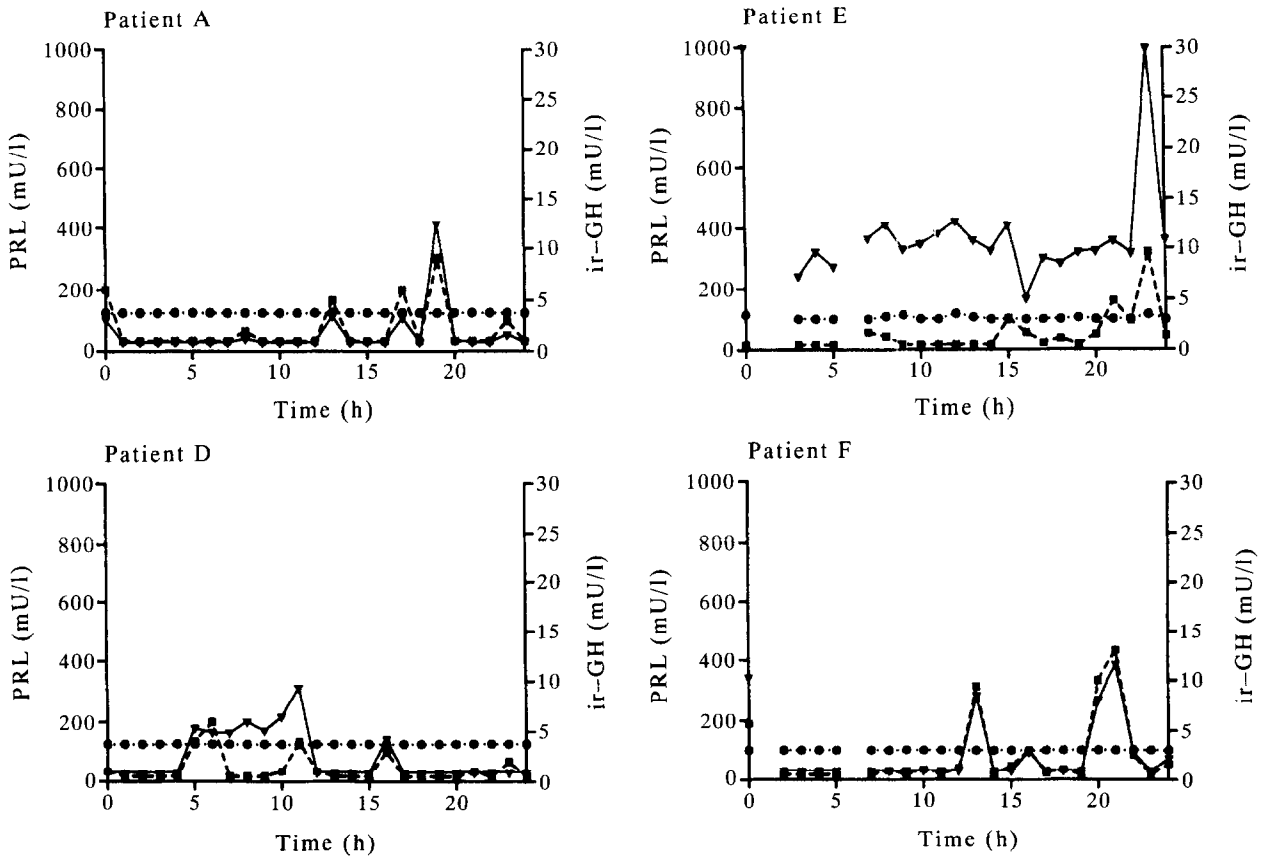

Fig. 4. Secretion of bioactive lactogenic hormone (▲), ir-PRL (●) and ir-GH (■) throughout 24 h in 4 patients who achieved 3 months of treatment with combined bromocriptine and octreotide. Hourly sampling was started at 9.00 a.m. in all 4 patients.

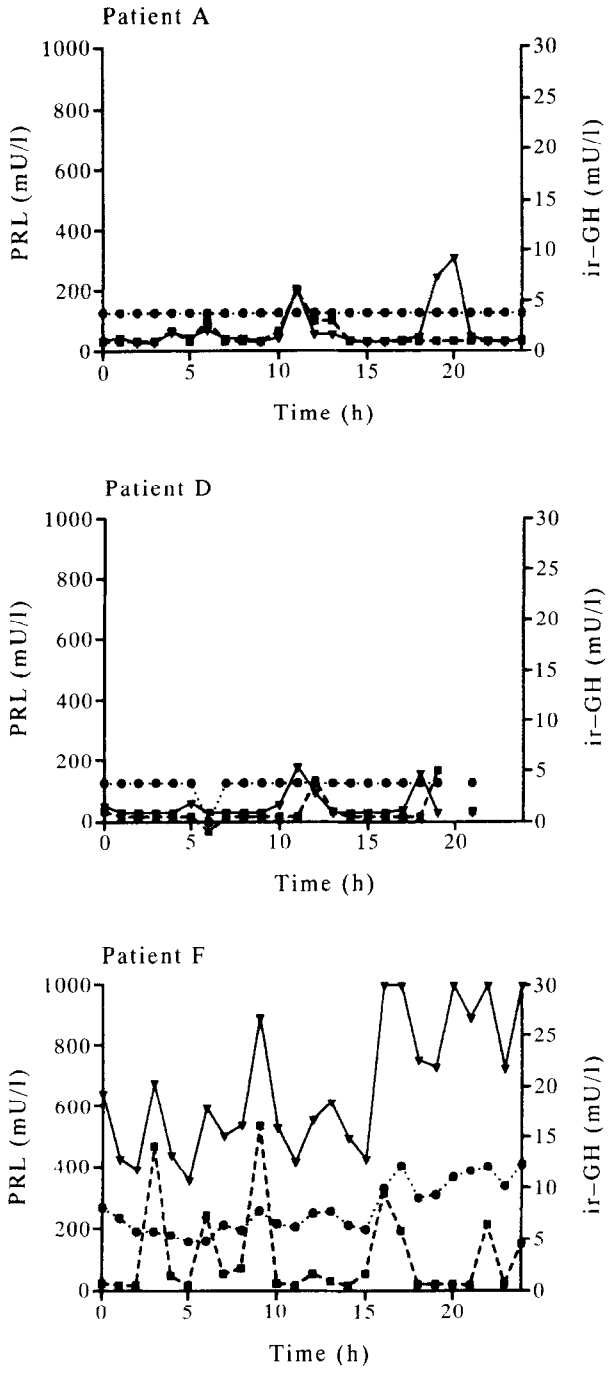

Fig. 5. Secretion of bioactive lactogenic hormone (▲), ir-PRL (●) and ir-GH (■) throughout 24 h in 3 advanced breast cancer patients after 6 months of treatment with bromocriptine and octreotide (patient A) or at tumour progression (patients D and F). Hourly sampling was started at 9.00 a.m. in all patients.

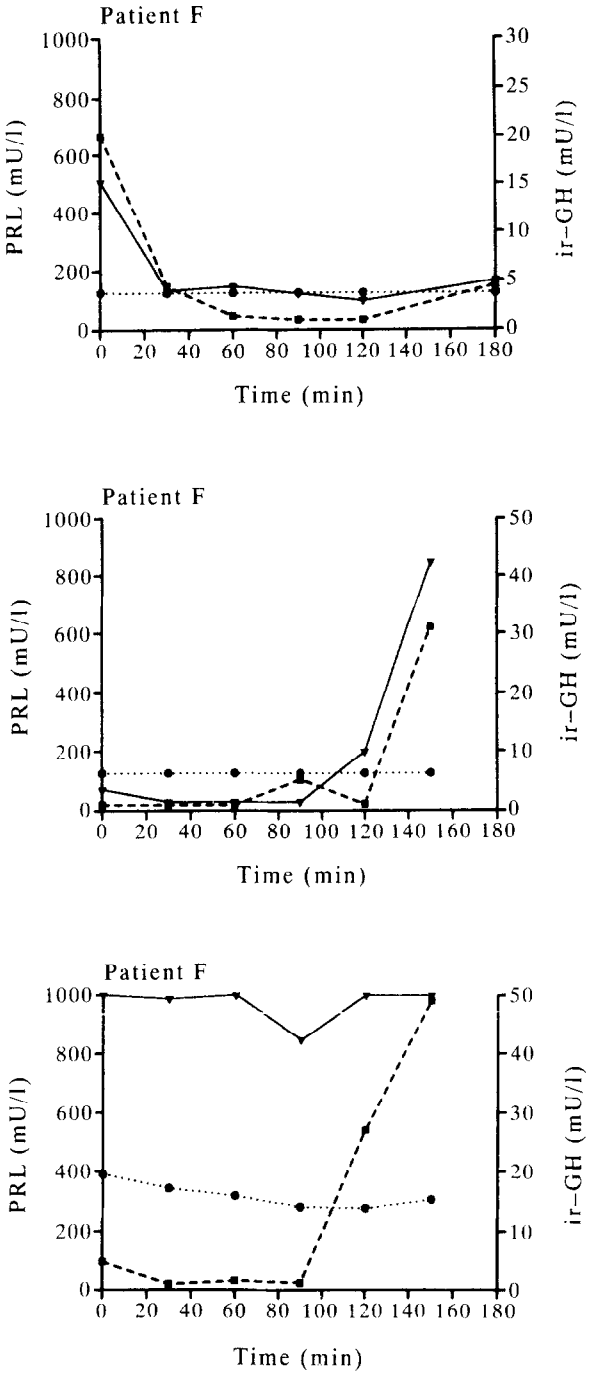

Fig. 6. Response of bioactive lactogenic hormone (▲), ir-PRL (●) and ir-GH (■) to pituitary stimulation by glucagon in patient F throughout therapy with bromocriptine and octreotide. Tests were performed at the start of treatment (top panel) and after 3 months (middle panel) and 6 months (lower panel).

bioacive lactogenic hormone present in the sera of advanced breast cancer patients [28].

It was clear from the profiles taken 2 weeks after the start of treatment that the combination of bromocriptine and octreotide was highly effective in suppressing ir-PRL secretion throughout the day. However, whilst the pulses of ir-GH secretion were greatly reduced, they were not completely abolished and the detectable BLH at this time corresponded completely to the ir-GH peaks. This finding is surprising because the route of octreotide administration was chosen because it caused the most effective and sustained suppression of GH levels in acromegaly [25]. As well as the suppression of basal ir-GH levels, the combination of bromocriptine and octreotide reduced the ir-GH response to pituitary stimulation by either insulin-induced hypoglycaemia or glucagon administration in some patients which is in line with the findings of the study by Manni *et al.* [22]. As in the pretreatment pituitary stimulation tests, ir-PRL was not increased and BLH corresponded completely to the ir-GH levels.

In 3 of the 4 patients who remained on bromocriptine and octreotide treatment for 3 and 6 months, ir-PRL remained profoundly reduced whilst ir-GH and consequently BLH con-

tinued to show decreased but detectable episodic secretion. In those patients who underwent pituitary stimulation, the responses of ir-GH and BLH varied but were generally blunted. The exception was patient F who, before treatment, had quite large pulses of ir-GH secretion which were not reduced to the same extent as in the other patients after 3 months of treatment. At the 6-month time point, however, this patient's ir-PRL, ir-GH and BLH levels were elevated to those seen before the start of the study. It is suspected that this patient stopped her treatment before being admitted for the final 6-month investigations and the rises seen in the lactogenic hormone levels are in accordance with the initial trials of octreotide in acromegaly which showed that withdrawal of treatment did not result in a rebound of ir-GH secretion but that the levels gradually returned to pre-treatment values [33]. It may be worth noting that Manni *et al.* [22] have reported one escape from ir-PRL suppression in their series of 10 breast cancer patients.

By profiling lactogenic hormone secretion we have shown that the combination of bromocriptine and octreotide is highly effective in reducing basal and stimulated ir-PRL and ir-GH levels in women with advanced breast cancer. This reduction is probably biologically significant because there is an accompanying decrease in lactogenic bioactivity. There was also an accompanying decrease in serum IGF-I content in all 6 of the patients after 2 weeks treatment. These lower levels were maintained after 3 months therapy and in 2/3 patients after 6 months. In patient A, however, there appeared to be an increase in serum IGF-I content such that it was higher than before the start of treatment. These data on serum IGF-I levels concur with those of Manni *et al.* [22].

It is impossible to state whether the combination of octreotide and bromocriptine is an effective treatment for advanced breast cancer because of the heterogeneity of the therapy administered to the patients. In view of the relatively low toxicity of the regime it would seem worth determining the efficacy of this combined approach in a larger group of less heavily pretreated patients. The route of administration should be carefully considered as it is clear from our studies that even continuous infusion does not completely suppress pulsatile secretion of ir-GH and associated bioactivity.

Finally, the mechanisms by which octreotide and bromocriptine exert their effects upon human breast tumour cells are, at present, unknown. There are a number of possible explanations. Reducing both PRL and GH may be sufficient to cause tumour regression. The reduction of serum IGF-I levels accompanying the GH decrease may inhibit tumour growth or octreotide itself may have direct inhibitory effect upon the breast tumours. Certainly, *in vitro* studies have shown a direct growth inhibitory effect upon human breast cancer cells in culture [34].

It is apparent from our studies that important additional information may be obtained from the detailed profiling of pituitary hormone secretion in breast cancer patients and measurement of hormone levels in single serum samples or only after pituitary stimulation does not take into account many of the factors governing lactogenic hormone secretion. In view of the relatively low toxicity of the combination of bromocriptine and octreotide, it would seem feasible to extend the clinical trials to include less heavily pretreated breast cancer patients at an earlier stage of disease in order to determine the true effectiveness of the regime.

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Preferential Antibody Targeting to Small Lymphoma Metastases in the Absence of the Primary Tumour

Udo Schmid, Volker Schirrmacher, Frank Momburg and Siegfried Matzku

Targeting of spontaneous liver metastases of the ESb.MP murine lymphoma was achieved with anti-CD2 monoclonal antibody (MAb) 12-15A, which does not react with normal liver tissue. Using quantitative autoradiography on whole body sections of animals that had received a standard dose of 1.1 MBq of ^{125}I-labelled monoclonal antibody, metastases accumulated up to > 90% of the injected dose per gram (id/g). The average uptake of primary tumour lesions was at a low level of 24 Bq/mg (corresponding to 2.2% id/g) because of highly non-uniform accumulation, while metastatic lesions were all above 50 Bq/mg. Uptake was particularly pronounced in animals tested after resection of the primary tumour: 85% of metastases showed levels above 300 Bq/mg, which was the upper limit of uptake in metastases of non-resected animals. These findings demonstrate the potential of the antibody approach with regard to attacking residual metastatic lesions after debulking.
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INTRODUCTION

THERE IS increasing evidence to show that monoclonal antibodies (MAb) are non-uniformly accumulated in varying tumour types [1–7], the pattern being dependent on tumour architecture and the nature of the target antigen. We set out to analyse whether small metastatic foci would show a more uniform accumulation, thereby reaching high uptake levels as observed in some segments of macroscopic tumour nodules, or whether they are secluded by inherent accessibility barrier(s). The ESb.MP murine lymphoma model [8] was chosen to address the question. ESb.MP metastasises reproducibly into the liver irrespective of the mode of implantation (i.e. intradermal or subcutaneous) and of surgical removal of the primary inoculum. Targeting of primary and secondary processes was successfully achieved with MAb 12-15A, while non-specific antibodies or fragments showed no marked accumulation [9]. MAb 12-15A [10] recognises an epitope of murine CD2 differentiation antigen and possibly also of the Fc receptor [11, 12], the antigen density being much higher on ESb.MP lymphoma cells than on murine lymphocytes [10]. Accumulation of labelled MAb 12-15A could be selectively directed towards lymphoma tissue by pretreatment of animals with the unlabelled MAb, the effect

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