study any substantial effect of age on local relapse rate in either group. The above-mentioned differences in local recurrence risk between the two study arms might be less important when looking at local recurrences which are the first or only sign of treatment failure (see Table 3). It should be appreciated that the total number of local relapses is small and indeed included some appearing together with or after distant disease manifestation. The possible impact of size and nodal status on treatment choice therefore is restricted.

The importance of the mentioned prognostic factors will be studied in a multivariate analysis, which will be performed after completion of the pathology review. Extensive intraductal component (EIC) and other pathology criteria will then also be studied. In a recent interim evaluation the impact of EIC does not appear to be large in this study probably because of the high boost dose and the large boost volume.

The result of salvage treatment after local recurrence in this study does not appear to be better in the BCT group if compared with the results after salvage therapy for local recurrence in the RM group. However, it is too early to draw definite conclusions because of the relatively small numbers of local recurrences. This disappointing finding, which differs from literature data, suggests that there is a fair chance that many of the tumours in the patients with recurrent disease are in both groups of the same type and biological behaviour, locally relapsing irrespective of the treatment given.

The poor result of the treatment of recurrences in the BCT group might also be explained in part by the postulate that in these patients early diagnosis of local recurrence is hampered by fibrosis in the boost region as was frequently seen in our BCT cases.

Patients' satisfaction with treatment was high in the BCT group. Proper evaluation of quality of life will be addressed in a separate study.

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Intermittent High-dose Tamoxifen as a Potential Modifier of Multidrug Resistance

Michael J. Millward, Brian M.J. Cantwell, Ernst A. Lien, James Carmichael and Adrian L. Harris

***In vitro* tamoxifen reverses multidrug resistance (MDR). To evaluate the clinical potential of using tamoxifen in this way, intermittent high-dose tamoxifen was combined with oral etoposide in 86 patients. At 320 mg/day tamoxifen for 6 days, mean plasma levels of tamoxifen in 11 patients increased from 453 ng/ml (range 269–664) on day 2 to 984 ng/ml (578–1336) on day 6. Of 31 patients who had plasma tamoxifen measured during the time of etoposide administration (days 4–6), 13 (43%) were over 1111 ng/ml (3 μmol/l), an active *in vitro* level. Potentially active levels of the principal metabolite, *N*-desmethyl tamoxifen, were also obtained but accumulation was slower. Emesis and thromboembolism were toxicities. Tamoxifen is a modifier of MDR, a role that warrants further clinical studies.**
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INTRODUCTION

THE RESISTANCE of malignant cells to anticancer agents is a major cause of treatment failure in cancer. The multidrug resistant phenotype (MDR) is a form of cellular resistance characterised by reduced intracellular drug accumulation related to a cell membrane glycoprotein termed the P-glycoprotein which may be induced following exposure to a variety of structurally and functionally dissimilar cytotoxics such as etoposide, vinca alkaloids and anthracyclines [1, 2] with resulting cross resistance. Expression of the MDR has been shown to be correlated with clinical drug resistance in pretreated lymphoma, myeloma, leukaemia and soft tissue sarcoma [3, 4] and has been reported prior to any cytotoxic exposure in several tumour types including sarcoma, renal carcinoma, carcinoid tumours and breast cancer [4–6]. Thus strategies to overcome the MDR have the potential to benefit both

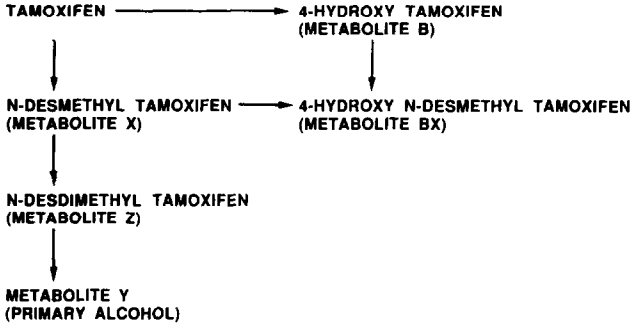

Fig. 1. Metabolic pathways of tamoxifen.

chemotherapy resistant patients and untreated patients with a variety of malignant diseases.

Tamoxifen can overcome MDR mediated doxorubicin resistance *in vitro* [7]. We have demonstrated that in doxorubicin resistant Chinese hamster ovary cells that enhancement of cytotoxicity occurred with tamoxifen levels as low as 1 μmol/l although more pronounced enhancement was seen at 5 and 10 μmol/l [7]. The metabolite 4-hydroxy tamoxifen was also capable of reversing resistance in this system and both tamoxifen and 4-hydroxy tamoxifen were able to increase doxorubicin cytotoxicity in the doxorubicin-sensitive parent line but to a lesser extent than in the resistant line. The precise mechanism by which tamoxifen modulated the MDR *in vitro* is not known but may not involve direct alteration of drug accumulation [8].

In the conventional clinical doses of 20–40 mg/day continuously steady state tamoxifen levels of 100–400 ng/ml (0.25–1.1 μmol/l) are achieved by 3–4 weeks [9]. Higher loading doses up to 160 mg/day allow these levels to be reached in 4 days [10]. Tamoxifen is principally metabolised by *N*-desmethylation and hydroxylation. Recently *N*-desmethyl tamoxifen has also been shown to be a potential enhancer of vinblastine cytotoxicity in MDR expressing human renal cell carcinoma cell lines [11]. This suggests that the administration of tamoxifen may result in potential modulation of the MDR both from the parent compound and from metabolism to *N*-desmethyl tamoxifen and 4-hydroxy tamoxifen. Following oral administration the levels of the *N*-desmethyl metabolite exceed those of the parent compound with chronic administration but steady state levels of this metabolite are not achieved until 6–8 weeks [9]. A number of other metabolites have been identified in lower concentrations in serum and other body fluids [12] (Fig. 1).

Tamoxifen has been widely used in breast cancer both in advanced disease and as adjuvant therapy. When used for prolonged periods as an adjuvant therapy less than 5% of patients experience significant toxicity [13] and there are no established dose-limiting side effects. To investigate the clinical potential of combining high doses of tamoxifen with chemotherapy we have treated 86 patients in two studies with different dose schedules of tamoxifen in combination with oral etoposide. We chose this cytotoxic as resistance to it is known to be affected by the MDR and it allowed an all oral regimen to be offered to extensively pretreated patients. Additionally an *in vitro* study had suggested that reversal of the MDR may lead to less potentiation of normal tissue toxicity when etoposide is used as the cytotoxic instead of doxorubicin [14].

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PATIENTS AND METHODS

Patients and study protocols

Patients with progressing solid tumours either resistant to conventional chemotherapy or of low chemosensitivity were eligible for these studies following oral informed consent. All the patients were treated at a single centre (Newcastle Upon Tyne). Adequate haematological parameters (white cell count $>3.0 \times 10^9$/l, platelets $>100\,000 \times 10^9$/l) were required but stable biochemical abnormalities of renal and hepatic function were permitted. In both studies the initial dose of etoposide was 300 mg/day for 3 days and cycles were repeated every 3 weeks. No maximum number of cycles was specified. Response to treatment and toxicity were graded by standard criteria.

In the first study patients received tamoxifen 40 mg three times daily (120 mg/day), for 3 days with oral etoposide each day. Plasma tamoxifen levels were performed on day 3 of the first cycle.

For the second study the tamoxifen dose was increased to 160 mg twice daily (320 mg/day) for 6 days with the oral etoposide given on days 4, 5 and 6. This dose of tamoxifen was chosen to try to obtain plasma levels of 1000 ng/ml during the period of etoposide administration. During the second study daily measurements over the first cycle of therapy were performed in 11 consenting patients selected by their ability to attend daily and spot levels during the first cycle in 20 other patients (18 on day 4, 2 on day 5). In the second and subsequent cycles spot levels were performed on day 4. A larger number of patients were enrolled in the second study to allow more detailed pharmacokinetic data to be collected and toxicity assessed at a dose that was predicted to produce plasma levels capable of modulating the MDR in *in vitro* systems [8].

Sample analysis

Plasma samples were taken 3–4 h after the morning dose of tamoxifen and stored at −20°C until analysis. We used a sample processing method and liquid chromatography system developed for the assay of tamoxifen and metabolites [12]. Briefly samples were mixed with an equal volume of 100% acetonitrile and the precipitated protein removed by centrifugation. 250 μl samples were injected into a small precolumn with a length of 3 cm and an internal diameter of 0.21 cm packed with 5 μm ODS material. The samples were on-column concentrated by equilibrating the precolumn with 50% acetonitrile in water containing 3 mmol/l acetic acid and 2 mmol/l diethylamine. The analytes were then directed into an analytical ODS-Hypersil column (0.21 × 10 cm) by changing the mobile phase followed by column switching. The composition of the mobile phase was 91% acetonitrile containing 1 mmol/l acetic acid and 0.67 mmol/l diethylamine, and the flow rate was 0.3 ml/min. Small adjustments were made in the acetonitrile concentration of the mobile phase to compensate for different composition of the extracts.

Tamoxifen and its metabolites were eluted in the following order: metabolite Y, metabolite B, metabolite BX, tamoxifen, metabolite Z and metabolite X. These compounds were post-column converted to fluorophors by ultraviolet illumination while passing through a quartz tube, and then monitored by a

Table 1. Study 2—patient details and outcome

Tumour type	No. of patients	No. with previous chemotherapy	Response CR	PR	SD	PD
Ovarian carcinoma	11	11	0	1	3	7
Soft tissue sarcoma	6	4	1	1	2	2
Colorectal carcinoma	6	3	0	0	1	5
Breast carcinoma	5	5	0	0	0	5
Melanoma	5	5	0	1	1	3
Mesothelioma	4	1	0	0	1	3
Non-small cell lung cancer	3	1	0	0	2	1
Gastric carcinoma	3	1	1	0	2	0
Head and neck SCC	3	3	0	0	0	3
Small cell lung cancer	3	3	0	1	0	2
Carcinoid	2	1	0	0	1	1
Pancreatic carcinoma	2	1	0	0	0	2
Renal carcinoma	2	2	0	0	1	1
Gall bladder carcinoma	1	1	0	0	0	1
Oesophageal carcinoma	1	1	0	0	0	1
Salivary gland carcinoma	1	0	0	0	1	0
Testicular teratoma	1	1	0	0	0	1
Total	59	44	2	4	15	37

SD required to be >3 months duration.
CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, SCC = squamous cell carcinoma.

fluorescence detector. Plasma samples with concentrations of above 800 ng/ml were reanalysed after dilution with acetonitrile.

The within-day precision (CV) of this assay for tamoxifen and metabolites Y, B, X and Z was 0.6–5.6% for levels between 10 and 800 ng/ml. Since our standard for metabolite BX is a mixture of the *cis* and *trans* isomers [12], the CV was not determined for this metabolite. This assay was originally developed for serum samples but we have observed that it works equally well with plasma.

RESULTS

Study 1

Clinical results, toxicity and pharmacology. 18 patients were entered into this study. The principal diagnoses were small cell lung cancer (7 patients), soft tissue sarcoma (5 patients) and mesothelioma (4 patients). 1 patient with peritoneal mesothelioma was previously untreated, the other patients had all received chemotherapy with at least one drug known to induce the MDR. The previously untreated patient with mesothelioma had a partial response of intra-abdominal mass for 19 weeks; no other patient responded. Grade 2 (WHO) mucositis occurred in one patient, grade 3 vomiting in 1 patient and nadir leukopenia grade 4 in 1 patient. These toxicities were not thought to be greater than expected with the etoposide schedule used in these heavily pretreated patients.

Tamoxifen levels were measured on day 3 of the first cycle in 6 patients. The mean was 205 ng/ml (0.55 μmol/l) and the levels ranged from 162–248 ng/ml (0.44–0.67 μmol/l).

Study 2

Clinical results. This study totalled 68 patients with a wide variety of tumour types. 59 patients were assessable for response (Table 1) and 9 were inevaluable. 6 patients had objective responses (Table 2) giving an overall response rate of 10.3% (95% confidence intervals 4%–21%) and 15 had stable disease of greater than 3 months duration. Of these patients 3 (ovarian carcinoma 2, non-small cell lung cancer 1) had stable disease for ≥35 weeks.

Toxicity. A total of 177 cycles of treatment were given and 154 cycles in 58 patients are evaluable for toxicity. 4 patients (7%) required dose reductions of etoposide or delays in commencing the next cycle because of myelosuppression. Grade 3 thrombocytopenia occurred in 1 patient, grade 3 leukopenia in another patient and grade 2 leukopenia in 7 patients. No infectious complications were seen. 2 patients had bleeding from intra-abdominal tumours without thrombocytopenia and 3 other patients required transfusion for anaemia but in none of these was chemotherapy induced myelosuppression thought to be solely responsible. Vomiting requiring therapy occurred in 7 patients. In 4 this commenced while taking tamoxifen alone, was considered to be directly related to the tamoxifen, and lead to discontinuation of therapy. 4 patients suffered thromboembolic events (femoral vein thrombosis 2, both recovered with heparin anticoagulation; brachial artery embolus 1, recovered with embolectomy and heparin anticoagulation; retinal artery embolus 1, no recovery). Further tamoxifen was not given to these patients. Other recorded toxicities, each seen in a single patient, were mucositis, diarrhoea, postural hypotension, visual disturbance with mood upset, non-specific dizziness and possible allergic reaction to tamoxifen (paroxysmal dyspnoea in a patient with lung metastases).

Tamoxifen pharmacology. The levels of tamoxifen and metabolites during the first cycle in the 11 patients who had daily measurements are shown in Table 3. On day 4 when the oral etoposide commenced the mean plasma tamoxifen level was 834 ng/ml (2.25 μmol/l) (range 575–1209 ng/ml; 1.55–3.26 μmol/l) and on the final day of oral etoposide was 984 ng/ml (2.65 μmol/l) (range 578–1336 ng/ml; 1.56–3.60 μmol/l). The plasma *N*-desmethyl tamoxifen level increased more slowly than tamoxifen and consequently the mean ratio of plasma tamoxifen to *N*-desmethyl tamoxifen was 4.2 on day 2 and 1.3 on day 6 (paired *t*-test $P = 0.0025$). On day 6 the mean sum of plasma tamoxifen plus *N*-desmethyl tamoxifen was 4.76 μmol/l (range 3.39–6.50). Other metabolites were detected in lower concentrations. Metabolite Z could not be accurately resolved from the much larger tamoxifen peak and was not quantified.

Of the 31 patients who had at least one measurement during the period of etoposide administration (days 4–6) in the first cycle, 13 (43%) recorded a tamoxifen level of over 1111 ng/ml (3 μmol/l). The maximum tamoxifen level recorded was 2982 ng/ml (8.0 μmol/l) on day 4 of the patient's second cycle. 14 patients had measurements of tamoxifen and *N*-desmethyl tamoxifen and 9 of these had measurements of the other metabolites performed on day 4 of the first two cycles (Fig. 2). In these patients the mean tamoxifen level was 954 ng/ml (2.57 μmol/l) (range 215–2365 ng/ml; 0.59–6.37 μmol/l) in cycle 1 and 1104 ng/ml (2.98 μmol/l) (range 523–1715 ng/ml; 1.41–4.62 μmol/l) in cycle 2. This difference is not significant (paired *t*-test $P = 0.18$). However, a significant increase in mean level was seen for *N*-desmethyl tamoxifen from 482 ng/ml (1.35 μmol/l) (range 255–962 ng/ml; 0.71–2.69 μmol/l) to 904 ng/ml (2.57 μmol/l) (range 663–1334 ng/ml; 1.88–3.74 μmol/l) ($P = 0.0001$). 8 of the 14 patients had a third cycle of therapy and levels on day 4 of that cycle were not significantly

Table 2. Study 2—details of responding patients

No.	Tumour type	Disease sites	Previous therapy	Response	Duration (weeks)
1	Gastric carcinoma	Liver	None	CR	29
2	Sarcoma (MFH)	Retroperitoneum	Doxorubicin	CR	70+
3	Small cell lung	Lung Lymph nodes	1. Doxorubicin/vincristine ifosfamide/etoposide 2. Chlorambucil/etoposide/ procarbazine/prednisolone	PR	23
4	Sarcoma (unclassified)	Retroperitoneum	Doxorubicin/cisplatin	PR	21
5	Melanoma	Cutaneous Lymph nodes Lung	CB10–277	PR	9
6	Ovarian carcinoma	Pelvic mass	1. Doxorubicin/cisplatin 2. Cisplatin/iproplatin	PR	21

MFH = malignant fibrous histiocytoma, CB10–277 = 1-*p*-carboxy-3,3-dimethylphenyltriazine.

Table 3. Tamoxifen and metabolite levels in first cycle of therapy (n = 11)

	Day 2	Day 3	Day 4	Day 5	Day 6
Tamoxifen	453 (56)	620 (93)	834 (66)	977 (66)	984 (84)
N-Desmethyl tam	124 (14)	209 (46)	388 (36)	534 (58)	753 (55)
4-Hydroxy tam	14.6 (1.1)	20.6 (3.9)	29.4 (2.8)	31.8 (3.4)	34.7 (5.3)
Metabolite Y	22.1 (4.1)	35.6 (8.1)	60.7 (11.0)	78.1 (15.6)	115 (17)
Metabolite BX	1.9 (0.7)	4.1 (1.6)	8.3 (2.2)	13.1 (2.9)	19.9 (4.3)

Tam = tamoxifen.
Mean (S.E.) ng/ml.

different from the second cycle with a mean tamoxifen level of 978 ng/ml (2.64 μmol/l) (range 570–1694 ng/ml; 1.54–4.56 μmol/l) and mean *N*-desmethyl tamoxifen 1016 ng/ml (2.84 μmol/l) (range 302–1563 ng/ml; 0.85–4.38 μm). Significant increases between cycle 1 and 2 were seen for metabolite Y (mean 74 ng/ml, range 10–49 to 149 ng/ml, range 38–298; $P = 0.01$) and metabolite BX (mean 9.2 ng/ml, range 10–28 to 24.4 ng/ml range 10–42; $P = 0.003$) but not for 4-hydroxy tamoxifen (mean 26 ng/ml, range 11–36 to 32 ng/ml, range 20–39; $P = 0.1$) and there was no further change in the 3 patients who had measurements of these metabolites in their third cycle.

As selected patients were prospectively requested to provide samples for plasma levels, they were not performed on all responding patients nor all patients experiencing significant toxicity and therefore no attempt was made to correlate either of these to tamoxifen or metabolite levels.

DISCUSSION

We have shown that the intermittent oral administration of tamoxifen in doses up to 320 mg/day for 6 days with etoposide is feasible and results in the attainment of circulating plasma levels of both tamoxifen and *N*-desmethyl tamoxifen that are close to those capable of reversing the MDR in preclinical systems. A steady state tamoxifen level was achieved by 5 days but, as could be predicted from the pharmacokinetics at conventional dose levels [9], *N*-desmethyl tamoxifen continued to accumulate over successive days and over two cycles. The levels of 4-hydroxytamoxifen achieved were 25–30 fold lower than tamoxifen so it is unlikely that this metabolite can be a potential contributor to resistance modification *in vivo*.

The potential tamoxifen related toxicities identified with high dose tamoxifen were emesis and thrombo-embolism. Vomiting directly related to tamoxifen occurred in 4 (5.8%) of patients receiving 320 mg/day and was of sufficient severity in these patients to require discontinuation of therapy. Although nausea is one of the most common reasons for women with breast cancer on adjuvant tamoxifen to discontinue it [13], severe vomiting is rare at conventional doses. The mechanism for this toxicity is unclear but a direct CNS effect has been suggested [13] and therefore the demonstration of P-glycoprotein in the cerebral microvasculature [15] may be relevant to the enhanced severity of this toxicity at a higher tamoxifen dose. Additionally tamoxifen has recently been shown to concentrate in normal brain tissue up to 40 times the serum level [16]. Tamoxifen has been reported to be associated with thrombosis in 7/220 (3.2%) of patients when used both as a single agent and with chemotherapy for metastatic breast cancer [17]. In adjuvant therapy the combination of tamoxifen and chemotherapy may cause thrombosis in up to 7.9% of patients [18]. We observed 4 cases of thrombo-embolism temporally related to tamoxifen suggesting the incidence (5.8%) at 320 mg/day given intermittently is unlikely to be significantly higher than with conventional doses.

The toxicity seen from etoposide was not greater than expected

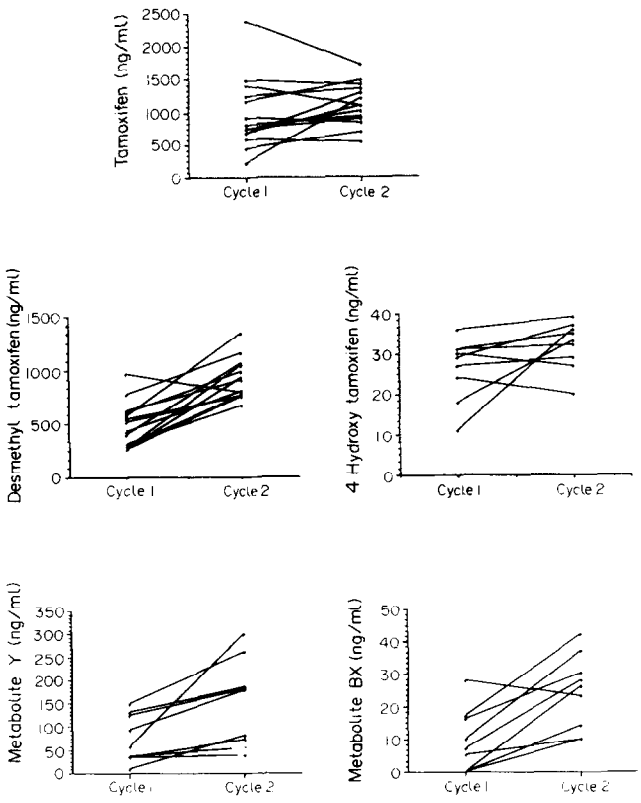

Fig. 2. Tamoxifen and metabolite levels on day 4 of cycles 1 and 2 (individual patient results).

but a precise assessment of the possible influence of high-dose tamoxifen on etoposide toxicity could not be made as patients did not receive a control cycle of etoposide alone. Similarly our study design did not allow us to attempt to measure any influence of high-dose tamoxifen on etoposide pharmacokinetics.

There have been few other studies attempting to use high-dose tamoxifen as a potential modifier of drug resistance. Figuerdo *et al.* [19] combined tamoxifen up to a dose of 100 mg/day and verapamil (up to 360 mg/day) with chemotherapy for extensive small cell lung cancer. No specific toxicity related to the tamoxifen or increased chemotherapy induced toxicity was found and at the highest tamoxifen and verapamil doses there was a possible increase in response rate. In that study tamoxifen was also started 3 days prior to chemotherapy and although tamoxifen levels were not performed, at the highest dose used they are unlikely to have exceeded 1 μmol/l. Trump *et al.* used increasing doses of tamoxifen for 13 days with 5 day infusions of vinblastine. Initial results [20] suggest that at plasma tamoxifen concentrations of >3.2 μmol/l unacceptable neurological toxicity occurs. This concentration corresponded with a dose of 150 mg/m^2 twice daily.

Although antitumour activity from the combination of etoposide and high-dose tamoxifen was seen in some patients, the overall response rate was low. This may be due to the use of oral etoposide which has variable bioavailability that is saturable at the dose used leading to lower than expected plasma etoposide levels [21]. Etoposide is also highly schedule dependent with more prolonged low-dose oral administration over 21–28 days showing promising response rates in tumours resistant to conventional schedules [22] suggesting that the 3 day schedule we used was not optimal. Resistance to etoposide is also now known to be not only mediated via the classical MDR with overexpression of P-glycoprotein but also through alteration in cellular levels of the target enzyme, topoisomerase II, termed "atypical MDR" [23] and there is no evidence that tamoxifen will modify this form of resistance directly. However, if cells also express classical MDR, resistance to etoposide may be modified by elevation of intracellular etoposide levels to obtain maximum interaction with topoisomerase II. It is also possible that in some patients high levels of the serum protein alpha-1-acid glycoprotein could have interfered with the potential of tamoxifen to modulate resistance. This protein has been shown to bind tamoxifen and *in vitro* addition of alpha-1-acid glycoprotein in amounts that can be present in some cancer patients can block MDR reversal [8].

There are other potential effects of tamoxifen that may be important in enhancing cytotoxic efficacy independent of the MDR. Tamoxifen can inhibit protein kinase C [24], an enzyme involved in the transmembrane signalling of growth factors and tumour promoters. This effect, however, is minimal at levels less than 20 μmol/l [24]. At lower levels tamoxifen can reduce the circulating plasma insulin-like growth factor 1, an autocrine growth factor in small-cell lung cancer and possibly breast cancer [25]. Tamoxifen also stimulates production of the growth-inhibitory transforming growth factor beta [26]. The observed consequences of adding high-dose tamoxifen to chemotheapy are therefore likely to have multiple underlying mechanisms.

Our results show tamoxifen is a potential modifier of drug resistance that can be given orally to achieve potentially active plasma levels without the limiting cardiac toxicity of calcium channel blockers. Additionally its principal metabolite is also active at achievable levels. The toxicity we observed in these studies was infrequent and further studies are in progress to assess the possibility of giving higher doses of tamoxifen on a 6 day schedule and over long periods with etoposide to better exploit this cytotoxic's schedule dependency.

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Goserelin Depot in the Treatment of Premenopausal Advanced Breast Cancer

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333 pre- and peri-menopausal patients with breast cancer entered a programme of open studies on the effect of goserelin. Of the 333 patients, 265 patients were entered into assessable efficacy studies. Efficacy data were analysed from 228 eligible patients receiving 3.6 mg of goserelin administered as a subcutaneous injection of a depot formulation once every 28 days. Mean serum luteinising hormone (LH) and oestradiol concentrations were suppressed by day 22 after the first injection. Subjective response occurred in 68.3% of patients assessed. Objective response (UICC criteria) occurred in 36.4% of patients and the lifetable median duration of response was 44 weeks. Responses were observed in all histological grades of tumour, and regardless of oestrogen receptor status. Treatment was well tolerated with no withdrawals due to possible adverse reactions of which hot flushes (75.9%) and loss of libido (47.4%) were commonly encountered. Goserelin provides an effective well tolerated medical alternative to ovarian ablation in the management of advanced breast cancer.
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INTRODUCTION

IT HAS long been known that certain hormones may have a profound influence on the growth of breast cancer, notably oestrogens and progestogens. In 1889 Schinzinger first suggested [1] that surgical castration could be used as a therapeutic manoeuvre in an attempt to slow the progression of advanced disease. Beatson, in 1896 reported [2] the first clinical responses to this form of treatment. Numerous reports of the results of bilateral oophorectomy in women with metastatic breast cancer have appeared in the medical literature and ovarian ablation either by oophorectomy or by irradiation of the ovaries, which was shown to be comparable, has become the established basis of hormonal manipulation in advanced pre-menopausal breast cancer. Response rates to castration have ranged from 21% to 37% [3–7].