Pergamon

Printed in Great Britain
0959–8049/96 $15.00+0.00

S0959-8049(96)00032-9

Special Paper

New Endocrine Therapies for Breast Cancer

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INTRODUCTION

It is 100 years since Beatson removed the ovaries of a 33-year old patient with advanced breast cancer in May 1895 [1]. She responded to treatment for 42 months which led later to the general acceptance of this form of therapy. The mechanism of the response was unknown at the time as the term 'hormone' was not coined until later [2].

In the 100 years since Beatson made his observation, a series of endocrine therapies have been introduced, many of which have later been discarded [3]. Since nearly all endocrine therapies give equivalent response rates and response durations, the reason for change has been the need to reduce the toxicity of treatments and to make them more widely applicable. Thus, the relatively toxic high-dose oestrogens have been replaced by the relatively non-toxic tamoxifen, adrenalectomy by aromatase inhibitors and androgens by protestogens.

THE NEED FOR NEW ENDOCRINE THERAPIES

The active and continuing search for new agents is fuelled by the considerations outlined in Table 1. We need agents with increased efficacy in advanced breast cancer, as adjuvants after surgery and for prevention. We also need to aim for even greater decreases in toxicity and to design agents which increase women's general health as well as having an antitumour effect. Improvements are required with respect to general health since breast cancer often arises during the period of a woman's life when the activity of the ovaries is declining, or has been artificially interrupted leading to menopausal symptoms and long-term increase in the risk of cardiovascular and skeletal problems. These problems can be reversed by oestrogen and progesterone replacement therapy. We are reluctant to use these hormones in the clinic because of fear of recurrence. It would be of great value if we could design an antitumour endocrine agent which also eliminated menopausal problems.

Table 1. Some aims of modern endocrine therapy for breast cancer

Increased efficacy	• Advanced disease	— response rate
		— response duration
	• Adjuvant therapy	— cure rate
		— delay in relapse
	• Prevention	— any activity if found
Decreased toxicity	• General	— gastrointestinal symptoms, asthaenia
	• Endocrine	— sweats/flushes
		— weight gain
Improved general health	• Cardiovascular	— less events
	• Skeleton	— less events
	• Uterus	— no proliferation

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Revised and accepted 12 Jan. 1996.

Although we may consider that current endocrine therapies are an improvement over older ones, there remains room for considerable improvement. The aim of this review is to consider the efficacy, toxicity and general health aspects of newer endocrine therapies which are in clinical trial, but which are not yet (or may never be) commercially available. The three most active areas of clinical trial are the new anti-oestrogens together with the new aromatase inhibitors and antiprogestins. Other areas which are regarded as endocrine therapies and which include the use of vitamin D analogues, retinoids and somatostatin analogues will not be covered in view of the paucity of clinical results to date. Nor will we mention LHRH analogues or progestins since there are few new data concerning these agents.

ANTI-OESTROGENS

The activities of agents which resemble the triphenylethylene anti-oestrogens such as tamoxifen were first assessed in the 1940s [4, 5] but it was not until the demonstration that tamoxifen was as active but less toxic than oestrogens and androgens that this type of therapy gained wide acceptance, first as treatment for advanced disease [6], then as an adjuvant, and more recently for the prevention of breast cancer [7]. Two main avenues have been taken in order to attempt to improve on tamoxifen. One is to chemically alter the non-steroidal triphenylethylene ring structure of tamoxifen or to produce new non-steroidal ring structures, e.g. the benzothiaphenes

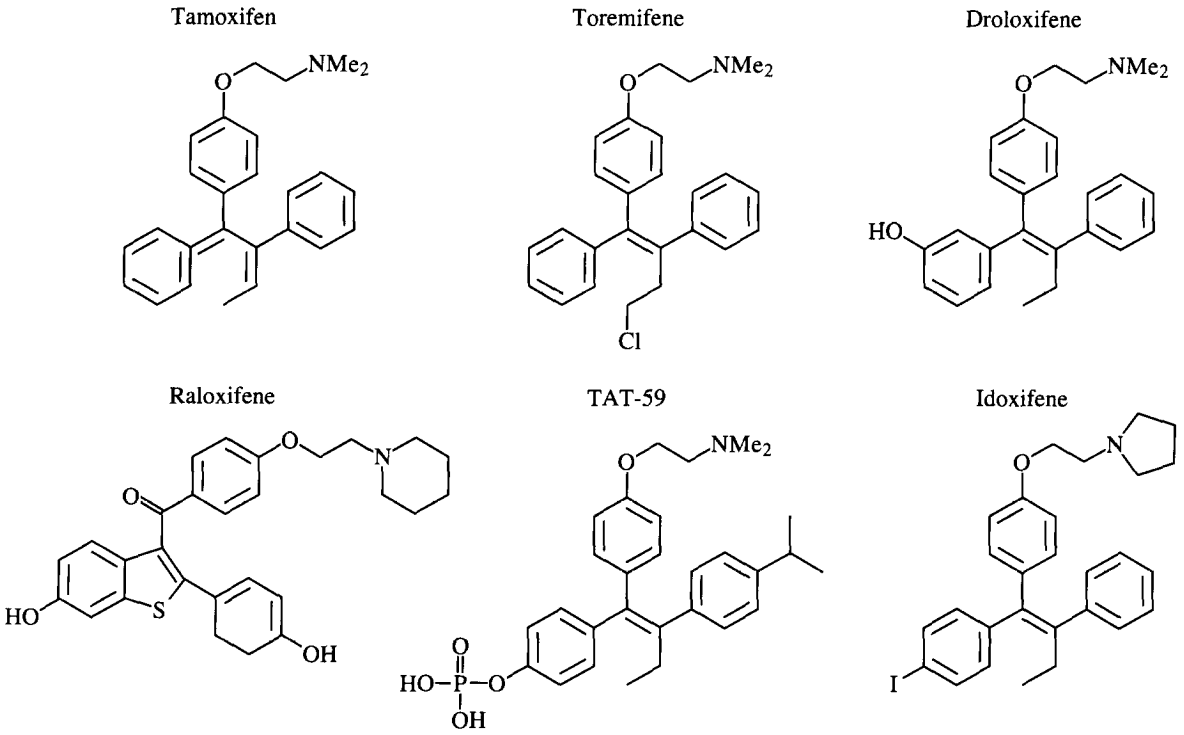
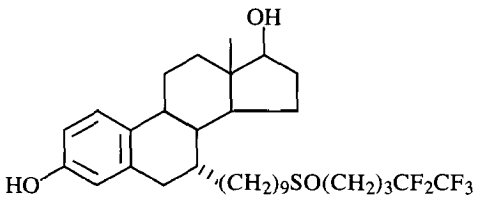


Figure 1. Structures of non-steroidal anti-oestrogens clinically available or in clinical trial.

(Figure 1). The second is to produce steroidal analogues of oestrogen with growth inhibiting activity (Figure 2).

The two types of antioestrogen, steroidal and non-steroidal, appear to have different mechanisms of action which may account for differences in their activity and side-effect profiles. The triphenylethylene anti-oestrogens bind to the oestrogen binding sites of oestrogen receptor (ER) monomers which then combine to form dimers. This process of dimerisation facilitates binding of the ER to specific oestrogen response elements (ERE) in the vicinity of oestrogen-regulated genes. The ER protein contains two trans-activating functions (TAFs) both of which are active when oestrogen binds to the molecule, resulting in the range of gene transcription and gene repression associated with the effect of oestrogen. Tamoxifen binding to ER results in activation of TAF1 in a manner similar to oestrogen, but activation of TAF2 is abrogated by tamoxifen. Thus, tamoxifen is a partial agonist because it activates TAF1 and an antagonist because it inhibits TAF 2 [8, 9].



ICI 182,780

Figure 2. Structure of the steroidal specific 'pure' anti-oestrogen ICI 182,780.

The activity of the steroidal anti-oestrogens appears to be different. For example, the specific antioestrogen ICI 182780 binds to ER, but because of the long side chain on the 7 alpha position of the molecule it appears to stearically hinder receptor dimerisation [10]. There is evidence that ER turnover is increased with an associated reduction of detectable ER molecules in the cell [11, 12]. In the absence of receptor dimerisation, binding of ER to EREs may be abolished or attenuated. *In vitro*, virtually no transcriptional activity of ER has been detected in cells treated with specific anti-oestrogens.

Non-steroidal anti-oestrogens (NSAEs)

Five NSAEs have completed their preclinical testing programme and are in clinical trial. The clinical trial programmes of TAT-59, raloxifene and idoxifene, are still in their early stages whereas for toremifene and droloxifene, phase III trials comparing each agent with best standard therapy (e.g. tamoxifen) are in progress.

The rationale for deciding to embark upon a clinical trial programme for a new NSAE depends upon laboratory studies indicating some measure of superiority over tamoxifen. Given preclinical evidence of superiority, the trial programme then needs to demonstrate that the new NSAE has some measure of superiority over standard therapy (i.e. tamoxifen) in the clinic. Areas which may need to be addressed in preclinical studies and clinical studies are shown in Table 2.

In preclinical studies, evidence of superiority over tamoxifen may be sought in one or more of the following areas; receptor binding, antitumour activity, the balance between tumour antagonism and peripheral agonism, whether there are potentially useful alternative mechanisms of action, and activity against tamoxifen-resistant cells. We will examine how each of the new agents fare in these areas compared with tamoxifen. The structures of each of the NSAEs are shown in Figure 1.

Table 2. Assessment of non-steroidal anti-oestrogens (NSAEs)

(A) In the laboratory
1. Oestrogen receptor binding
2. Tumour antagonism
3. Peripheral antagonist/agonist ratio
4. Alternative mechanisms of action
5. Activity against tamoxifen-resistant cell lines

(B) In the clinic
1. Activity as first-line therapy
2. Activity in tamoxifen-resistant tumours
3. Side-effect profile
4. Utility of peripheral antagonist/agonist ratio
5. Pharmacokinetics

All are based on the triphenylethylene structure of tamoxifen with the exception of raloxifene which is a benzothiaphene.

Oestrogen receptor binding. All the NSAEs have greater affinity for the ER relative to tamoxifen and a greater binding to ER relative to oestrogen compared with tamoxifen with the exception of toremifene (Table 3) [13–17].

Tumour antagonism. Preclinical antitumour activity is most often tested against human mammary tumour cell lines (usually MCF-7 cells) both in culture and when transplanted into athymic nude mice. In addition, activity is usually tested against carcinogen-induced mammary tumours in rodents. Some of the available data are summarised in Table 4 [13, 14, 16–25]. In some studies no direct comparison with tamoxifen was made and these are not cited. The antitumour activities of toremifene and raloxifene *in vitro* and *in vivo* were less or equally active as tamoxifen, whereas those of droloxifene and TAT-59 were apparently more active. Idoxifene was apparently more active than tamoxifen but not to the same extent as droloxifene and TAT-59.

Peripheral agonist/antagonist ratio. All of the NSAEs are partial agonists, and it is customary to look for compounds which have low or reduced agonist activity since it is thought that high activity may result in reduced antitumour effects. However, increased agonist activity may be beneficial with respect to the skeletal and cardiovascular systems. The relative agonist/antagonist effects of the five non-steriodal anti-oestrogens in the immature rat uterus assay are shown in Table 5 [14, 16, 17, 23, 26–28]. Antagonist activity is assessed by percentage reduction in weight of the oestrogen primed uterus caused by the antioestrogen, and agonist activity is assessed by growth stimulation of the uterus by the antioestrogen in the absence of oestrogen priming. In general, the new compounds have less agonist and more antagonist activity than tamoxifen; raloxifene and idoxifene appear to be the most antagonistic and least agonistic.

Table 3. Binding to oestrogen receptors (ER) relative to tamoxifen and to oestrogen of NSAEs in clinical trial

	Binding to ER relative to tamoxifen	% binding to ER relative to oestrogen	[Ref.]
Tamoxifen	—	5	[13]
Toremifene	×1	5	[13]
Droloxifene	×10	7.5	[14]
Raloxifene	?	>100	[15]
TAT-59	×10	10	[16]
Idoxifene	×2.5	12.5	[17]

Alternative mechanisms of action. It is known that high concentrations of non-steroidal anti-oestrogens cause cell death in ER-negative as well as ER-positive cell lines *in vitro*. The mechanism of non-receptor mediated cell death is not well understood, but may be related to calmodulin antagonism [13], inhibition of protein kinase C activity or to antioestrogen binding sites, which seem to be present in cells irrespective of their ER status. Whether these activities are associated with response to NSAEs *in vivo* is not known, although if they were, responses would be expected irrespective of receptor status, which is rarely found clinically. Nevertheless, interest in increasing the degree of calmodulin antagonism is being investigated. For example, idoxifene is four times more active as a calmodulin antagonist than tamoxifen [29]. It remains to be seen whether more potent antagonists can be synthesised which, if active, should increase tumour response rates.

Activity against tamoxifen-resistant cell lines. In vitro evidence that a new NSAE is effective against tamoxifen-resistant cell lines would indicate a use for such an agent in tamoxifen-resistant tumours in the clinic. Few data are available for NSAEs in tamoxifen-resistant lines, but Jarman (personal communication) has shown that idoxifene is 10 times more active against the tamoxifen-resistant cell line RL-3 than tamoxifen itself, and this observation has led to a clinical trial of this agent in patients who have failed tamoxifen. Clinical trials assessing cross-resistance of NSAEs are outlined in the section on 'Activity of newer NSAEs in tamoxifen-resistant tumours'.

Clinical data

As judged by receptor binding, antitumour activity and agonist activity, toremifene appears to be very similar to tamoxifen in preclinical studies. Droloxifene looks much more active than tamoxifen in all three assays, although there are no data on whether it has an alternative mechanism of action or whether it is active in tamoxifen failures. We have been unable to find full reports on raloxifene, but it looks highly active with respect to ER binding and oestrogen antagonism in the rat uterus assay, although disappointing in animal model systems. Both TAT-59 and idoxifene appear to have excellent antioestrogen profiles. The question now arises of whether these preclinical data are reflected in the clinical experience with each drug. Data on the first- and second-line activity, side-effect profile, peripheral antagonist/agonist ratios and the pharmacokinetics of the NSAEs are summarised below as far as they have been tested.

New non-steroidal anti-oestrogens as first-line therapy. The current results of the trials of NSAEs as first-line therapy for advanced disease are summarised in Table 6. All the studies were performed in patients with ER-positive or unknown tumours, and all were previously untreated with endocrine therapy for advanced disease. There were no significant differences in response rates between tamoxifen and toremifene in

Table 4. Preclinical antitumour activity of non-steroidal anti-oestrogens compared to tamoxifen (TAM)

	Human cell lines *in vitro*	Human cell lines in the nude mouse	Carcinogen-induced rat tumours
Toremifene	< × 1 [18,19]	? NT	DMBA = to TAM [13]
Droloxifene	× 10 [20]	> TAM [21]	R3230AC > TAM [14,21]
Raloxifene	?	NT	NMU < TAM [22]
			DMBA < TAM [23]
TAT-59	~ × 10 [16]	> TAM [24]	DMBA > TAM [16]
Idoxifene	×1.5 [17]	> TAM [25]	NMU > TAM [17]

NT, not tested against TAM. DMBA, dimethylbenz(a)anthracene. NMU, nitrosomethylurea.

Table 5. Agonist/antagonist activity of non-steroidal anti-oestrogens in the immature rat uterus assay

	% Agonism	% Antagonism	[Ref.]
Tamoxifen	50	50	[26]
Toremifene	43	?	[27]
Droloxifene	35	~20	[14]
			[28]
Raloxifene	5	83	[23]
TAT-59	?	>TAM	[16]
Idoxifene	15	85	[17]

TAM, tamoxifen.

Table 6. Results of studies using newer non-steroidal anti-oestrogens as first-line endocrine therapy for advanced breast cancer

Drug [Ref.]	Dose (mg/day)	No. of patients	Response CR/PR (%)
Toremifene [32]	20	14	21
(Phase II trials	60	93	52
summarised)	240	38	68
Toremifene [30]	240	31	29
Tamoxifen	40	31	44
(Phase III trial)			
Toremifene [31]	60	221	21
	200	212	22
Tamoxifen	20	215	19
(Phase III trial)			
Droloxifene [33]	20	84	30
(Randomised phase	40	88	47
II trial)	100	96	44
TAT-59 [59]	10	15	15
(Randomised Phase	20	11	55
II trial)	40	13	31

the randomised trials that have been published to date [30, 31]. These data conflict with several phase II studies where toremifene showed higher response rates than tamoxifen at doses of 60 and 240 mg/day [32]. These high response rates have not been seen in the phase III studies using comparable doses.

The results of a major international phase II trial of droloxifene were reported recently [33]. Patients were randomised to receive 20, 40 or 100 mg of droloxifene per day. This study comprised a large number of patients and reported significantly higher and impressive response rates at the two higher doses compared to the lower one (Table 6). Time to progression in higher dose groups was also significantly longer. Preliminary data on TAT 59 look promising. Phase II studies with raloxifene are in progress and it is too early for idoxifene to have been assessed as a first-line agent.

Activity of newer NSAEs in tamoxifen-resistant tumours. New endocrine therapies are usually tested after tamoxifen failure. This clinical situation is particularly interesting with respect to NSAEs, since we discover whether drugs which are thought to have similar mechanisms of action show cross-resistance or cross-sensitivity.

With the exception of one small study [34], the response rate (CR + PR) to toremifene after tamoxifen failure is 5% or lower (Table 7) [30, 34–40]. However, a number of patients had prolonged stabilisations of disease in these studies although it is difficult to assess numbers of these because short and long durations were combined in most. If we define at least 6 months as a 'no change' (NC) response [41], we estimate that NC may be 20–25%.

There were no responses in a small study using raloxifene as a second-line treatment [39]. However, responses and NC were seen with droloxifene and idoxifene after tamoxifen failure, suggesting that TAT 59 'phenyl' ring subsitutions of the tamoxifen molecule may produce non-cross-resistant NSAEs. However, responses were lower than expected for standard second-line therapy, such as megestrol acetate, sug-

Table 7. Response to NSAEs in tamoxifen-resistant breast cancer

	Dose (mg)	No. of patients	CR+PR %	NC %	(Duration of NC)	[Ref.]
Toremifene	200	9	33	35	(NK)	[34]
	240	34	0	26	(5–27 months)	[35]
	240	50	4	44	(> 2 months)	[36]
	200	102	5	23	(med 7.8 months)	[37]
	240	23	0	22	(med 6.0 months)	[30]
Droloxifene	100	26	15	19	(> 6.0 months)	[38]
Raloxifene		14	0	NK		[39]
TAT–59	10–40	33	27	4	('Long')	[59]
Idoxifene*	20	14	14	21	(> 5–12 months)	[40]

NC, no change. NK, not known.
*Given mainly as third-line endocrine therapy.

gesting some cross-resistance despite these chemical modifications.

Side-effect profiles of NSAE. If new NSAEs have comparable activity to tamoxifen but have reduced side-effects, then these are of clinical interest and could replace tamoxifen. Some of the more common side-effects are shown in Table 8 [32, 33, 39, 42]. Although no precise data are available for idoxifene and few for TAT-59, there appears to be little difference with respect to tamoxifen and the new NSAEs. Differences between drugs may be true differences, but could also be explained by the assiduousness with which toxicity was sought, or the size of the study (e.g. the raloxifene data was based on only 14 patients).

Utility of the peripheral agonist/antagonist ratio. A major aim, stated in studies of new NSAEs, is for less agonistic and more antagonistic molecules. The aim is laudable with respect to the tumour and the endometrium but, in terms of women's general health, more agonistic activity towards bone and the cardiovascular system would probably be beneficial.

All five new NSAEs have agonist activity *in vivo* since they reduce gonadotrophin levels and increase SHBG (sex hormone binding globulin) (with the possible exception of idoxifene). With the exception of raloxifene, there appears to be no data available on the effects of new NSAEs on bone density, lipids and the endometrium. Raloxifene had equivalent activity to Premarin on bone and reduces low density lipid cholesterol significantly, but has no significant effect on high density lipid cholesterol. Raloxifene also showed no stimulatory effect on the endometrium and if this compound has good antitumour activity, it may be an attractive choice in the clinic [43].

NSAE pharmacokinetics. Toremifene has similar pharmacokinetics to those of tamoxifen [44]. It takes longer for idoxifene to reach steady-state concentrations (6–12 weeks) than tamoxifen. The terminal half-life of idoxifene in patients on prolonged therapy is 23 days, which is longer than tamoxifen [40]. Therapeutic levels of droloxifene are reached within the first day of therapy, in contrast to tamoxifen, where therapeutic concentrations are reached after 11 days, but the terminal half-life of droloxifene is short at 25 h [45, 46]. This means that droloxifene may be more suitable than tamoxifen when considering the approaches to sequencing with other endocrine therapies or chemotherapies. The pharmacokinetics of raloxifene do not appear to have been published, making the available pharmacokinetic data for the new NSAEs incomplete.

Table 8. Incidence of common side-effects with new NSAEs

	Hot flushes (%)	Lassitude (%)	Nausea/vomiting (%)	[Ref.]
Tamoxifen	30	10	10	[42]
Toremifene	19	10	8	[32]
Droloxifene	29	26	29	[33]
Raloxifene	43	36	14	[39]
TAT-59	10	3	3	[59]
Idoxifene	"similar to TAM"			

TAM, tamoxifen.

Summary. We can say, from very large and extensive clinical phase III studies, that the preclinical data have been borne out with regard to the similarity in effectiveness of toremifene compared to that of tamoxifen. The preclinical studies produced no data on cross-resistance, but the clinical studies appear to show cross-resistance between the two molecules with respect to their antitumour effects. The small number of responses that were seen could well have been related to the withdrawal of tamoxifen [47]. We have been unable to find data on toremifene with respect to bone, lipids and the endometrium. One possible advantage of toremifene is that it does not appear to produce DNA adducts in the standard assays.

Droloxifene appeared active in the preclinical screens and also appears to be so in the clinic, although there are, as yet, no clinical phase III data available. It has some cross-sensitivity with tamoxifen and its short half-life may make it particularly useful in alternating schedules. In preclinical tests, it looks less agonistic than tamoxifen, but does show agonist activity in the areas reported to date (reduced LH and FSH, increased SHBG), but more data are required.

In rat uterus assays, raloxifene was shown to be only weakly agonistic, but paradoxically has proved to be a clinically useful agent for the treatment of osteoporosis and has a favourable effect on lipids. The animal data may predict the clinical effect on the uterus since raloxifene (in preliminary studies) is said to have little agonist effect on this organ [43].

Chemical data available on TAT-59, a Japanese NSAE, suggest it may be very active. Idoxifene has been specifically designed to be active in tamoxifen failures and is now in trial in this clinical situation. Its preclinical and early clinical activity look promising.

STEROIDAL ('PURE') ANTIOESTROGENS

Substitutions of the oestrogen molecule at various positions can produce compounds with antioestrogenic activity [48–52]. The oestrogen molecule was chosen as a basis for further development of anti-oestrogens because it was felt by Wakeling and Bowler [53] that further alteration of the triphenylethylene molecule was unlikely to lead to strikingly better anti-oestrogens.

This new generation of anti-oestrogens have been described as 'pure' or specific anti-oestrogens since they have little or no agonist activity in preclinical studies. Clinical data, in addition to preclinical data, are only available for the steroidal antioestrogen ICI 182,780 and these are summarised in Tables 9 and 10 [26, 54–63].

ICI 182,780 binds to ER with the same affinity as oestradiol [26]. It is superior to tamoxifen when tested against human mammary tumour cell lines *in vitro* [26] and cell lines transplanted into nude mice [54]. It is also active in tamoxifen-resistant cell lines *in vitro* [56–58] and when transplanted into nude mice [63] (Table 9).

Limited data are available from clinical studies (Table 10), but ICI 182,780 inhibits tumour proliferation and significantly reduces ER content when administered for 1 week before tumour resection [11]. It is active against tamoxifen-resistant metastatic human tumours in women and *in vitro* [60–62]. As predicted from the nude mouse model, ICI 182,780 administration appears to result in a particularly long duration of tumour suppression. The median duration of response is, as yet, unknown since it has not been reached after 22 months of a phase II study, where tamoxifen-resistant tumours were treated with ICI 182,780 [61]. Preliminary data

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