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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 Thus, the relatively toxic high-dose oestrogens have been replaced by the relatively non-toxic tamoxifen, adrenalectomy by aromatase inhibitors and androgens by protestogens.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

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

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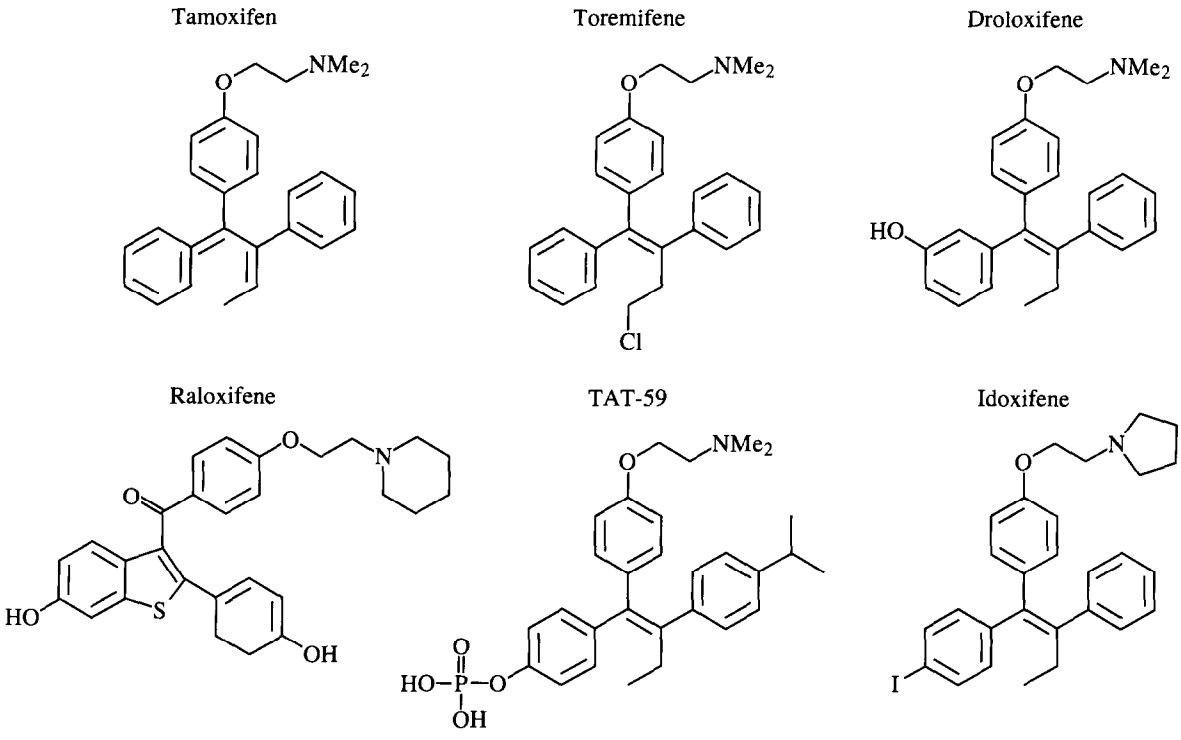
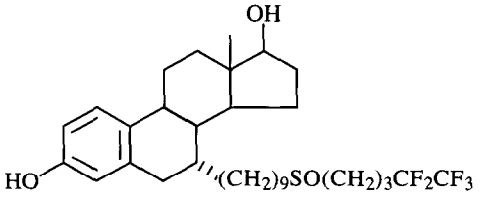


Figure 1. Structures of non-steroidal anti-oestrogens clinically available or in clinical trial.

(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

Table 9. Preclinical data for ICI 182,780 in comparison with tamoxifen

Assessment	Effect	[Ref.]
Oestrogen receptor binding	Equal to oestrogen	[26]
Tumour antagonism	— MCF-7 cells *in vitro* twice as active	[26]
	— MCF-7 cells in nude mice, growth suppressed twice as long	[54]
	— Animal tumours?	
Peripheral antagonist/agonist ratio	— Immature rat uterus assay—complete antagonist. No agonist activity	[26]
	Reduced cancellous bone in rats	[55]
	— No effect on bone in rats	[65]
	— No effect on gonadotrophins	[56]
Alternative mechanisms of action	— None reported	
Activity against tamoxifen-resistant cell lines	— Yes	[56–58]

Table 10. Clinical results with ICI 182,780

Assessment	Effect	[Ref.]
Activity first line	— No studies	
	— Reduces tumour proliferation and ER before surgery	[11]
Activity in tamoxifen-resistant tumours	— PR 7/19(37%), NC 6/19 (32%)	[61]
	— 19 patients treated	
	— Median duration of response >22/12	
	— Active *in vitro*	[62]
Side-effects	— None of note	[61]
Peripheral antagonist/agonist ratio	— ? No effect on gonadotrophins or SHBG	[61]
	— Inhibition of endometrial proliferation	[65]
	— Bone–no data	[65]
Pharmacokinetics	— Given by monthly depot	[61]
	— Therapeutic levels present throughout month	

indicate no agonist or antagonist effects of ICI 182,780 on gonadotrophins, or SHBG, although uterine proliferation is inhibited [61, 64]. There are no clinical data with respect to the effect of ICI 182,780 on bone.

Thus, in preliminary studies, ICI 182,780 appears to be highly active, with little toxicity or agonist activity and the preclinical data appear to be predicting the clinical effects. The effect of ICI 182,780 on bone remains to be determined. From the limited data available from animal studies, there was no effect [65] or a negative effect [55] and clinical studies are required to resolve this issue. Thus, the specific antagonist ICI 182,780 may be an important new approach to antioestrogen therapy. Overviews of the laboratory and clinical development of these compounds have been published recently [66, 67].

AROMATASE INHIBITORS

Aromatase inhibitors have been in the clinic for over 20 years [68]. However, the toxicity profile of the major drug available (aminoglutethimide) led to it being used mainly as a third-line endocrine agent after second-line progestogen treatment. The major and most disturbing side-effect of weight gain with progestogen use led to a continued interest in aromatase inhibitors, the aim being to produce a non-toxic, easily administered compound with equivalent or better activity than aminoglutethimide, and which did not cause weight gain. The structure of aromatase inhibitors in the clinic and in clinical trials are shown in Figure 3.

The aromatase inhibitors fall into two major groups: non-steroidal and steroidal compounds which appear to have different mechanisms of action. Following the recognition that aminoglutethimide was a non-specific, reversible inhibitor of several cytochrome P450s including aromatase, the quest began for more selective and more potent non-steroidal compounds. Rogletimide was an early example which showed improved enzyme (and pharmacological—no CNS effects) selectivity, but only comparable potency (Figure 3). Also, like aminoglutethimide, pyridoglutethimide is a potent liver enzyme inducer and enhances its own metabolism. Fadrozole represented a major advance in potency (*ca* 500-fold) and selectivity, but the latter is not complete and 18-hydroxylase inhibition becomes apparent towards the upper end of the aromatase inhibitory dose response curve in women. Vorozole, letrozole and anastrozole (all triazole derivatives) combine potency and high selectivity for aromatase and have no discernible effects on adrenal function at the maximally effective aromatase inhibiting doses. The latter three drugs have been shown to reduce circulating oestradiol levels in postmenopausal women to the limits of detection of the most sensitive assays currently available (Table 11) [69–75]. Although they are intrinsically reversible enzyme inhibitors, the long plasma half-lives of letrozole and anastrozole enable continuous enzyme inhibition to be achieved with simple once daily dosing. The question arises as to whether greater suppression of aromatase activity as is shown by such low hormone levels will result in greater response to treatment. An important issue for further research is the apparent lack of cross-resistance between various methods of aromatase inhibition (see Table 12) [76–80].

The steroidal substrate analogue 4 hydroxyandrostenedione (Lentaron), was one of the first examples to be described and is the first to be developed. It has high enzyme selectivity, but poor oral bioavailability due to its high first-pass metabolism and, it is, therefore, provided as a parenteral formulation (twice monthly). Weak 'hormonal' (androgenic) effects are discernible in animals in the form of gonadotrophin suppression and in humans in the lowering of SHBG, although the latter was only seen with large twice daily oral dosing. Plomestane and exemestane represent second generation steroidal type of inhibitors and offer the potential for oral dosing.

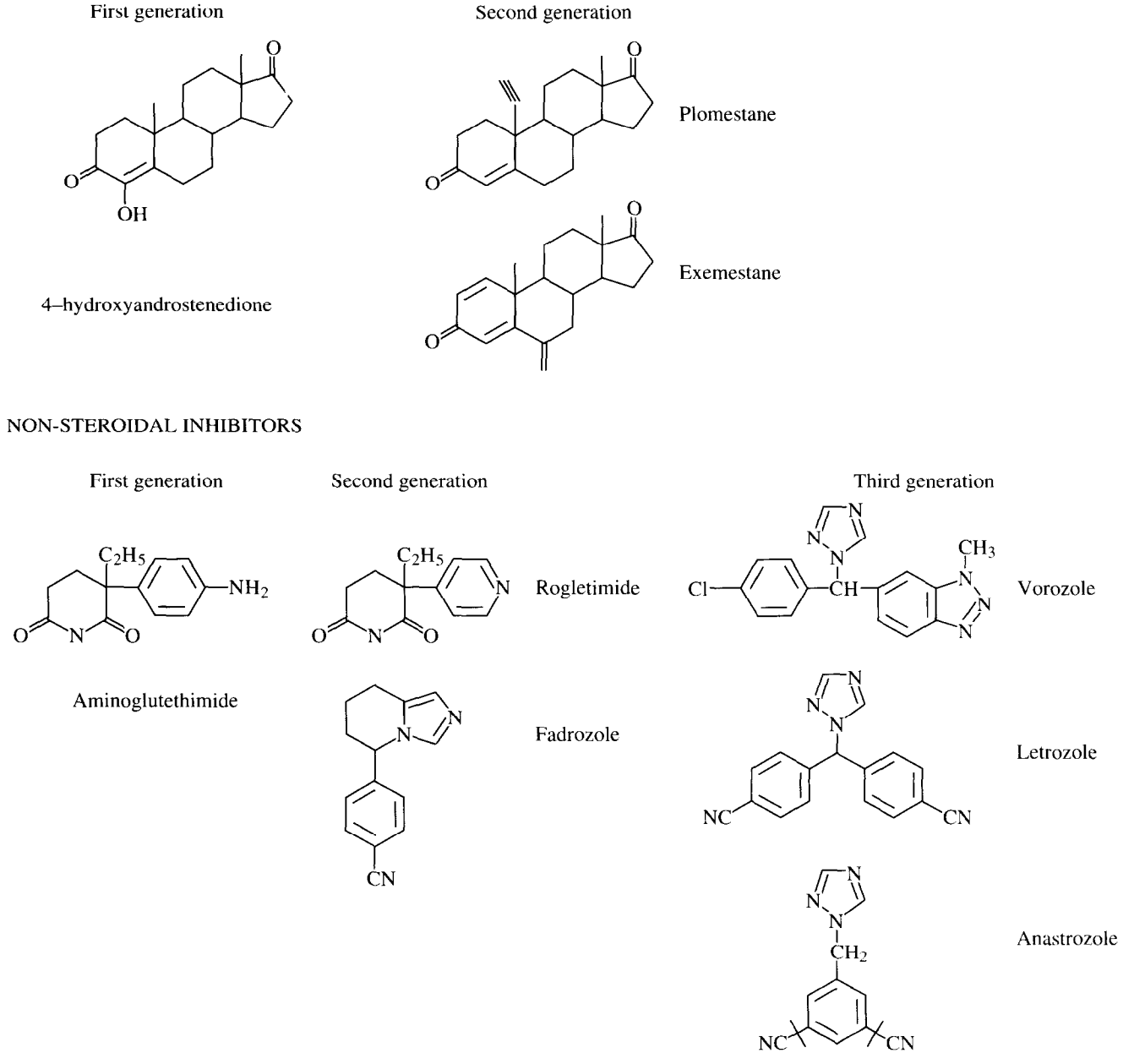


Figure 3. Structural formulae of aromatase inhibitors clinically available or in clinical trial.

All three compounds interact covalently with the aromatase enzyme during the first oxidation cycle and cause irreversible inhibition. This provides prolonged peripheral aromatase inhibition in spite of rapid plasma clearance of the drug (cross-reactivity of plomestane and its metabolites complicates simple oestrogen measurements and has slowed its development).

None of the second and third generation aromatase inhibitors in trial have the toxicity associated with aminoglutethimide. The major side-effects are mild gastrointestinal disturbance and hot flushes in approximately 40% of patients [72, 73, 81–83].

A major advantage of the new aromatase inhibitors compared with progestogens is the difference in weight gain. In a recently reported three-arm phase III trial of anastrozole 1 mg or 10 mg compared with megestrol acetate 160 mg, more than 30% of patients treated with megestrol acetate had weight gain of 5% or more and over 10% had a weight gain of 10% or more. Furthermore, the weight gain experienced with megestrol acetate increased over time (Figure 4) [83].

The results of many of the clinical trials with the new aromatase inhibitors are shown in Table 13 [72, 73, 81–87]. Except for the randomised trials, the response rates should be viewed with caution as the response rate in small phase II studies can be greatly influenced by patient selection. With the exception of the comparison of tamoxifen with fadrazole [82], all aromatase inhibitors have been tested as second- or third-line treatments in ER-positive or ER unknown patients.

Three phase II trials of exemestane are currently ongoing in

Table 11. Suppression of serum oestradiol, oestrone and oestrone sulphate by second and third generation aromatase inhibitors

	Dose (mg/day)	Oestradiol (%)*	Oestrone (%)*	Oestrone sulphate (%)*	[Ref.]
Exemestane (FCE 24304)	25	28	35	39	[69]
Pyridoglutethimide	1600	50	17	—	[70]
Fadrozole (CGS 16949A)	2–16	65	27	30	[71]
Vorozole (R83842)	5	11	45	31	[72]
Letrozole (CGS 20267)	0.1–2.5	21†	21†	—	[73]
Letrozole (CGS 20267)	0.1–5	<10†	<10†	<10†	[74]
Anastrozole (ZD 1033)	1	15†	15†	8†	[75]

*Percentage of baseline. †At or below detection limit of assay.

Table 12. Studies which demonstrated that additional suppression of serum oestradiol by a second oestrogen lowering agent may result in further response to therapy

First treatment	Second treatment	Responders /total no.	(%)	[Ref.]
AMG*	Formestane	23/112	(21)	[76]
Formestane	AMG	2/7	(28)	[77]
Hypophy†	AMG	4/9	(44)	[78]
AMG	Hypophy	5/25	(20)	[79]
LHRH	Formestane	4/6	(66)	[80]
AMG 250 mg/day	AMG 1000 mg/day	4/17	(23)	[76]

*AMG, aminoglutethimide. †Hypophy, hypophysectomy/ adrenalectomy.

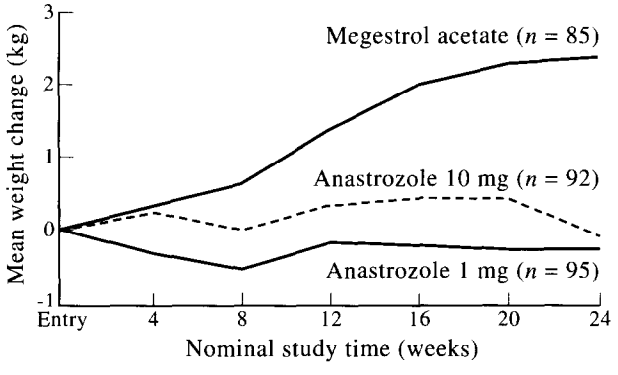


Figure 4. Weight gain over time for megestrol acetate.

the United States and Europe in tamoxifen or megestrol acetate failures, and one European study of exemestane in aminoglutethimide failure. With the exception of the latter study [88], data are not available at present.

Two dose-finding randomised phase II studies of pyridoglutethimide were reported recently which suggests that the optimal dose is 600 or 800 mg [81, 84]. In a phase II study, fadrozole was shown to be active after tamoxifen failure [85]. In a Swiss phase III study, it was shown to be equivalent in efficacy and toxicity to tamoxifen [82] and in two phase III studies was shown to be as effective as megestrol acetate [87].

Vorozole was shown to be active after tamoxifen failure [72] and is now in phase III evaluation versus aminoglutethimide. Letrozole was shown to be active in two phase II studies [73, 74] and is now also in a phase III trial in a three-arm study comparing letrozole 0.5 mg and letrozole 2.5 mg against megestrol acetate.

Two phase III studies of anastrozole (Arimidex) have been completed and the early results reported for each one [83, 89]. The results of a European trial are shown in Table 13. In both studies, anastrozole 1 mg and anastrozole 10 mg were compared with megestrol acetate 160 mg. The lower dose of anastrozole (1 mg) was sufficient to suppress serum oestradiol to below the detection limit of the assay used (3 pmol/l). The dose of 10 mg was also tested, based on the hypothesis that there may be some additional effect, possibly in suppression of intratumoural aromatase activity, or further depression of serum oestradiol (i.e. well below the detection limits of most assays). Both trials were performed in patients who had failed tamoxifen and who had ER-positive or ER unknown tumours, and the results of both trials were identical. There were no significant differences in response rates between the two doses of anastrozole or between either dose of anastrozole and megestrol acetate. Thus, the dose of anastrozole to be used in the clinic will be 1 mg per day. The once a day dosing and lack of weight gain with anastrozole make it more attractive than megestrol acetate for treatment of patients failing therapy with tamoxifen, and a candidate for adjuvant therapy.

Antiprogestins

Progestins are currently the most commonly used second-line endocrine therapy for advanced breast cancer. Antiprogestins have been in clinical trial for some years, but have not become serious contenders for standard treatments mainly because of their toxicity and apparent low efficacy.

The precise mechanism whereby antiprogestins inhibit receptor-positive tumour cells is not clear. They may act as antiprogestins [90], anti-oestrogens [91, 92] or oestrogens [93]. The antiprogestins which have entered the clinic, mifepristone (RU486) and onapristone (ZK 98299) (Figure 5) also bind glucocorticoid (GR) and androgen receptors. In fact, the affinity of RU486 for GR is more than three times that of dexamethasone. The newer antiprogestin ZK 98299 has only a 21% affinity for GR compared with dexamethasone, but a lower affinity for PR. Newer compounds with higher affinity for PR and lower affinity for GR are being developed (e.g. Org 31710 [94]).

Recent studies, using cells transfected with oestrogen and progesterone reporter vectors containing a variety of promotors and expression vectors for PR (A and B isoforms) and

Table 13. Response rates in phase II and (where available) phase III studies with second and third generation aromatase inhibitors

	Dose (mg/day)	No. of patients	% CR+PR	NC*	[Ref.]
Pyridoglutethimide	600	21	14	34 (?)	[81]
	800	21	10	86 (?)	
	400	46	4	—	[84]
	800	46	14	—	
Fadrozole	1.4	80	23	45 (?)	[85]
Fadrozole	1.2	18	11	55 (?)	[86]
	2	19	5	58 (?)	
	4	19	11	68 (?)	
Fadrozole†	2	86	16	48 (?)	[82]
versus					
Tamoxifen	20	90	24	48 (?)	
Fadrozole	2	?	11	25 (?)	[87]
versus					
Megestrol acetate	160		16	20 (?)	
Fadrozole	2		13	24 (?)	
versus					
Megestrol acetate	160	?	12	30 (?)	
Vorozole‡	1–5	24	33	17 (≥6/12)	[72]
Letrozole‡	0.5–2	21	33	24 (≥3/12)	[73]
Anastrozole†	1	135	10	24 (≥6/12)	[83]
Anastrozole	10	118	13	21	
Megestrol acetate	160	125	13	22	

*Minimum duration of NC (NC, no change). †Phase III study. ‡Randomised phase II studies.

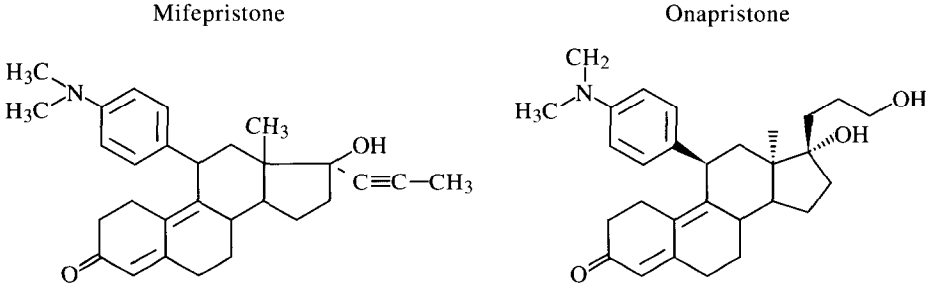


Figure 5. Structural formulae of the antiprogestins mifepristone and onapristone.

ER, have helped elucidate one mechanism of action of antiprogestins. These experiments indicate that there is extensive inhibitory cross-communication between ER and PR. Both progestins (R5020) and antiprogestins (RU486) have been shown to act as potent ligand dependent repressors of ER activity when bound to either isoform of PR [92]. Some studies indicate that ZK 98299 may act by blocking PR binding to DNA [95], but this mechanism of action is controversial. Jeng and associates [93] have demonstrated that RU 486 stimulated the growth of MCF-7 cells at a concentration of 10^{-6} M which could be blocked by 40H-tamoxifen and ICI 164,384, suggesting some oestrogenic activity.

Whatever their mechanism of action, mifepristone and onapristone inhibit the proliferation of human PR-positive mammary tumour cell lines [96, 97], the growth of human tumour cells in nude mice [98] and carcinogen-induced animal tumours [99, 100] (Table 14). One alternative mechanism of action which is of interest is the demonstration that ZK 98,299 can induce differentiation in some human mammary tumour cell lines [101]. In addition, RU486 has been shown to be active in oestradiol insensitive cell lines [102] (Table 14).

RU486 was initially tested, clinically, in tamoxifen-resistant advanced breast cancer, but few responses were seen (Table 15) [103–106]. In order to test RU486 under the best possible circumstances, Eisenhauer and associates [105] gave RU486 to patients with PR-positive tumours, previously untreated with endocrine therapies. Unfortunately, the response rate was low (CR+PR 9%) and 59% of patients

Table 14. Preclinical studies with mifepristone and onapristone

Assessment		Mifepristone (RU486)	Onapristone (ZK 98.299)
Receptor binding		Binds PR and induces DNA binding [92]	Binds PR, no DNA binding
Tumour antagonism:	Cell lines	Inhibits MCF-7 cells [96]	Inhibits MCF-7 cells [97]
	Nude mice	NT	T 61 tumours inhibited [98]
	Animal tumours	DMBA inhibited [99]	DMBA inhibited [99]
			NMU = to tamoxifen [100]
Peripheral antagonist/agonist ratio		NK	NK
Alternative mechanism of action		Binds ER [101]	Induction of differentiation [101]
Activity against resistant cell lines		Active in E2 insensitive lines [102]	NK

NT, not tested. NK, not known.

Table 15. Response to antiprogestins in clinical studies

Drug	Dose (mg/day)	No. of patients	CR+PR (%)	NC (Duration) (%)	[Ref.]
Mifepristone	200	22	18	?	[103]
Mifepristone	200	11	9	?	[104]
Mifepristone*	200	22	9	41 (?)	[105]
Onapristone	100	90	10	42 (>3/12)	[106]

*Previously untreated for advanced disease and PR positive.

reported lethargy. Onapristone has shown activity in phase II trials (Table 15), but because of induction of abnormal liver function tests, this antiprogestin has been withdrawn from further clinical development. Future development of antiprogestin therapy must await more specific, less toxic compounds.

SUMMARY AND CONCLUSIONS

How do the new endocrine therapies stand up to the aims of modern endocrine therapy outlined in Table 1? We wish to see increased efficacy, decreased toxicity and improved general health in women taking a new agent.

None of the new non-steroidal anti-oestrogens have shown unequivocal evidence of improved efficacy in the clinic to mirror their improved profiles over tamoxifen in preclinical studies. We know that toremifene is equivalent to tamoxifen, but we do not have any phase III data from the other four compounds in development. The specific steroidal antioestrogen, ICI 182,780, looks very promising, but is early in its developmental programme. The new aromatase inhibitors are likely to prove equal to tamoxifen or progestagens, but it is disappointing that improved oestrogen suppression has not led, to date, to improved efficacy. No comment can be made about adjuvant or preventative therapy for any of the new agents, although trials are planned for the new aromatase inhibitors in this clinical situation. Currently, the antiprogestins are disappointing and we will need to wait a considerable time for new agents in preclinical testing to reach the clinic.

Many of the new agents are associated with decreased toxicity. It is likely that the NSAEs will be equitoxic with tamoxifen. The steroidal antioestrogen looks particularly nontoxic as do the new aromatase inhibitors, and thus we have an advance in terms of reduced toxicity.

The effects of the new agents on the uterus, lipids and bone are in the early stages of testing. Raloxifene, ICI 182,780 and the new aromatase inhibitors are expected to have no proliferative effects on the endometrium, but only the new NSAEs are expected to have beneficial cardiovascular and skeletal effects. If the steroidal anti-oestrogens and new aromatase inhibitors become adjuvant therapies of choice, other agents to prevent osteoporosis and cardiovascular events may also have to be administered.

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