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NEW ENDOCRINE DRUGS FOR TREATMENT OF ADVANCED BREAST CANCER

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Abstract

Current status of endocrine treatment for breast cancer is reviewed. Several new aromatase inhibitors as well as antiestrogens are being introduced for clinical trials. As different drugs within the same drug group may possess different biochemical actions, apart from being useful drugs for treatment of breast cancer, these drugs may also extend our knowledge about the endocrinology of breast cancer.

Key words: Endocrine therapy, breast cancer, antiestrogens, aromatase inhibitors, LH-RH analogues

Endocrine treatment of postmenopausal breast cancer has improved during the last two decades due to the introduction of tamoxifen and aminoglutethimide. More recently, treatment with progestins in high doses has been successfully applied to postmenopausal patients, and tamoxifen as well as medical castration by LH-RH agonists have become feasible treatment alternatives to castration in premenopausal women.

Tamoxifen and aminoglutethimide have been interpreted as prototypes of two classes of drugs, antioestrogens and aromatase inhibitors. Aminoglutethimide blocks the main oestrogen production pathway in postmenopausal women (peripheral conversion of androstenedione into estrone), tamoxifen is thought to act by binding to the oestrogen receptor inside the tumour cell blocking oestrogen stimulation. Aromatase inhibitors and antioestrogens are currently the main new drugs for endocrine treatment of postmenopausal breast cancer, initiated by the successful use of aminoglutethimide and tamoxifen.

However, the picture may be more complicated than that. Tamoxifen is not a pure antioestrogen, but it also possesses certain oestrogenic activities, shown in vivo to stimulate the synthesis of certain plasma proteins as SHBG and CBG in an oestrogen-like manner (1). Also, tamoxifen has been shown to bind to components other than the oestrogen receptor inside the tumour cell (2) and recent works suggest tamoxifen could interact with cellular oestrogen uptake and intracellular oestrogen metabolism (3). Aminoglutethmide, apart from being an aromatase inhibitor also inhibits several adrenal enzymes. This is probably of little importance for its mechanism of action (4). However, aminoglutethimide stimulates estrone sulfate metabolism, and this effect may be beneficial (5, 6), as it aggravates plasma estrone sulfate (E_1S) suppression [7]. Thus, it may be feasible to group different endocrine drugs into drugs acting by modifying oestrogen disposition (synthesis and/or metabolism) and drugs thought to act directly on endocrine mechanisms inside the tumour cell.

Drugs acting on breast cancer by modifying oestrogen disposition

Before discussing drug influence on oestrogen disposition, different oestrogen production pathways that may be targets of drug influence are reviewed.

Oestrogen production. In premenopausal women, except for the first days of the menstrual cycle, the ovary is the major source of circulating oestrogens. In postmenopausal women, most oestrogens are produced in peripheral tissue by so-called aromatization of circulating androgens (Fig. 1). Plasma androstenedione (Δ^4-A) and testosterone (T) levels are about 2–4 nmol/l and 1 nmol/l respectively in postmenopausal women (8, 9). About 2/3 of circulating Δ^4-A seems to have an adrenal origin, the rest is secreted by the ovary (10). Fifty per cent of plasma testosterone

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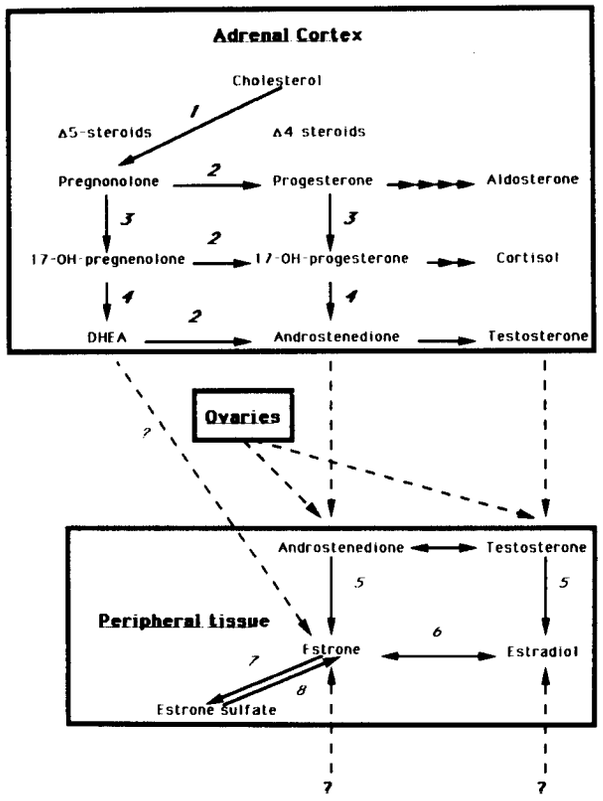

Fig. 1. Pathways of postmenopausal oestrogen synthesis. While a detailed outline of glandular synthesis of oestrogen precursors is given for the adrenals only, steroid synthesis occurs by similar pathways in the ovaries. 1 = cholesterol side chain cleavage enzyme (desmolase), 2 = 3β-ol-dehydrogenase-Δ^5- to Δ^4-isomerase, 3 = 17-hydroxylase, 4 = $C_{17,20}$-lyase, 5 = aromatase, 6 = 17β-hydroxysteroid dehydrogenase, 7 = estrone sulfotransferase and 8 = estrone sulfate sulfatase. The '?' illustrates the possibility of yet unknown pathways of oestrogen synthesis.

derives from adrenal secretion, with a similar amount produced by the ovaries (10). Between 1–2.5% of circulating Δ^4-A and 0.4–0.5% of circulating T are aromatized into estrone (E_1) and estradiol (E_2) respectively (Lønning et al. Unpublished data). While there is general agreement that aromatization of Δ^4-A to E_1 is the major oestrogen production pathway in postmenopausal women, it is not clear whether aromatization of Δ^4-A to E_1 and T to E_2 together may account for the total amount of oestrogens produced in postmenopausal women or whether alternative production pathways may exist. One study suggested a direct production pathway of E_1 from DHEA without plasma Δ^4-A as an intermediate, this pathway was suggested to account for 20–25% of circulating E_1 (11). This hypothesis has not been explored by others. The finding of persistent oestrogens in patients receiving treatment with aminoglutethimide despite a 95–98% aromatase inhibition in vivo (12) indirectly suggests alternative production pathways to exist. No direct evidence supports hypothesis of dietary oestrogen contribution. Findings support a hypothesis that reduced plasma estrogen levels in patients on special diets may be explained by alterations in enterohepatic cycling of endogenous steroids (13). Further, as ingested oestrogens will mainly undergo first-pass metabolism and thus appear as urinary metabolities but not unconjugated E_1 or E_2 in plasma (14), a high intake of dietary oestrogens might be expected to result in a higher total body oestrogen production rate when this parameter is measured in urine instead of plasma. This is not the case. If alternative oestrogen production pathways occurs, this could have important therapeutic implications.

It is not clear to what extent different plasma oestrogens as E_2, E_1 and E_1S each may contribute to the intratumour oestrogens. Nor is it clear to what extent local oestrogen production in tumour-surrounding tissue (15) or inside the tumour itself (16) may be of biological importance.

Drugs suppressing plasma oestrogens in premenopausal women. LH-RH analogues have recently been introduced as a 'medical castration' of premenopausal women. Hormone suppression as well as clinical response rates seem to be similar to what may be achieved by radiological or surgical castration (17, 18).

Drugs suppressing plasma oestrogens in postmenopausal women. Drugs known to suppress postmenopausal plasma oestrogen levels act by a) reducing substrates (androgens) available for aromatization to oestrogens, b) inhibiting aromatization of androgens to oestrogens, and/or c) stimulating oestrogen metabolism (Table 1).

Administration of LH-RH-analogues to postmenopausal women was recently shown to suppress plasma T with a slight effect on plasma E_2 (19). This finding may contrast with previous findings of similar plasma E_1 and E_2 levels in castrated and normal postmenopausal women (10, 20), but it may be due to recent improvement in sensitivity for estradiol measurements. Noteworthily, ovarian irradiation was shown 25 years ago to cause a slight reduction in urinary oestrogen excretion also in postmenopausal women (21). Summarizing different studies with LH-RH analogues in postmenopausal women with advanced breast cancer, a poor response rate of 8% (8/95) is found (17, 22–24). However, while use of LH-RH analogues as single drug treatment for postmenopausal breast cancer women is not warranted, this treatment causes few side effects, and the question whether it might improve the response rate to other endocrine treatment modalities if added in concert, has yet to be addressed.

Ketoconazole and trilostane are known inhibitors of adrenal 17α-hydroxylase and the 3β-ol-dehydrogenase-Δ^5- to Δ^4-isomerase respectively (Table 1). Both drugs have been suggested to be aromatase inhibitors (25, 26), but this hypothesis has not been explored in vivo by tracer studies. Ketoconazole has been found to inhibit placental aromatase in vitro, although studies of its potency compared to other aromatase inhibitors have yielded somewhat conflicting results (26–28). Studies in breast cancer patients

Table 1

Oestrogen production inhibitors used for treatment of breast cancer

LH-RH analogues	– suppress ovarian steroid production
Glucocorticoids	– suppress adrenal steroid production
Trilostane	– ihibits Δ^5-Δ^4 steroid conversion in adrenals
	– aromatase inhibition???
Ketoconazole	– inhibits the adrenal 17-hydroxylase
	– aromatase inhibitor in vitro
Testololactone	– aromatase inhibitor
Aminoglutethimide	– aromatase inhibitor
	– increases estrone sulfate metabolism
4-Hydroxyandrostenedione	– aromatase inhibitor
CGS 16949A	– aromatase inhibitor
Pyridoglutethimide	– aromatase inhibitor
R76713	– aromatase inhibitor
(only tested on normal volunteers so far)	

have revealed a significant suppression of plasma androgens, a slight suppression of plasma E_2, but no significant effect on plasma E_1 (29, 30); thus, there is no evidence of any significant aromatase inhibition in vivo. One study reported trilostane treatment to cause oestrogen suppression despite elevation of plasma androstenedione (25), but the results presented in this study do not allow conclusions about aromatase inhibition. An insufficient suppression of adrenal androgen output in patients on trilostane as well as ketoconazole treatment may be explained by the fact that the adrenal enzymes blocked are involved in cortisol biosynthesis; inhibition of cortisol biosynthesis may in turn stimulate pituitary ACTH excretion.

Ketoconazole given as sole drug treatment was reported to cause a low response rate (0/12) among postmenopausal breast cancer patients (30). Trilostane given with glucocorticoids provoked an objective response in 25/161 patients (16%) (31–33), this might well be caused by the glucocorticoids alone. Due to a high frequency of side effects (mainly gastrointestinal), neither ketoconazole nor trilostane may be suitable for combined drug therapy.

Glucocorticoids suppress adrenal steroidogenesis. Results from different studies suggest glucocorticoid administration may cause a more efficient suppression of plasma oestrogens than treatment with adrenal enzyme inhibitors as ketoconazole and trilostane when these drugs are given as single drug treatment (25, 30, 34). Noteworthily, administration of glucocorticoids will suppress adrenal secretion of DHEA as well as Δ^4-A and T (35–37), DHEA is produced mainly by adrenal secretion with little contribution from the postmenopausal ovaries (10, 38). If a direct production pathway from DHEA to E_1 exists, possibly suppression of adrenal DHEA secretion could explain why addition of glucocorticoids to aminoglutethimide 250 mg/day seems to enhance oestrogen suppression compared to treatment with aminoglutethimide alone (39). Glucocorticoid administration has been shown to cause less suppression of plasma oestrogens than that seen during aminoglutethimide treatment (34), and a response rate of about 10–20% (40) is clearly inferior to a response rate of about 30% to aminoglutethimide treatment. Why surgical adrenalectomy, on the other hand, causes a significant better response rate than glucocorticoids (41) with oestrogen suppression and clinical response rates similar to aminoglutethimide (42), is not clear.

If the magnitude of oestrogen suppression and clinical response rates among postmenopausl women treated with LH-RH analogues, trilostane, ketoconazole, glucocorticoids and the aromatase inhibitor aminoglutethimide are compared, a dose–response relationship between oestrogen suppression and response rate to the different treatments is suggested. However, such a comparison should be carefully interpreted. One investigation comparing oestrogen suppression among responders and non-responders to aminoglutethimide treatment showed no significant difference in hormone suppression in the two groups (43), and different drugs may have a different influence on local tissue and intra-tumour oestrogen disposition which might not be reflected in alterations in plasma steroid levels.

It is not clear whether combined treatment with different drugs acting on oestrogen production may be beneficial. Adding glucocorticoids to aminoglutethimide treatment obviously reduces adrenal Δ^4-A as well as DHEA secretion and it seems to cause a better suppression of plasma oestrogens (39). However, there is no direct evidence to suggest that glucocorticoids improve the response rate to aminoglutethimide among patients with relapsing disease (Table 2). On the other hand, recent results, suggesting that prednisone might improve the response rate to tamoxifen in breast cancer, raise new and interesting questions about the mechanism of action of glucocorticoids in breast cancer (44).

Two aromatase inhibitors, aminoglutethimide and Δ^1-testololactone have previously been evaluated for treatment of postmenopausal breast cancer. Currently, at least 3 new aromatase inhibitors are undergoing clinical trials (Fig. 2, Table 1). Before reviewing recent investigations on

Table 2

Response rates among postmenopausal women with breast cancer to treatment with different aromatase inhibitors

Drug	Schedule	Response		Reference
		n	%	
Aminoglutethimide	1 g/day + HC	410/1411	29%	38
	250 mg/day – HC	22/114	19%	69, 70
	250 mg/day + HC	25/101	25%[a]	71
		16/85	19%[b]	71
Testololactone	200 mg/day	5/118	4%	67
	1 000 mg/day	18/123	14%	67
	2 000 mg/day	15/121	12%	67
4-OH-androstenedione	500–1 000 mg/week i.m.	14/52	27%	22
	500 mg/day oral	8/29	28%	9

[a] All patients included.
[b] Patients receiving aminoglutethimide as first-line therapy excluded.

these drugs, some 'background controversies' should be considered:

When aminoglutethimide, the 'classic' aromatase inhibitor, is administered as a 'conventional dose' of 1 g a day with glucocorticoids it causes a response rate about

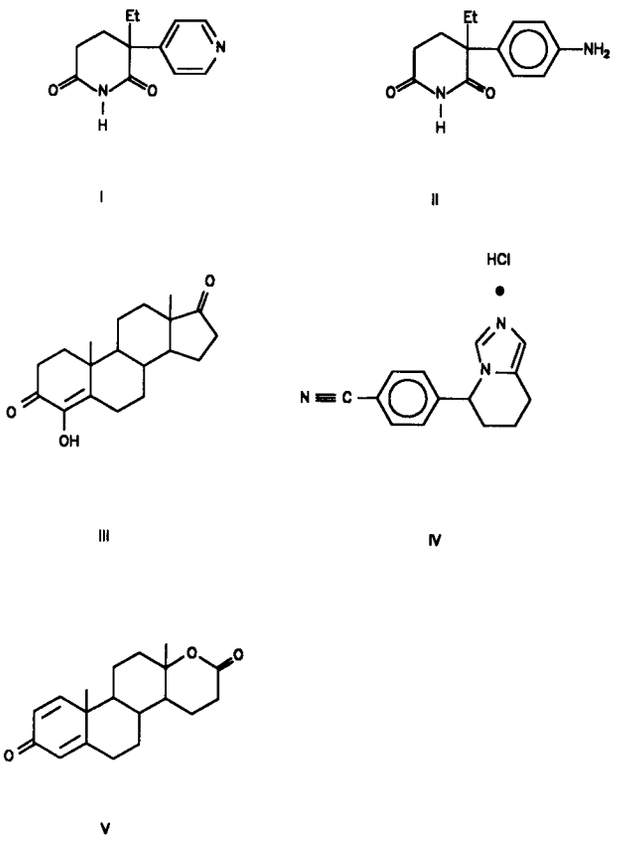

Fig. 2. Chemical structure of different aromatase inhibitors used for treatment of breast cancer. I = pyridoglutethimide, II = aminoglutethimide, III = 4-hydroxyandrostenedione, IV = CGS 16949A and V = Δ^1-testololactone.

30% among unselected postmenopausal breast cancer patients. About 50% of patients known to have a hormone-receptor positive tumour will respond (4). When administered as a 'low dose' drug treatment of 250 mg/day with or without glucocorticoids, a response rate of 19% has been achieved for patients with relapsing disease (Table 2). This finding is surprising, considering the fact that aminoglutethimide 250 mg/daily inhibits aromatase in vivo and suppresses plasma E_1 and E_2 to the same degree as what may be achieved by the high-dose treatment (35, 37). On the other hand, aminoglutethimide was recently shown to cause a 2-fold increase of E_1S clearance rate (5, 6) As this effect seems to be dose-related (6), it might contribute to a different response rate between the two different aminoglutethimide dose schedules. New aromatase inhibitors should be compared with aminoglutethimide 1 000 mg/day in phase III trials to assure an optimal effect of aminoglutethimide.

Testololactone (Δ^1-testololactone) is a weak androgen previously used for treatment of advanced breast cancer. Later, this drug was found to be an aromatase inhibitor, and treatment with testololactone seems to inhibit in vivo aromatization by about 90% (45, 46). Surprisingly, treatment with testololactone seems to cause an objective response in 10–15% of patients only (47). This finding questions the efficacy of aromatase inhibition alone as endocrine treatment for breast cancer.

The steroid derivative 4-hydroxyandrostenedione (4-OHA) is a potent aromatase inhibitor in vitro (48), and it suppresses plasma E_1 and E_2 in vivo (49, 50). One study found 4-OHA to inhibit aromatization in primates in vivo (51), but no such study has been conducted in man as yet. 4-OHA is currently the only new aromatase inhibitor that has undergone phase II trials. This drug has few side effects except for a tendency to local complications when administered by the i.m. route (52). The response rates to this drug when administered as i.m. or oral drug schedules

have been reported to be 27–28% (52, 53). Interestingly, 4-OHA is not only a competitive aromatase inhibitor, it also inactivates the aromatase enzyme itself (27, 48).

CGS 16949A is a new, non-steroidal aromatase inhibitor currently undergoing phase II trials. The drug has been shown to suppress plasma E_1, E_2 and E_1S in a dose-dependent matter up to a dose of 2 mg/daily (54). This drug, however, does not seem to be a pure aromatase inhibitor. At the drug level which causes a maximum suppression of plasma oestrogens, it also seems to cause some inhibition of the adrenal 18-hydroxylase, causing a reduction in adrenal aldosterone output (55). CGS 16949A was claimed to suppress plasma E_1 and E_2 as well as E_1S to a similar extent as previously reported with aminoglutethimide (54, 56). Such a direct comparison, however, should be carefully interpreted. The endocrine investigations on these two drugs were conducted 7 years apart, and mean pretreatment E_1S values were twice as high among the patients treated with aminoglutethimide as among the patients treated with CGS 16949A (54, 56). Current studies are in progress to assess aromatase inhibition and oestrogen production rates among patients receiving CGS 16949A therapy.

Pyridoglutethimide is a drug chemically related to aminoglutethimide (57, 58). A phase I trial has been undertaken. The drug was found to cause significant suppression of plasma E_2 (59). Further, pyridoglutethimide seems to be an enzyme inducer in man (ibid). Whether this drug causes stimulation of E_1S metabolism similar to what is seen during aminoglutethimide treatment has yet to be assessed.

R76713 is a new, potent, non-steroidal aromatase inhibitor. When this drug was given to male volunteers, a significant suppression of plasma E_2 was seen (60). No results have so far been reported for postmenopausal women.

Anti-oestrogens

Tamoxifen, the 'classic' anti-oestrogen, is currently first-line endocrine treatment due to its low frequency of side effects (61). Randomized studies have revealed the response rate to tamoxifen to be similar to what may be achieved by aminoglutethimide (62, 63).

Currently, several new anti-oestrogens are undergoing phase I/II trials (Fig. 3, Table 3). While most patient series contain a small number of patients, evidence so far suggests that drugs such as trioxifene mesylate (8, 64) and toremifene (65) induce response rates similar to tamoxifen. While trioxiphene mesylate was found to cause more side

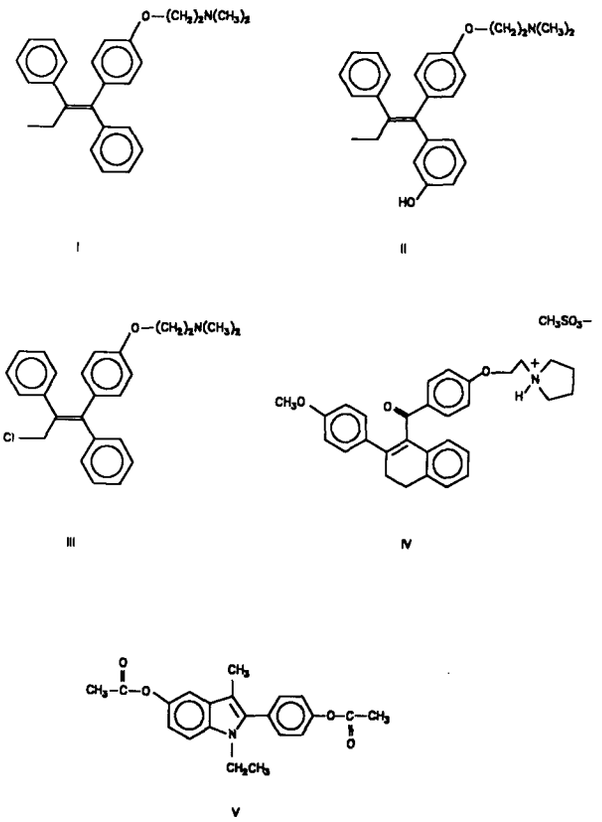

Fig. 3. Chemical structure of different anti-oestrogens used for treatment of breast cancer. I = tamoxifen, II = droloxifene, III = toremifene, IV = trioxifene mesylate and V = zindoxifene.

Table 3

Response rates to new anti-oestrogens in phase II trials

Drug	Dose	Response		Reference
		n	%	
Trioxifene mesylate	5–20 mg b.d.	27/52	52%[a]	31
	20 mg b.d.	2/17	12%[b]	31
	0.5–100 mg/m²/day	9/36	25%	–
Toremifene	60 mg o.d.	25/46	54%	63
Zindoxifene	10–100 mg o.d.	0/25	0%	60

[a] Patients not receiving previous TAM.
[b] Patients receiving previous treatment with TAM.

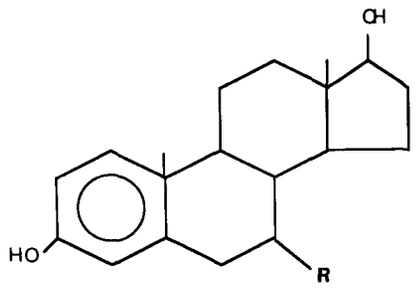

Fig. 4. Chemical structure of the new steroidal anti-oestrogens. Different compounds are produced by the introduction of different groups in the 7-position.

effects than tamoxifen (64), toremifene as well as droloxifene (66) are currently undergoing trials. While in vitro investigations suggested zindoxifene to be a suitable anti-oestrogen for in vivo use, a first phase I/II trial found an unexpected low response rate among patients treated with this drug (67).

Most anti-oestrogens possess weak oestrogen agonistic properties. Whether this is detrimental or not has been discussed; it may be of different importance for treatment in the advanced situation and during long-term adjuvant therapy. An interesting new group of steroidal anti-oestrogens synthesized by substitution in the 7-position on the steroid nucleus have been claimed to be pure oestrogen antagonists in vitro ((68). Fig. 4); these drugs will probably soon go into phase I trials.

Another aspect of new anti-oestrogens is the clinical importance of pharmacokinetic properties. Tamoxifen has an exceptionally long half-life; detectable plasma drug levels are found weeks following cessation of therapy (61). Due to the low toxicity of most anti-oestrogens it is seldom necessary to remove the drug quickly on account of side effects. Thus, during steady state therapy a slow turnover will have few practical implications. However, when cyclic drug treatment is to be given, such a slow drug turnover might be disadvantageous and, from a theoretical point of view, anti-oestrogens with a rapid turnover could be more feasible.

Conclusive remarks

In the next few years several new aromatase inhibitors as well as anti-oestrogens will enter phase I and phase II trials, the most successful drugs will then be compared with aminoglutethimide and tamoxifen in phase III trials. Such trials will need a very careful planning for several reasons:

The large number of patients to be included in such a trial should be considered. To discriminate between a response rate of 20 and 30% for two different drugs at a 95% probability level and with a chance of a type II error less than 10%, several hundred patients would have to be included in each treatment arm.

Another interesting aspect is whether resistance or lack of response to one drug also implies resistance to similar drugs within the same group; trials should be undertaken to address this question. Considering aromatase inhibitors, the possibility of combining different drugs should be assessed. If aminoglutethimide acts partly by inducing E_1S metabolism, it could be beneficial to add aminoglutethimide to patients progressing or relapsing on treatment with other aromatase inhibitors.

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REFERENCES

1. Sakai F, Cheix F, Clavel M, et al. Increases in steroid binding globulins induced by tamoxifen in patients with carcinoma of the breast. J Endocrinol 1978; 76: 219.
2. Sutherland RL, Murphy LC. The binding of tamoxifen to human mammary carcinoma cytosol. Eur J Cancer 1980; 16: 1141.
3. Pasqualini JR, Gelly C. Effect of tamoxifen derivatives on the conversion of estrone sulfate to estradiol in the MCF-7 mammary cancer cell line. Cancer Lett 1988; 40: 115.
4. Lønning PE, Kvinnsland S. Mechanisms of action of aminoglutethimide as endocrine therapy of breast cancer. Drugs 1988; 35: 685.
5. Lønning PE, Johannessen DC, Thorsen T. Alterations in the production and clearance rate of oestrone and oestrone suphate in breast cancer patients treated with aminoglutethimide. Br J Cancer 1989; 60: 107.
6. Lønning PE, Kvinnsland S, Thorsen T, Ueland PM. Alterations in the metabolism of estrogens during treatment with aminoglutethimide in breast cancer patients: preliminary findings. Clin Pharmacokinet 1987; 13: 353.
7. Lønning PE, Johannessen DC, Thorsen T, Ekse D. Effects of aminoglutethimide on plasma estrone sulfate not caused by aromatase inhibition. J Steroid Biochem 1989; 33: 541.
8. Lee RW, Buzdar AU, Blumenschvein GR, Hortobagyi N. Trioxifene mesylate in the treatment of advanced breast cancer. Cancer 1986; 57: 40.
9. Vermeulen A, Verdonck L. Sex hormone concentrations in postmenopausal women. Clin Endocrinol 1978; 9: 59.
10. Vermeulen A. The hormonal activity of the postmenopausal ovary. J Clin Endocrinol Metab 1976; 42: 247.
11. Longcope C, Bourget C, Flood C. The production and aromatization of dehydroepiandrosterone in post-menopausal women. Maturitas 1982; 4: 325.
12. Santen RJ, Santner S, Davis B, Veldhuis J, Samojilik E, Ruby E. Aminoglutethimide inhibits extraglandular estrogen production in postmenopausal women with breast cancer. J Clin Endocrinol Metab 1978; 47: 1257.
13. Goldin BR, Gorbach S. Effect of diet on the plasma levels, metabolism, and excretion of estrogens. Am J Clin Nutr 1988; 48: 787.
14. Longcope C, Gorbach S, Goldin B, Woods M, Dwyer J, Warram J. The metabolism of estradiol; oral compared to intravenous administration. J Steroid Biochem 1985; 23: 1065.

15. O'Neill J, Elton RA, Miller WR. Aromatase activity in adipose tissue from breast quadrants: a link with tumour site. Br Med J 1988; 296: 741.
16. James VHT, Reed MJ, Adams EF, et al. Oestrogen uptake and metabolism in vivo. Oestrogens and the human breast, Edinburgh, 22–24 Sept, 1988.
17. Santen RJ, Manni A, Harvey H. Gonadotropin releasing hormone (GnRH) analogus for the treatment of breast and prostatic cancer. Breast Cancer Res Treat 1986; 7: 129.
18. Williams MR, Walker KJ, Turkes A, Blamey RW, Nicholson RI. The use of an LH-RH agonist (ICI 118630, Zoladex) in advanced premenopausal breast cancer. Br J Cancer 1986; 53: 629.
19. Dowsett M, Cantwell B, Lal A, Jeffcoate SL, Harris AL. Suppression of postmenopausal ovarian steroidogenesis with the luteinizing hormone-releasing hormone agonist goserelin. J Clin Endocrinol Metab 1988; 66: 677.
20. Rader MD, Flickinger GL, De Villa GO, Mikuta JJ, Mikhail G. Plasma estrogens in postmenopausal women. Am J Obstet Gynecol 1973; 116: 1069.
21. Nissen-Meyer R, Sanner T. The excretion of oestrone, pregnanediol and pregnanetriol in breast cancer patients. II. Effects of ovariectomy, ovarian irradiation and corticosteroids. Acta Endocrinol 1963; 44: 334.
22. Harris AL, Carmichael J, Cantwell BMJ, Dowsett M. Zoladex: endocrine and therapeutic effects in post-menopausal breast cancer. Br J Cancer 1989; 59: 97.
23. Plowman PN, Nicholson RI, Walker KJ. Remission of postmenopausal breast cancer during treatment with the luteinising hormone releasing hormone agonist ICI 118630. Br J Cancer 1986; 54, 903.
24. Waxman JH, Harland SJ, Coombes RC, et al. The treatment of postmenopausal women with advanced breast cancer with buserelin. Cancer Chemother Pharmacol 1985; 15: 171.
25. Beardwell CG, Hindley AC, Wilkinson PM, St John J, Bu'lock D. Hormonal changes in postmenopausal women with breast cancer treated with trilostance and dexamethasone. Clin Endocrinol 1985; 23: 413.
26. Wouters W, De Coster R, Goeminne N, Beerens D, van Dun J. Aromatase inhibition bythe antifungal ketoconazole. J Steroid Biochem 1988; 30: 387.
27. Ayub M, Levell MJ. Structure-activity relationship of the inhibition of human placental aromatase by imidazole drugs including ketoconazole. J Steroid Biochem 1988; 31: 65.
28. Mason JI, Murry BA, Olcott M, Sheets JJ. Imidazole antimycotics: inhibitors of steoid aromatase. Biochem Pharmacol 1985; 34: 1087.
29. De Coster R, Caers I, Haelterman C, Debroye M. Effect of a single administration of ketoconazole on total and physiologically free plasma testosterone and 17β-oestradiol levels in healthy male volunteers. Eur J Clin Pharmacol 1985; 29: 489.
30. Harris AL, Cantwell BMJ, Dowsett M. High dose ketoconazole: endocrine and therapeutic effects in postmenopausal breast cancer. Br J Cancer 1988; 58: 493.
31. Beardwell CG, Hindley AC, Wilkinson PM, Todd IDH, Ribeiro GG, Bu'lock D. Trilostane in the treatment of advanced breast cancer. Cancer Chemother Pharamcol 1983; 10: 158.
32. Coombes RC, Powles TJ, Muindi J, et al. Trilostane therapy for advanced breast cancer. Cancer Treat Rep 1985; 69: 351.
33. Williams CJ, Barley V, Blackledge G, et al. Multicenter study of trilostane: A new hormonal agent in advanced postmenopausal breast cancer. Cancer Treat Rep 1987; 71: 1197.
34. Harris AL, Dowsett M, Smith IE, Jeffcoate S. Hydrocortisone alone vs. hydrocortisone plus aminoglutethimide: A comparison of the endocrine effects in postmenopausal breast cancer. Eur J Cancer Clin Oncol 1984; 20: 463.
35. Harris AL, Dowsett M, Smith IE, Jeffcoate SL. Endocrine effects of low dose aminoglutethimide alone in advanced postmenopausal breast cancer. Br J Cancer 1983; 47: 621.
36. Samojlik E, Santen RJ, Adrenal suppression with aminoglutethimide: III. Comparison of plasma Δ4- and Δ5-steroids in postmenopausal women treated for breast carcinoma. J Clin Endocrinol Metab 1978; 47: 717.
37. Vermeulen A, Paridaens R, Heuson JC. Effects of aminoglutethimide on adrenal steroid secretion. Clin Endocrinol 1983; 19: 673.
38. Maroulis GB, Abraham GE. Ovarian and adrenal contributions to peripheral steroid levels in postmenopausal women. Obstet Gynecol 1976; 48: 150.
39. Dowsett M, Harris AL, Stuart-Harris R, et al. A comparison of the endocrine effects of low dose aminoglutethimide with and without hydrocortisone in postmenopausal breast cancer patients. Br J Cancer 1985; 52: 525.
40. Rose DP. Postmenopausal oestrogen production and its inhibition. In: Stoll BA, ed. New aspects of breast cancer. London: Will. Heineman Med Books Ltd, 1982; vol 5.
41. Dao TL, Tan E, Brooks V. A comparative evaluation of adrenalectomy and cortisone in the treatment of advanced mammary carcinoma. Cancer 1961; 14: 1259.
42. Santen RJ, Worgul TJ, Samojlik E, et al. Randomized trial comparing surgical adrenalectomy with aminoglutethimide plus hydrocortisone in women with advanced breast cancer. N Engl J Med 1981; 308: 545.
43. Santen RJ, Worgul TJ, Lipton A, Aminoglutethimide as treatment of postmenopausal women with advanced breast carcinoma. Ann Intern Med 1982; 96: 94.
44. Rubens RD, Tinson CL, Coleman RE, et al. Prednisone improves the response to primary endocrine treatment for advanced breast cancer. Br J Cancer 1988; 58: 626.
45. Barone RM, Shamonki IM, Siiteri PK, Judd HL. Inhibition of peripheral aromatization of androstenedione to estrone in postmenopausal women with breast cancer using Δ^1-testololactone. J Clin Endocrinol Metab 1979; 49: 672.
46. Dunaif A, Longcope C, Canick J, Badger T, Crowley WF. The effects of the aromatase inhibitor Δ^1-testololactone on gonadotropin release and steroid metabolism in polycystic ovarian disease. J Clin Endocrinol Metab 1985; 60: 773.
47. Volk H, Deupree RH, Goldenberg IS, Wilde RC, Carabas RA, Escher GC. A dose response evaluation of delta-1-testololactone in advanced breast cancer. Cancer 1974; 33: 9.
48. Brodie AMH, Garrett WM, Hendrickson JR, Tsai-Morris CH, Marcotte PA, Robinson CH. Inactivation of aromatase *in vitro* by 4-hydroxy-4-adrostene-3,17-dione and 4-acetoxy-4-androstene-3,17-dione and 4-acetoxy-4-androstene-3,17-dione and sustained effects *in vivo*. Steroids 1981; 38: 693.
49. Dowsett M, Cunningham DC, Stein RC, et al. Dose-related endocrine effects and pharmacokinetics of oral and intramuscular 4-hydroxyandrostenedione in postmenopausal breast cancer patients. Cancer Res 1989; 49: 1306.
50. Dowsett M, Goss PE, Powles TJ, et al. Use of the aromatase inhibitor 4-hydroxyandrostenedione in postmenopausal breast cancer: optimazation of therapeutic dose and route. Cancer Res 1987; 47: 1957.
51. Brodie AMH, Longcope C. Inhibition of peripheral aromatization by aromatase inhibitors, 4-hydroxy-and 4-acetoxy-androstene-3,17-dione. Endocrinology 1980; 106: 19.
52. Goss PE, Powles TJ, Dowsett M et al. Treatment of advanced postmenopausal breast cancer with an aromatase inhibitor,

4-hydroxyandrostenedione: phase II report. Cancer Res 1986; 46: 4823.
53. Cunningham D, Powles TJ, Dowsett M, et al. Oral 4-hydroxyandrostenedione, a new endocrine treatment for disseminated breast cancer. Cancer Chemother Pharmacol 1987; 20: 253.
54. Santen RJ, Demers LM, Adlercreutz H, et al. Inhibition of aromatase with CGS 16949A in postmenopausal women. J Clin Endocrinol Metab 1989; 68: 99.
55. Dowsett M, Lal A, Stein RC, Coombes RC. Dose-related endocrine study of aromatase inhibitor CGS 16949A. 13th Congr Eur Soc Med Oncol, Lugano Oct 30–Nov 1, 1988.
56. Samojlik E, Santen RJ, Worgul TJ: Plasma estrone-sulfate assessment of reduced estrogen production during treatment of metastatic breast carcinoma. Steroids 1982; 39: 497.
57. Daly MJ, Jones GW, Nicholls PJ, Smith HJ, Rowlands MG, Bunnett MA. Synthesis and biochemical evaluation of analogues of aminoglutethimide based on phenylpyrrolidine-2,5-dione. J Med Chem 1986; 29: 520.
58. Foster AB, Jarman M, Leung C-S, et al. Analogues of aminoglutethimide: selective inhibition of aromatase. J Med Chem 1985; 28: 200.
59. Haynes B, Griggs LJ, Jarman M, Coombes RC, Lønning PE, Powles TJ. Pharmacokinetics of pyridoglutethimide, a new aromatase inhibitor. 30th Ann Meeting Br Ass Cancer Res, Glasgow, April 10–12, 1989.
60. Bruynseels J, Vanderpas H, van Roy P, De Coster R, Amery WK, Janssen PAJ. R76713: aromatase blocker. Single-dose effect studies in volunteers. Sixth NCI-EORTC symposium on new drugs in cancer therapy. Amsterdam, March 7–10, 1989.
61. Furr BJA, Jordan VC. The pharmacology and clinical uses of tamoxifen. Pharmacol Ther 1984; 25: 127.
62. Lipton A, Harvey H, Santen RJ, et al. Randomized trial of aminoglutethimide versus tamoxifen in metastatic breast cancer. Cancer Res 1982; 42 (Suppl): 3434s.
63. Smith IE, Harris AL, Morgan M, et al. Tamoxifen versus aminoglutethimide in advanced breast carcinoma: a randomized trial. Br Med J 1981; 283: 1432.
64. Witte RS, Pruitt B, Tormey DC, et al. A phase I/II investigation of trioxifene mesylate in advanced breast cancer. Clinical and endocrine effects. Cancer 1986; 57: 34.
65. Valavaara R, Pyrhonen S, Heikkinen M, et al. Toremifene, a new antiestrogenic compound for treatment of advanced breast cancer. Phase II study. Eur J Cancer Clin Oncol 1988; 24: 785.
66. Stamm H, Roth R, Huber H-J, et al. Preliminary data on a phase-I trial of the new antiestrogen droloxifene: tolerance, pharmacokinetics and metabolism. Contr Oncol 1986; 23: 73.
67. Stein RC, Dowsett M, Cunningham DC, et al. Phase I/II study of the antiestrogen, zindoxifene (D16726) in the treatment of advanced breast cancer. In document.
68. Wakeling AE, Bowler J. Novel antioestrogens without partial agonist activity. J Steroid Biochem 1988; 31: 645.
69. Murray R, Pitt P. Low-dose aminoglutethimide without steroid replacement in the treatment of postmenopausal women with advanced breast cancer. Eur J Cancer Clin Oncol 1985; 21: 19.
70. Stuart-Harris R, Dowsett M, Bozek T, et al. Low dose aminoglutethimide in treatment of breast cancer. Lancet 1984; 2: 604.
71. Harris AL, Cantwell BMJ, Carmichael J, et al. Phase II study of low dose aminoglutethimide 250 mg/day plus hydrocortisone in advanced postmenopausal breast cancer. Eur J Cancer Clin Oncol 1989; 25: 1105.