

# Cross-resistance to different aromatase inhibitors in breast cancer treatment

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## Abstract

Recent studies have documented the biochemical and clinical efficacy of novel aromatase inhibitors. These drugs belong to one of two classes: non-steroidal and steroidal drugs, known to be different with respect to enzyme binding site and their effect on the aromatase enzyme. Several studies have now confirmed a lack of complete cross-resistance to drugs of the two classes. While some of these observations may be explained by a more potent aromatase inhibition caused by some aromatase inhibitors compared to others, this is not always the case. Such observations therefore focus on the importance of alternative mechanism of action like a differential influence on the intratumour aromatase enzyme by the different drugs. Current and future studies should aim at exploring the mechanism of cross-resistance and evaluate optimal use of different aromatase inhibitors in sequence or probably, also in concert.

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## Introduction

The development of aromatase inhibitors for breast cancer therapy emerged from two different sources. The first evidence that such drugs could inhibit oestrogen synthesis and cause tumour regression in postmenopausal breast cancer came from the discovery that amino-glutethimide (initially introduced for breast cancer therapy in an attempt to achieve a 'medical adrenalectomy') suppressed plasma oestrogen levels despite sustained plasma androgens (Samojlik *et al.* 1977). This observation was followed by direct confirmation of *in vivo* aromatase inhibition in patients treated with the drug (Santen *et al.* 1978). At the same time pioneering work on substrate (androgen) analogues as aromatase inhibitors (Brodie *et al.* 1977) demonstrated in 1984 (Coombes *et al.* 1984) that a steroidal aromatase inhibitor, 4-hydroxyandrostenedione, was clinically effective in breast cancer patients. The development of two different classes of aromatase inhibitors, steroidal (or 'irreversible', substrate-site binding type I) and non-steroidal (haem-binding, type II) inhibitors, provided the rationale for exploring lack of cross-resistance between drugs belonging to the two classes.

Due to toxic side-effects of aminoglutethimide (Lønning & Kvinnsland 1988) and the fact that 4-hydroxyandrostenedione has to be administered parentally to be fully effective (MacNeill *et al.* 1995), much effort has been spent on developing novel drugs.

Whilst efforts to develop novel analogues of aminoglutethimide (MacNeill *et al.* 1992) and the use of the dextro-isomer of aminoglutethimide (Geisler *et al.* 1998b) have given disappointing results, another class of non-steroidal aromatase inhibitors, the triazoles, has been successfully developed, with several highly potent, specific and non-toxic substances (Wall *et al.* 1993, Buzdar *et al.* 1996, Dombernowsky *et al.* 1998). In addition, exemestane (6-methylandrostenedione) has been developed as a potent aromatase inhibitor for oral use (Johannessen *et al.* 1997, Giudici *et al.* 1998). An important question to answer at this stage is whether we should search for the single, most effective drug, or whether using different aromatase inhibitors with a different chemistry administered in sequence or together actually may prove advantageous.

## The importance of potent oestrogen suppression

Recent results from clinical trials suggest a relationship between the degree of oestrogen suppression and clinical benefits (response rate and duration). Anastrozole and letrozole, two of the novel aromatase inhibitors belonging to the triazole class, both inhibit *in vivo* aromatisation by 97-98% (Dowsett *et al.* 1995, Geisler *et al.* 1996b). This contrasts with aminoglutethimide, which causes about 91% inhibition (MacNeill *et al.* 1992). Recently, a randomised study revealed treatment with letrozole

2.5 mg daily to be superior to aminoglutethimide 500 mg daily (Gershanovich *et al.* 1998). While the full mechanism of the anti-tumour action of progestins administered in high doses to breast cancer patients is not understood (Lundgren 1992), it is now clear that megestrol acetate 160 mg daily suppresses plasma oestrogen levels by 75-80% in postmenopausal women (Lundgren *et al.* 1996). Recent studies have shown that letrozole given as 2.5 mg daily produces a better response rate compared with megestrol acetate (Dombernowsky *et al.* 1998). Similarly, anastrozole 1 mg daily produces more prolonged remissions and improved survival when compared to the progestin (Buzdar *et al.* 1998). These results further support the hypothesis that enhanced plasma oestrogen suppression is associated with an improved clinical response. On the other hand, letrozole 0.5 mg daily, which also inhibits *in vivo* aromatisation by 98% (Dowsett *et al.* 1995), was found inferior to megestrol acetate with respect to response rate (Dombernowsky *et al.* 1998), suggesting alternative mechanisms may be involved.

### Observations on sequential use of different aromatase inhibitors

With a few exceptions, aromatase inhibitors have been used for second- or third-line treatment. Accordingly, when evaluating responses to treatment with a second aromatase inhibitor in patients failing on therapy with another aromatase inhibitor, the second aromatase inhibitor in general is administered as a third-line (or later) regimen. While the literature dealing with response rates to different forms of second-line endocrine therapy is substantial, there are few reports dealing with the response rates to third-line hormone therapy in large groups of patients (Garcia-Giralt *et al.* 1992, Iveson *et al.* 1993a, Murray & Pitt 1995). Although such data on this issue have to be interpreted carefully due to selection of patients, the general impression is that a substantial number of patients obtain stabilization of disease and a limited number achieve an objective response (complete response (CR)+partial response (PR)) to third-line (or later) regimens. Finally, it should be recognised that the response rates to conventional second-line endocrine regimens have been lower in recent, peer-reviewed trials with strict response criteria and confirmation of responses (Buzdar *et al.* 1998, Dombernowsky *et al.* 1998, Gershanovich *et al.* 1998) (Table 1) than those reported in earlier trials.

The limited numbers of trials conducted on sequential use of different aromatase inhibitors may be divided into two groups: trials evaluating the effect at relapse of adding a second aromatase inhibitor to the first one, and studies

**Table 1** Response rates (CP+PR) to contemporary endocrine therapies in advanced breast cancer

| Treatment             | Dose (mg/day) | Response rate (%)                    |
|-----------------------|---------------|--------------------------------------|
| Anastrozole           | 1             | 10.3 <sup>a</sup>                    |
| Anastrozole           | 10            | 8.9 <sup>a</sup>                     |
| Megestrol acetate     | 160           | 7.9 <sup>a</sup>                     |
| Letrozole             | 0.5           | 12.8 <sup>b</sup> /16.7 <sup>c</sup> |
| Letrozole             | 2.5           | 23.6 <sup>b</sup> /19.5 <sup>c</sup> |
| Megestrol acetate     | 160           | 16.4 <sup>b</sup>                    |
| Aminoglutethimide     | 500           | 12.4 <sup>c</sup>                    |
| Exemestane third-line | 25            | 6.6 <sup>d</sup>                     |

<sup>a</sup>Buzdar *et al.* (1996); <sup>b</sup>Dombernowsky *et al.* (1998);  
<sup>c</sup>Gershanovich *et al.* (1998); <sup>d</sup>Lønning *et al.* (1998).

evaluating a second aromatase inhibitor as monotherapy following relapse on the first regimen.

### Effects of adding a second aromatase inhibitor on relapse

So far, the experience with two aromatase inhibitors used in concert following relapse on one of the drugs is limited to small groups of patients in whom aminoglutethimide was added to treatment with formestane when progressing on the latter regimen. Thus, among 11 patients progressing on formestane given as 250 mg/2 weeks, 3 achieved a second response when aminoglutethimide 1000 mg daily was added (Lønning *et al.* 1992, Geisler *et al.* 1996a). Notably, combined treatment with formestane and aminoglutethimide has been shown to achieve a better aromatase inhibition compared with each drug on its own (Jones *et al.* 1992, MacNeill *et al.* 1992, 1994) and adding aminoglutethimide to formestane caused a further fall in plasma levels of oestradiol, as well as oestrone and oestrone sulphate (Lønning *et al.* 1992, Geisler *et al.* 1997). This decrease was most profound for plasma oestrone sulphate, which may be because aminoglutethimide enhances the metabolism of this oestrogen conjugate (Lønning *et al.* 1987, 1989).

### Treatment with a second aromatase inhibitor as monotherapy following progression on another aromatase inhibitor

This concept has been evaluated in five different trials. Of these, four have evaluated use of a steroidal aromatase inhibitor following relapse on a non-steroidal agent, while one study evaluated use of a non-steroidal inhibitor

following relapse on a steroidal drug. Two studies evaluated the use of formestane (250 mg every second week) in patients relapsing on aminoglutethimide (Murray & Pitt 1995, Geisler *et al.* 1996a). One study evaluated use of exemestane administered as a 'high-dose regimen' of 200 mg daily in patients relapsing on aminoglutethimide (Thürlimann *et al.* 1997), and one study has evaluated exemestane 25 mg daily in patients relapsing on either aminoglutethimide or novel aromatase inhibitors such as anastrozole, letrozole or vorozole (Lønning *et al.* 1998). Finally, one study has evaluated anastrozole (1 mg daily) in patients progressing on treatment with formestane (Coombes *et al.* 1999).

These studies all show a lack of complete cross-resistance to a steroidal and a non-steroidal aromatase inhibitor.

The two studies evaluating use of formestane in patients progressing on aminoglutethimide revealed an objective response in 23/112 (21%) (Murray & Pitt 1995) and 3/30 (10%) (Geisler *et al.* 1996a) patients respectively.

The study by Thürlimann and colleagues (1997) revealed 20/78 (26%) patients progressing on aminoglutethimide to respond to treatment with exemestane 200 mg daily. However, endocrine studies have revealed that exemestane administered as 25 mg once daily causes maximal oestrogen suppression without causing the androgenic side-effects seen with doses of 200 mg daily (Johannessen *et al.* 1997). A drug dose of 25 mg daily is currently recommended for clinical use. The use of this exemestane regime in patients failing on treatment with a non-steroidal inhibitor has been evaluated in a large international study (Protocol OEXEO17), enrolling a total of 241 patients. Results are currently becoming available. Importantly, to test the hypothesis that any beneficial effect of exemestane in this patient group results not only from a substantial total body aromatase inhibition but also from specific effects of a steroidal aromatase inhibitor at the tumour level, this study also enrolled patients progressing on novel, potent inhibitors like anastrozole, letrozole and vorozole. Whilst a limited number of patients achieved an objective response to therapy (7%), stabilisation of disease for  $\geq 6$  months was seen in a further 18%, producing a total of 25% benefiting from the therapy (Lønning *et al.* 1998). These figures are somewhat lower, but not very different from contemporary results obtained with other novel aromatase inhibitors given as second-line therapy (Tables 1 and 2). Interestingly, a similar response rate and number of patients achieving a stabilisation of disease of  $\geq 6$  months duration was seen among those previously exposed to either novel potent non-steroidal aromatase inhibitors or aminoglutethimide.

So far, only one study has evaluated the response to a non-steroidal aromatase inhibitor (anastrozole) after

relapse on a steroidal one (formestane). This study revealed that 5/12 relapsing patients who had previously responded to formestane obtained stable disease for  $\geq 6$  months when exposed to anastrozole (Coombes *et al.* 1999).

**Table 2** 'Benefits' (CP+PR+ stabilised disease for 6 months) to contemporary endocrine therapies in advanced breast cancer.

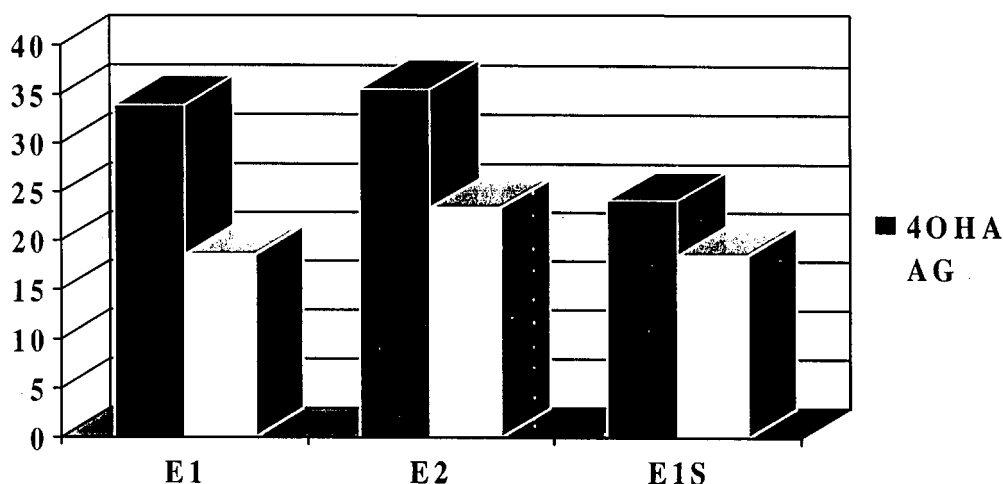
| Treatment             | Dose (mg/day) | Response rate (%)                |
|-----------------------|---------------|----------------------------------|
| Anastrozole           | 1             | 35 <sup>a</sup>                  |
| Anastrozole           | 10            | 32 <sup>a</sup>                  |
| Megestrol acetate     | 160           | 34 <sup>a</sup>                  |
| Letrozole             | 0.5           | 27 <sup>b</sup> /33 <sup>c</sup> |
| Letrozole             | 2.5           | 35 <sup>b</sup> /35 <sup>c</sup> |
| Megestrol acetate     | 160           | 32 <sup>b</sup>                  |
| Aminoglutethimide     | 500           | 29 <sup>c</sup>                  |
| Exemestane third-line | 25            | 25 <sup>d</sup>                  |

<sup>a</sup>Buzdar *et al.* (1996); <sup>b</sup>Dombernowsky *et al.* (1998);

<sup>c</sup>Gershanovich *et al.* (1998); <sup>d</sup>Lønning *et al.* (1998).

## Interpretations of the findings

The clinical efficacy of adding aminoglutethimide following progression on formestane monotherapy could be explained by enhanced aromatase inhibition (Jones *et al.* 1992, MacNeill *et al.* 1992) in addition to induced metabolism of oestrone sulphate (Lønning *et al.* 1989). In this respect, it may also be recalled that some patients progressing on aminoglutethimide 'low-dose treatment' (250 mg daily) may subsequently respond to treatment with the 'high-dose regimen' (1000 mg daily) (Murray & Pitt 1985). Enhanced aromatase inhibition could also be the explanation why patients relapsing on aminoglutethimide subsequently respond to therapy with exemestane (MacNeill *et al.* 1992, Geisler *et al.* 1998a). Likewise, anastrozole is a more potent aromatase inhibitor compared with formestane (Jones *et al.* 1992, Geisler *et al.* 1996b). Contrary, formestane is not a more potent aromatase inhibitor compared to aminoglutethimide. Comparing plasma hormone levels measured in patients during treatment with aminoglutethimide and formestane in our laboratory with similar biochemical assays revealed a better plasma oestrogen suppression with aminoglutethimide compared with formestane (Geisler *et al.* 1996a, 1997, Geisler *et al.* 1998b) (Fig. 1), reflecting the hypothesis that enhanced oestrogen suppression may explain responses to formestane after therapy with aminoglutethimide. However, some cautions should be



**Figure 1** Plasma levels of oestrone (E1), oestradiol (E2) and oestrone sulphate (E1S) in patients on treatment with formestane (4OHA) and aminoglutethimide (AG). Values expressed as percentage of pretreatment levels in individual patients. From Geisler *et al.* (1996a,b, 1997) and Geisler *et al.* (1998b).

raised when comparing plasma oestrogen measurements during treatment with aromatase inhibitors in general and during treatment with drugs belonging to the steroidal class in particular. The problem of sensitivity of methodology is illustrated in several publications (Iveson *et al.* 1993b, Johannessen *et al.* 1997) which show that a substantial number of patients treated with potent aromatase inhibitors have plasma oestrogen levels suppressed down to the sensitivity limit of the assay. The development of a highly sensitive assay to measure oestrone sulphate (Lønning & Ekse 1995) means that 98-99% suppression of the plasma level of this oestrogen conjugate may be assessed in the majority of patients. Data so far suggest that neither formestane nor exemestane is aromatised. However the doses of these drugs administered (formestane 250 mg/2 weeks, exemestane 25 mg daily) is about 10- to 20-fold higher than the endogenous synthesis of androstenedione (1-2 mg/day; calculated from a mean plasma concentration of 2-4 nmol/l and a total body clearance rate of 2000-2500 l/day) (Horton & Tait 1966, Lønning *et al.* 1990). Considering 1-4% of circulating androstenedione is aromatised to oestrone in untreated postmenopausal patients and only 0.1% is aromatised during treatment with a potent aromatase inhibitor, even very minor metabolites of formestane or exemestane could significantly influence the results if they cross-react with the RIA antibodies. This has been documented with exemestane: while measurement of plasma oestrogen levels by RIA in patients on treatment with exemestane revealed suppression to about 30% of control values, following HPLC purification of the samples, suppression to about

5-16% of control values was recorded (Johannessen *et al.* 1997).

The finding that patients relapsing on potent non-steroidal inhibitors such as anastrozole and letrozole may benefit from therapy with exemestane is not likely to be explained by a superior total body aromatase inhibition, as the drugs seem to be equally effective in this respect (Dowsett *et al.* 1995, Geisler *et al.* 1996b, Geisler *et al.* 1998a).

To explain these observations, and also the finding that patients may respond to formestane after aminoglutethimide there is a need to consider hormone as well as drug disposition at the tumour level. So far, there are a limited number of observations on changes in tumour oestrogen concentrations in patients on treatment with aromatase inhibitors (Reed *et al.* 1991, de Jong *et al.* 1997). An interesting observation in this respect was a study conducted by Miller (1991). In this study, he showed that *in vitro* oestrogen synthesis in human tumour specimens could be inhibited by formestane in a dose-dependent manner, but some samples appeared to be resistant to the drug. While the mechanism of this resistance is currently unknown, the findings suggest that some tumours may be resistant to certain drugs.

### Implications for further development of aromatase inhibitors

It would be of great interest to learn the mechanisms of lack of cross-resistance to different aromatase inhibitors. Bearing in mind the modest effects of chemotherapy in advanced breast cancer in general (Clavel & Catimel

1993, Porkka *et al.* 1994), it is mandatory to extend the duration of control with endocrine therapy as long as possible. MCF-7 cells have been shown to adapt to oestrogen deprivation *in vitro* and such sensitised cells may achieve growth stimulation by oestradiol down to a concentration of  $10^{-14}$ - $10^{-15}$  mol/l (Masamura *et al.* 1995). If a similar phenomenon exists *in vivo*, additional clinical benefits may be achieved through more potent total body aromatase inhibition and oestrogen suppression. Some of the observations reviewed here support such a hypothesis. On the other hand, not all observations may be explained by this means, suggesting alternative mechanisms are involved. *In vivo* studies suggest intratumour aromatisation may contribute to the intratumour oestrogen levels to a variable extent (Reed *et al.* 1989, Miller 1994), and there is evidence that aromatisation in some tumour tissues (for reasons yet unexplained) may be less sensitive to the pharmacological effects of certain aromatase inhibitors (Miller 1994). While the aromatase enzyme in peripheral and tumour tissue is the same, regulation of enzyme expression by growth factors and cytokines may vary (Reed *et al.* 1993), and there are limited data concerning intratumour oestrogen levels in patients during treatment with aromatase inhibitors (Reed *et al.* 1991, de Jong *et al.* 1997, Miller *et al.* 1997). Thus, a major target for future studies in this field is to assess alterations in tumour oestrogen concentrations as well as growth factor expression in patients receiving treatment with aromatase inhibitors in different sequences as well as in concert.

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