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Review

Lack of complete cross-resistance between different aromatase inhibitors; a real finding in search for an explanation?

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ABSTRACT

While third-generation aromatase inhibitors (anastrozole, letrozole and exemestane) are successfully implemented as adjuvant and first-line therapy for hormone-sensitive breast cancer in postmenopausal women, important questions remain to be addressed. An issue of particular interest is the question about lack of complete cross-resistance between steroidal and non-steroidal compounds. Although the studies reporting this phenomenon in general contain a small number of patients, the findings across the different reports seem consistent. While several potential mechanisms have been suggested, so far we lack scientific proof what mechanisms may be responsible for this finding. Finally, we do not know whether lack of cross-resistance actually signals an improved efficacy for certain compounds or may be due to alternative mechanisms of action. Neither do we know whether some tumours are more sensitive to particular drugs. This paper summarizes clinical findings up to now with respect to lack of cross-resistance and discuss potential mechanisms involved.

1. Introduction

Contrasting the first-generation aromatase inhibitor aminoglutethimide,[1,2] the third-generation aromatase inhibitors; anastrozole, letrozole and exemestane, have revealed superiority with respect to relapse-free survival compared to tamoxifen in the adjuvant setting.[3–10] This mirrors what has been observed in metastatic disease. Here, third generation compounds revealed superiority compared to tamoxifen as first-line treatment[11] and megestrol acetate as well as aminoglutethimide for second-line therapy.[12–14] In contrast, the results obtained with aminoglutethimide as well as the second-generation compounds fadrozole and for mestane (4-hydroxyandrostenedione) revealed clinical efficacy for these compounds similar to tamoxifen and megestrol acetate.[15] Thus, the third-generation aromatase inhibitors differ from their predecessors with respect to treatment efficacy.

The three third generation aromatase inhibitors differ between each other with respect to pharmacological characteristics. The compounds may be divided into two distinct classes; anastrozole and letrozole are both non-steroidal compounds (imidazole derivatives), contrasting the steroidal structure of exemestane, a derivative of the aromatase substrate androstenedione (Fig. 1). The steroidal

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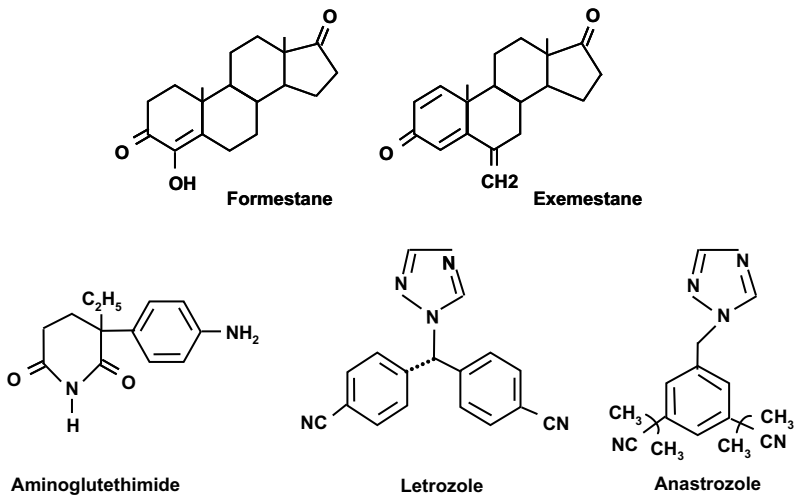

Fig. 1 – Chemical structure of the non-steroidal first generation agent aminoglutethimide and the second-generation steroidal compound formestane together with the three third-generation compounds currently in clinical use (anastrozole, letrozole and exemestane).

and non-steroidal compounds bind to different parts of the aromatase enzyme. In addition, the binding kinetics varies, with non-steroidal compounds binding reversible, while the steroidal compounds bind irreversibly. Another contrast relates to the weak androgen-agonistic effects of the steroidal compounds. Finally, the two non-steroidal compounds anastrozole and letrozole differ with respect to endocrine efficacy *in vivo*.

The clinical importance of these differences remains open. We know there is lack of cross-resistance between compounds of the two classes (Section 2 below), at least when a steroidal compound is administered second-line to a non-steroidal compound. However, we may not say at this stage exactly which mechanism may be responsible. In addition, we do not know whether this signals an improved clinical efficacy for steroidal versus non-steroidal compounds. Alternatively, the observations could be due to different mechanisms of action, including variation with respect to individual tumour sensitivity toward particular compounds. Except from one study in second-line therapy for metastatic disease, so far no results from head to head comparisons are available.

This paper summarize the results of clinical studies evaluating lack of cross resistance between aromatase inhibitors of the different classes and discuss potential mechanisms which may contribute to sensitivity to different compounds.

2. Clinical evidence revealing lack of cross-resistance between steroidal and non-steroidal aromatase inhibitors

The results from different cross-over studies in metastatic disease are summarized in[16–29] Table 1. As may be seen, most studies have looked at the efficacy of a steroidal compound in patients becoming resistant to therapy with a non-steroidal agent. While the studies in general included a limited number of patients, some general conclusions may be drawn. First, while the objective response rate in general is low, a significant number of patients obtain stable disease for 6 months or more; thus, the number of patients "benefiting" from treatment (having an objective response, alternatively, stable disease for 6 months or longer) in total may average 25–30%. While stable disease for >24 weeks in general is interpreted as a therapeutic effect in trials on endocrine agents in breast cancer, some care should be taking interpreting the results from non-randomised studies. Some hormone-sensitive tumours are slowly progressing, and the possibility exist some of these observations merely reflect this phenomenon.

Notably, in most of these studies a first/second generation compound is followed by a steroidal second-generation compound (4-hydroxyandrostenedione, formestane), or a third-generation non-steroidal compound is followed by exemestane. In two studies aminoglutethimide is followed by exemestane,[19,21] while in two studies letrozole or anastrozole is followed by formestane.[17,18] Although these studies each enrolled a limited number of patients and the results should be interpreted carefully, the results do not suggest aggravated aromatase inhibition to be a key issue explaining the findings. This view is supported by clinical observations including responses or durable stable disease on formestane after failing letrozole or anastrozole[17,18] and similar clinical benefit for exemestane among patients previously exposed to the weaker inhibitor aminoglutethimide compared to letrozole or anastrozole.[21]

Considering treatment with a non-steroidal agent after failing a steroidal compound, this topic will be discussed separately in Section 3.6.

Recently, indirect evidence in favour of lack of complete cross-resistance between third-generation non-steroidal aromatase inhibitors (anastrozole and letrozole) and exemestane was indirectly substantiated by the EFECT study.[30] In this double-blind randomized study a total of 693 patients failing on a non-steroidal aromatase inhibitor were treated either with exemestane 25 mg daily by the oral route or fulvestrant 250 mg i.m. injections. The objective response rate as well as stable disease was similar in the two arms. In addition, no difference was recorded with respect to time to progression either.

Table 1 – Cross-over studies metastatic setting.

1st AI	2nd AI	*n*	OR	CB	TTP	Reference
Non-steroidal	Steroidal					
Aminoglutethimide	Formestane	112	21.0%	43.0%	NR	[16]
Aminoglutethimide	Formestane	10	20.0%	50%	NR	[29]
Letrozole	Formestane	9	11.0%	55.0%	NR	[17]
Anastrozole	Formestane	21	4.8%	65.5%	6.5 months	[18]
Aminoglutethimide	Exemestane	78	26.0%	39.0%	5.0 months 19	
AG,ANA,LET,VOR	Exemestane	241	6.6%	24.3%	4.0 months	[21]
Anastrozole	Exemestane	50	8.0%	44.0%	5.0 months	[20]
ANA, LET	Exemestane	23	NR	43.5%	5.1 months	[22]
Anastrozole	Exemestane	12	NR	NR	4.4 months	[23]
ANA, LET	Exemestane	114	5.0%	46.0%	4.5 months	[24]
ANA, LET	Exemestane	31	19.4%	54.8%	3.2 months	[25]
ANA, LET	Exemestane	30	0.0%	46.6%	4.0 months	[26]
ANA, LET	Exemestane	60	20.0%	38.3%	3.2 months	[27]
Steroidal	Non-steroidal	781				
Formestane	Anastrozole	21	0.0%	62.0%	NR	[28]
Exemestane	Let/(Ana)	18	22.0%	55.0%	9.3 months	[22]
Exemestane	Anastrozole	11	NR	NR	1.9 months	[23]

Abbreviations: AI, aromatase inhibitor/inactivator; OR, objective responses (complete responses and partial responses); CB, clinical benefit (objective responses and stable disease for $\geqslant$6 months); AG, aminoglutethimide; ANA, anastrozole; LET, letrozole; VOR, vorozole; NR, not reported.

3. Potential mechanism explaining lack of cross-resistance

3.1. *Do particular biological characteristics or previous therapy influence lack of cross-resistance between non-steroidal and steroidal aromatase inhibitors?*

An important topic is whether certain biological parameters may predict a particular benefit of aromatase inhibitors in comparison to other types of endocrine therapy. Thus, Ellis et al.[31] reported a higher response rate for letrozole compared to tamoxifen as pre-operative therapy. They recorded a particular benefit for letrozole among patients with HER-1/HER-2 over-expressing tumours. With respect to estrogen receptor expression, most interestingly their data suggested a particular benefit for letrozole among patients with tumours expressing a moderate level of ER expression (Allred score around 4). In the ATAC study, Dowsett et al.[32] found a particular benefit for anastrozole compared to tamoxifen among patients with ER positive but PgR negative tumours. While the authors did not quantify ER levels, PgR negative tumours have been known for 3 decades to express lower levels of the ER compared to PgR positive tumours on average.[33] However, central receptor assessment on a sub-cohort of patients from the ATAC study did not reveal a particular benefit for anastrozole among patients with PgR negative tumours,[34] and similar findings have been recorded with respect to exemestane[8] as well as letrozole.[35] Data from these studies[34,35] revealed an inferior outcome among HER-2 positive compared to HER-2 negative patients whether exposed to tamoxifen or an aromatase inhibitor, but with no differential effect with respect to the different options.

Considering the 3 largest studies evaluating a steroidal aromatase inhibitor subsequent to a non-steroidal compound,[16,19,21] data do not suggest any particular advantage for ER positive compared to ER unknown patients. While the largest international study[21] reported a higher incidence of overall benefit among patients exposed to endocrine therapy but no chemotherapy earlier compared to patients who had received previous chemotherapy, this may reflect patient selection. In the study by Murray and Pitt on patients with metastatic breast cancer,[16] previous response to a non-steroidal agent (aminoglutethimide) predicted response to subsequent therapy with formestane; except from that, no predictive factors specific to treatment with aromatase inhibitors have been identified.

3.2. *Different pharmacological (endocrine) efficacy between different agents?*

Contemporary studies have reported mean plasma levels of estradiol in postmenopausal women in the range of 15 – 25 pM.[36,37] Assuming aromatase inhibitors to suppress plasma levels by more than 90%, to detect such a suppression radioimmunoassays (or GC–MS methods) need a sensitivity limit not exceeding 2 pM. In the 1980s and early 1990s, when most studies on aminoglutethimide as well as the second-generation compounds were conducted, with the exception of the radioimmunoassay for estradiol at the Royal Marsden Hospital[38] and, in the middle 1990s, assays validated in some other centres like our own,[39] the methods employed did not reach such sensitivity limits. In general, the results from early endocrine assessments should not be compared to results achieved by contemporary standards. The most sensitive contemporary methods express detection limits for estradiol, estrone and estrone sulphate of about 0.7 pM, 1.1 pM and 0.6 pM, respectively.[40] Even with such methods, we may not detect exact plasma estrogen levels among patients for whom we may assume a degree of aromatase inhibition around 98% as suggested from tracer studies (see below).

To overcome the problem, tracer techniques have been developed. Following infusion or injection of a mixture of ^{3}H labelled androstenedione and ^{14}C labelled estrone, the degree of aromatase inhibition may be determined from the isotope ratio in the estrogen metabolites.[41–43] In collaboration with professor Dowsett's group at the Royal Marsden Hospital we developed a tracer technique able to detect >99.1% aromatase inhibition in the majority of patients.[44] Using this method, we subsequently classified different aromatase inhibitors based on their overall ability to inhibit *in vivo* aromatization. The results are presented in Table 2.[44–51]

The key message from these studies is the ability of the third-generation compounds to inhibit *in vivo* aromatisation by >98%, contrasting a 90% or lower inhibition achieved with the first/second generation compounds. Importantly, these findings corroborate the clinical observations that these third-generation compounds each improve outcome compared to tamoxifen in the metastatic as well as adjuvant setting, contrasting the effects if the first/second generation compounds (see Section 1).

While the inhibition efficacy of the third-generation compounds compared to the first- and second-generation is obvious, no conclusion should be based on indirect evidence comparing marginal differences in-between the different third-generation aromatase inhibitors. The number of patients in each study is small (about 12), and the inter-individual variation may explain some differences.[49] Thus, we may not tell whether exemestane may differ from either anastrozole or letrozole with respect to total body aromatase inhibition. Yet, considering anastrozole and letrozole, a direct head to head comparison has been performed, evaluating *in vivo* aromatase inhibition with the two compounds in the same patients.[51] Doing so,[51,52] letrozole 2.5 mg daily was found consistently to be a more potent aromatase inhibitor compared to anastrozole 1 mg daily in each of the patients studied (Fig. 2). Notably, a second study, randomising patients to primary medical treatment with either letrozole or anastrozole, has confirmed a difference between the two compounds with respect to plasma estrogen level suppression.[37]

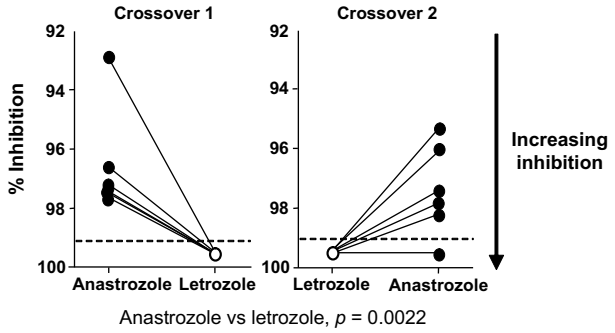

Fig. 2 – In a cross-over study evaluating the effects of anastrozole versus letrozole on *in vivo* aromatase inhibition, letrozole was a more potent drug in all patients independent of whether anastrozole was administered prior to letrozole (left side) or letrozole was administered first. Results obtained from original paper[51] as presented elsewhere.[52]

In conclusion, there is little evidence suggesting enhanced aromatase inhibition may explain an effect of steroidal compounds after a non-steroidal aromatase inhibitor.

3.3. *Lack of effective uptake in tumour tissue of particular compounds?*

Notably, intratumour estrogen levels may not necessarily reflect plasma levels. For estrogen (and progesterone) receptor expressing tumours in particular, intratumour levels of estradiol in general exceeds plasma- as well as normal tissue levels.[53] The reasons could be local (intratumoral) estrogen synthesis, in as much as malignant breast tissue often express the aromatase enzyme[54,55]; alternatively, there may be enhanced uptake from the circulation.[56]

We now have data from two studies evaluating plasma and intra-tumour estrogen suppression among patients treated with anastrozole or letrozole as primary medical treatment for locally advanced breast cancers.[57,58] These protocols were single studies; no head to head comparison has been performed. Thus, the results should be interpreted carefully.

Table 2 – Effects of different aromatase inhibitors and inactivators on whole-body aromatisation.[a]

Compound	Drug dose in mg	% of aromatase inhibition	Reference
Aminoglutethimide	250 quid	90.6%	[46]
Rogletimide	200 bid; 400 bid; 800 bid	50.6%; 63.5%; 73.8%	[46]
Fadrozole	1 bid; 2 bid	82.4%; 92.6%	[44]
Formestane (p.o)	125 od; 125 bid; 250 od	72.3%; 70.0%; 57.3%	[47]
Formestane (i.m)	250 2w; 500 2w	84.8%; 91.9%;	[45]
Formestane	500w	92.5%	[48]
Form + AG	500w/250 qid	93.8%	[48]
Anastrozole	1 od; 10 od	96.7%; 98.1%	[49]
Anastrozole	1 od	97.3%	[51][b]
Letrozole	2.5 od	>99.1%	[51][b]
Exemestane	25 od	97.9%	[50]

a All values are determined by the same assay at the Academic Department of Biochemistry, Royal Marsden Hospital, London, UK (head: Prof. M. Dowsett) and the Breast Cancer Research Group at the Haukeland University Hospital in Bergen, Norway (head: Prof. P.E. Lønning).

b Direct head-to-head, intra-patient, cross-over trial; Abbreviations: *od*, once daily; *bid*, twice daily; *qid*, four times daily; *w*, weekly; *2w*, every 2 weeks.

Table 3 – Suppression of plasma and breast tumour tissue estrogens by anastrozole and letrozole expressed in% of pre-treatment values

	Anastrozole		Letrozole	
	P	T	P	T
Oestradiol	84.9	89.0	87.8	97.6
Oestrone	81.0	83.4	84.3	90.7
Oestrone sulphate	93.5	72.9	98.0	90.1

Data for Refs. [57,58] .

Yet, summarizing *in vivo* plasma and tumour estrogen suppression (percentage) from the two studies, there is no evidence suggesting an inferior suppression of intratumour compared to plasma estrogen levels (Table 3). While estradiol seems to be somewhat less suppressed in plasma compared to tumour tissue, the opposite finding occurs with respect to estrone sulphate. The reason – most likely – is due to technical issues. While plasma levels of estrone sulphate exceeds plasma levels of estrone and estradiol by a factor of 5 – 10 versus 10–20, respectively[59], in the tumour tissue estradiol levels in general are much higher the levels recorded in plasma, while tissue levels of estrone sulphate are low.[57,58]

While we lack similar data with respect to intratumour estrogen levels during treatment with exemestane, to this day there is little evidence suggesting lack of a local effect caused by poor drug uptake to be a mechanism for resistance to particular aromatase inhibitors *in vivo*.

3.4. *Aromatase enzyme variants; different sensitivity toward different compounds?*

Aromatase is expressed (albeit to a low degree) in most normal tissue compartments (see Ref. [60]). While only one aromatase gene exist, it has an interesting regulation, in as much as the gene contains at least 10 different promoters.[61] With exception of a single promoter (pII) located at the beginning of exon II, these promoters all are located within the untranslated exon I. They get stimulated by different ligands and are differentially used across the different tissue compartments. Aromatase expression is mainly regulated through the exon 1.4 promoter in the normal breast tissue. In contrast, aromatase expression in breast cancer is mainly regulated by the 1.3 and PII, indicating a regulatory shift during the malignant transformation (see references to original works in[61,62]).

While a number of polymorphisms in the aromatase gene has been identified, only 4 out of these are located in the coding exons.[63] We have limited knowledge with respect to their potential biological effects, although one of the coding polymorphisms, M364T, is associated with a profound reduced expression of immunoreactive protein and reduced enzyme function.[63] Considering the three other exon polymorphisms, they seem to be associated with a modest reduction in protein expression. Interestingly, a recent study suggested an intronic variant (rs4646) to be associated with an improved prognosis among patients treated with letrozole for metastatic breast cancer.[64] However, we have no information whether this polymorphism may influence outcome among patients treated with any of the other aromatase inhibitors. The possibility exist it may be associated with outcome among patients treated with any type of endocrine treatment (including tamoxifen), or it may be a general prognostic factor independent of treatment. Clearly, more data are needed to address this important topic.

3.5. *Importance of irreversible aromatase inhibition?*

Contrasting non-steroidal aromatase inhibitors, which bind to the heme part of the enzyme, steroidal compounds bind to the substrate-binding pocket of the enzyme.[65] In addition, steroidal compounds bind irreversible due to covalent bonds, meaning new enzyme protein needs to be synthesized.[66–68] Thus, Professor Miller and his team in Edinburgh revealed a dose dependent inhibition of the different aromatase inhibitor compounds on breast cancer homogenates as well as cultured breast fibroblasts.[69,70] Following wash-out of the culture medium, they showed complete reversibility of the inhibition by non-steroidal compounds, while the enzyme remained inactivated following removal of the steroidal compounds formestane and exemestane. The mechanism of irreversible inhibition has recently been investigated in detail; interestingly, the irreversible inhibition created by exemestane was found to reduce the half-life of the aromatase protein by more than 50%.[71–73] However, pharmacological studies on the endocrine effects of formestane, conducted by the Royal Marsden group, suggest protein recovery may be fairly rapid (days) *in vivo* following elimination of the drug.[38,74] Finally, formestane seems to inhibit *in vivo* aromatization to the same degree as aminoglutethimide (formestane), and letrozole and anastrozole to the same degree as exemestane, respectively (Table 2). Based on these findings, the contribution of irreversible inhibition to aromatase inhibition *in vivo* remains unclear, and its potential contribution to the lack of cross-resistance between the different compounds remain open.

3.6. *Androgen agonistic effects?*

Contrary to the non-steroidal compounds, steroidal compounds like exemestane exhibit androgen agonistic activities *in vivo*[75] expressed as a dose-dependent suppression of sex hormone binding globulin (SHBG). This effect on SHBG is not related to estrogen suppression per se, as it is not observed among the non-steroidal compounds. Notably, it is not caused by exemestane itself but through its main *in vivo* metabolite 17hydroexemestane.[76] Interestingly, when looking at formestane, this compound does not express androgen

agonistic activities on sex hormone binding globulin when administered by the parenteral route; it is, however, observed after oral administration, probably due to the excessive first pass effect on the liver.[74,77]

While the androgen agonistic activity of exemestane is modest, it may be of clinical importance. Most breast cancers contain androgen receptors above 10 fmol per mg protein,[78] and overexpression of androgen receptors as well as stimulation of MCF-7 cells with dihydrotestosterone counteracts estradiol stimulation.[79] Androgens were used for breast cancer therapy before the contemporary era of tamoxifen use[80], and the finding in experimental systems that estrogen deprivation sensitizes breast cancer cells to androgen growth inhibition[81,82] raises the possibility that patients under treatment with aromatase inhibitors may be sensitized to the anti-tumour effects of androgens.

In contrast, those studies applying formestane second to a non-steroidal compound each administered formestane parenterally. However, a lack of a first-pass effect on liver synthesis of SHBG may not exclude an androgen-agonistic effect on tumour tissue.

A most important question remains, however: Is the lack of cross-resistance in general due to a better anti-tumour effect of the steroidal compared to the non-steroidal compound or does it simply reflect a different mechanism of action? If the second explanation is the correct one, we should expect to see lack of cross-resistance to non-steroidal drugs after failure with steroidal compounds.

Looking at the studies administering a steroidal drug upfront to a non-steroidal compound (Table 1), three small studies have so far been reported. With respect to formestane given upfront of anastrozole[28], clinical benefit may be explained by a more potent anti-aromatase effect of anastrozole compared to formestane. Interestingly, however, the two studies reporting use of letrozole or anastrozole following exemestane ended up with somewhat contradictory results. Due to the low number of observations, these results need to be interpreted carefully indeed. In the study by Bertelli et al.[22], the responses and benefits of the non-steroidal compound following exemestane were related to letrozole (one patient only had anastrozole and did not respond), while in the study by Mayordomo et al.[23] lack of effect of the non-steroidal compound was related to anastrozole. As mentioned previously, letrozole consistently inhibits *in vivo* aromatisation[51] and also suppresses intratumour estrogen levels more potently compared to anastrozole (Table 3). While the possibility exist that letrozole, but not anastrozole, may work after exemestane due to more potent estrogen suppression, more data on this topic is welcomed. Indirect evidence supporting or rejecting this hypothesis will be obtained from the MA27 and FACE trials, comparing anastrozole to exemestane and anastrozole to letrozole, respectively, in the adjuvant setting.

3.7. *Differential effects on growth factors and gene expression profiles?*

Little is known with respect to potential differential effects of steroidal and non-steroidal aromatase inhibitors on growth factors *in vivo*. Most interesting, in a joint study[83] Dr. Chow's group in Hong Kong and Dr. Toi's team in Tokyo reported a reduction in HER-2 immunoexpression during primary medical therapy with aromatase inhibitors. However, no differential effect between exemestane and letrozole was recorded. Working on acquired resistance to different aromatase inhibitors in cell culture, Dr. Chens team reported exemestane resistance to be associated with up-regulation of amphiregulin.[84] Exploring mRNA expression with use of microarrays, the same group found a different gene expression profile in cells acquiring resistance to exemestane compared to non-steroidal aromatase inhibitors[85]; however, they also recorded a different gene expression profile between cells becoming resistant to letrozole versus anastrozole.

While these results all suggest more research into the effects of different aromatase inhibitors *in vitro* as well as *in vivo* with respect to downstream gene regulation, at this stage no final conclusion may be drawn.

4. Conclusions

At this stage we may conclude there is a lack of cross-resistance between non-steroidal and steroidal aromatase inhibitors provided a non-steroidal inhibitor is given upfront. To some degree, this resembles what may be happening with respect to aromatase inhibitors versus progestins administered as high-dose regimens. Megestrol acetate 160 mg daily was found of equal clinical efficacy to aminoglutethimide when administered as second-line endocrine therapy,[86] and megestrol acetate and aromatase inhibitors do not express cross-resistance.[87,88] While the mechanism of action of progestins against breast cancer is incompletely understood,[87] megestrol acetate when given as 160 mg daily suppresses adrenal androgen production and, subsequently plasma estrogen levels, to a degree comparable to what was recorded for aminoglutethimide.[89]

From the evidence examined, we may not conclude what is the main mechanism behind lack of cross-resistance toward different aromatase inhibitors. What we know is that it can not be explained by an enhanced chemical efficacy, in as much as it is also observed between compounds expressing total body aromatase inhibition to a similar degree. While we do not know the importance of irreversible aromatase inhibition in this respect, possible explanation could be the androgen agonistic effects expressed by the steroidal compounds.

In contrast, we only have limited data suggesting benefit for non-steroidal inhibitors after steroidal compounds. Here, an intriguing question is whether this could be related to more potent aromatase inhibition as observed with letrozole. We need to learn more about the potential biochemical importance of pharmacogenomics with respect to potential lack of cross-resistance between aromatase inhibitors. Not only may it potentially explain the observations recorded; it could potentially help selecting patients for optimal therapy by selecting individual drugs not only in metastatic but also in the early adjuvant setting.

Conflicts of interest statement

The author has received speaker's honoraria and participated in Advisory Boards for Astra-Zeneca, Novartis and

Pfizer Inc., the three manufacturers of commercial aromatase inhibitors.

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