# review

# The potency and clinical efficacy of aromatase inhibitors across the breast cancer continuum

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The strategy of using estrogen suppression to treat breast cancer led to the development of aromatase inhibitors, including the third-generation nonsteroidal compounds anastrozole and letrozole, and the steroidal compound exemestane. Aromatase inhibitors potently inhibit aromatase activity and also suppress estrogen levels in plasma and tissue. In clinical studies in postmenopausal women with breast cancer, third-generation aromatase inhibitors were shown superior to tamoxifen for the treatment of metastatic disease. Studies of adjuvant therapy with aromatase inhibitors include (i) head-to-head studies of 5 years of the aromatase inhibitor versus 5 years of tamoxifen monotherapy; (ii) sequential therapy of 2–3 years of tamoxifen followed by an aromatase inhibitor (or the opposite sequence) versus 5 years of tamoxifen monotherapy; (iii) extended therapy with an aromatase inhibitor after 5 years of tamoxifen; and (iv) sequential therapy with an aromatase inhibitor versus aromatase inhibitor monotherapy. Recent results from the Arimidex, Tamoxifen, Alone or in Combination and Breast International Group 1–98 trials advocate using an aromatase inhibitor upfront. This article examines the clinical data with aromatase inhibitors, following a brief summary of their pharmacology.
**Key words:** anastrozole, aromatase inhibitor, breast cancer, estrogen, exemestane, letrozole

## introduction

Estrogen suppression through oophorectomy was first shown to cause antitumor effects in premenopausal women with breast cancer more than a century ago [1]. Later, in the 1950s, estrogen suppression through adrenalectomy and hypophysectomy was shown to have antitumor effects in postmenopausal women [2–5]. At that time, the adrenal gland was thought to be the site of estrogen synthesis in postmenopausal women; now, we know that the adrenal gland provides androgens (estrogen precursors) subject to peripheral conversion (aromatization) into estrogens. Subsequent efforts to cause estrogen suppression through 'medical adrenalectomy', using glucocorticoids [6, 7], turned out to have limited success from a clinical perspective, precipitating clinical trials exploring different adrenal enzyme inhibitors, including ketoconazole, for this purpose. The clinical results, however, were not encouraging [8, 9], probably because of suboptimal suppression of androgen secretion.

A more successful approach to estrogen suppression was achieved with use of an unsuccessful antiepileptic aminoglutethimide (Elliptene, later named Orimethene). Because of its adrenal toxicity [10], this drug was used for breast cancer treatment, revealing antitumor efficacy resembling that of other contemporary treatment options, including tamoxifen

[11–13]. Again, because of its adrenal toxicity, aminoglutethimide was generally administered with replacement glucocorticoids [14]. Notably, aminoglutethimide monotherapy resulted in estrogen deprivation, resembling the effect obtained when administered with glucocorticoids, despite a significant elevation of adrenal-secreted androstenedione [15].

Subsequent translational studies revealed aminoglutethimide to act as an aromatase inhibitor [16, 17]. In parallel, the group of Harry and Angela Brodie worked experimentally on androstenedione derivatives as substrate-binding blockers of the aromatase enzyme [18, 19], identifying 4-hydroxyandrostenedione (later named formestane) [20].

The major aim in developing novel (second-generation) aromatase inhibitors such as formestane and, later, fadrozole (CGS 16949A) was to achieve compounds devoid of the toxic side-effects of aminoglutethimide. However, some of these compounds, such as anastrozole, letrozole, and exemestane (considered third-generation compounds), subsequently revealed more potent aromatase inhibition compared with the first- and second-generation compounds, and they have completely replaced previous compounds for clinical use. This article examines clinical results with these compounds, following a brief summary of their pharmacology.

## the aromatase enzyme and estrogen disposition in postmenopausal women

While the aromatase enzyme has been studied for decades, its crystallographic structure was first reported in 2009 [21]. The gene

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harbors at least 10 different promoters [22], subject to different ligand stimulations in different tissue compartments [23–26].

Ovarian estrogen production ceases at menopause. Postmenopausal estrogens are synthesized from circulating androgens, mainly androstenedione, which is converted into estrone [27, 28]. In addition, a minor pathway includes aromatization of circulating testosterone into estradiol [29]. Plasma (and tissue) estradiol seems to have a dual origin, some arising from the direct aromatization of testosterone, with the rest synthesized from the reduction of estrone. While the adrenal gland is the main contributor of circulating androgens, conflicting evidence indicates a minor contribution of circulating androgens from the postmenopausal ovary [30, 31].

Interestingly, it has been known for more than two decades that estradiol levels are elevated in tissue, in particular breast cancer tissue, compared with plasma [32–37]. This in general has been attributed to local expression of the aromatase enzyme [38, 39]. However, there may be alternative explanations. In a recent study, we found elevated tissue concentrations of estradiol but reduced levels of estrone in breast tumors compared with the surrounding normal breast tissue [40]. Moreover, elevated estradiol concentrations were seen among estrogen receptor (ER)-positive tumors only [40]. Thus, recent results [41] suggest a strong correlation between intratumor estradiol levels and plasma estradiol but also intratumoral ER expression as well as negative to dehydrogenase 2 but positive to dehydrogenase 7 levels, indicating that intratumor estradiol may arise from estrone reduction. Such a hypothesis actually fits well with observations that circulating estrogen levels correlate with intratumoral expression of estrogen-regulated genes [42] and subsequent breast cancer risk [43] as well as to time to relapse [44] in hormone-sensitive breast cancer. These findings may have significant implications in future therapeutic strategies aiming at tumor-specific manipulation of estrogen synthesis.

## pharmacology and *in vitro* aromatase inhibition

Aromatase inhibitors may be separated into two distinct classes: steroidal and nonsteroidal compounds. Characteristically, steroidal inhibitors bind to the substrate-binding site in an irreversible manner [45], leading to degradation of the protein–drug complex. The exact binding mechanism is still a subject of research [46]. The two steroidal compounds extensively evaluated for clinical use were the second-generation compound formestane (4-hydroxyadrostenedione) and the third-generation compound exemestane.

In contrast to steroidal inhibitors, nonsteroidal compounds bind to the P450 site of the aromatase complex [45]. These compounds may be divided into compounds of the aminoglutethimide class and those of the imidazole/triazole class. Apart from aminoglutethimide itself, the aminoglutethimide class includes compounds such as rogletimide [47] as well as the separate L-enantiomer of aminoglutethimide [48]; due to toxicity, none of these compounds are in clinical use. The imidazole/triazole class includes the second-generation compound fadrozole [49, 50] and the third-generation compounds anastrozole and letrozole.

*In vitro* evaluations of aromatase inhibitors have been carried out using placental as well as ovarian microsomal fractions [51], breast cancer homogenates, and breast-derived fibroblasts [52]. Briefly, the results may be summarized as follows: all second- and third-generation compounds express a much higher potency compared with aminoglutethimide in experimental systems [52]. Second, the relative potency in general is higher for compounds of the imidazole/triazole class compared with steroidal compounds [52], consistent with the need for higher drug doses of the latter compounds in the clinic. Third, comparing the third-generation triazole derivatives anastrozole and letrozole, letrozole showed a significantly higher potency with respect to aromatase inhibition in breast homogenates and breast fibroblasts [52]. Finally, while in general the novel third-generation compounds possess a higher potency compared with the second-generation compounds, some *in vitro* studies actually revealed a somewhat higher potency for fadrozole compared with letrozole [53]. This underscores the fact that *in vivo* efficacy depends not on direct potency alone but is also subject to influence by other parameters, in particular pharmacokinetics.

## aromatase inhibition *in vivo* and plasma and tissue estrogen suppression

A major problem with evaluating the biochemical potency of aromatase inhibitors *in vivo* has been the problem of method sensitivity. Over the years, some groups, in particular the team headed by Professor Mitch Dowsett at the Royal Marsden Hospital and our team, have consistently worked at improving radioimmunoassays for this purpose [54–56]. While others [57] have developed gas chromatography/tandem mass spectrometry methods, so far such methods have been available for estradiol only and not for estrone or estrone sulfate, and the detection limit remains above what may be optimally achieved by radioimmunoassays. With a recent improved radioimmunoassay revealing detection limits for plasma estradiol, estrone, and estrone sulfate of 0.67, 1.14, and 0.55 pM, respectively [56], based on mean plasma estrogen values in postmenopausal women ($\sim$15, 80, and 400 pM, respectively), in theory, we may detect an average suppression of plasma estradiol, estrone, and estrone sulfate of 95.5%, 98.6%, and 99.9%, respectively. Notably, reanalyzing [37] plasma samples from patients treated sequentially with letrozole and anastrozole [58] using these improved assays, plasma estradiol levels were still suppressed below detection limit in 11 of 12 patients during treatment with letrozole and 5 of 12 patients on anastrozole; similar findings have been recorded by others [54]. For steroidal compounds such as exemestane, because of their steroid chemical structure and the potential for minor metabolites to interact in the radioimmunoassays, plasma estrogen analysis requires pre-purification of the samples with use of high-pressure liquid chromatography (HPLC) [59] or related methods.

A more sensitive method is to assess *in vivo* aromatase inhibition with tracer techniques [60, 61]. In a joint program with the Royal Marsden Hospital, aminoglutethimide, together with the different second- and third-generation compounds in clinical use was systematically examined with respect to

aromatase inhibition *in vivo* (Table 1) [49, 58, 62–67]. Notably, a distinct difference was observed between aminoglutethimide and the second-generation compounds, causing aromatase inhibition in general not exceeding 90%, and the novel third-generation compounds anastrozole, exemestane, and letrozole, causing 98% inhibition or better.

While these studies were conducted by the same investigators and with use of standardized methods, one should be careful making indirect comparisons within the group of third-generation inhibitors. Notably, there is substantial interindividual variation with respect to the degree of aromatization. Interestingly, when letrozole and anastrozole were compared in the same patients in a crossover study [58], letrozole was found to be a more potent inhibitor of *in vivo* aromatization in all patients; subsequent plasma estrogen analysis also revealed that letrozole treatment resulted in significantly better suppression for each estrogen fraction [37]. In addition, comparing the results of a study with letrozole and a similar study with anastrozole that used the same methods to analyze tissue estrogens, highly sensitive radioimmunoassay following HPLC purification showed greater suppression of each tissue estrogen fraction with letrozole than with anastrozole (97.6% versus 89.0% for estradiol, 90.7% versus 83.4% for estrone, and 90.1% versus 72.9% for estrone sulfate) [36, 37].

All in all, these findings indicate that exemestane, anastrozole, and letrozole are the most potent aromatase inhibitors, with a somewhat higher potency for letrozole 2.5 mg daily compared with anastrozole 1 mg daily. No conclusion may be drawn regarding exemestane potency versus the other two compounds. The potential contribution of aromatase inhibitor potency to the lack of cross-resistance between steroidal and nonsteroidal compounds is discussed later.

## clinical use of aromatase inhibitors in metastatic disease

Implementing treatment with aromatase inhibitors in the adjuvant setting was not possible without careful and systematic evaluation of their efficacy and toxicity in metastatic disease. As these investigations were conducted more than a decade ago, the results of these studies are provided as supplementary data, available at *Annals of Oncology* online.

## presurgical treatment of primary breast cancer

Presurgical treatment (previously termed neoadjuvant therapy) has gained increasing use in breast cancer. While most studies have explored the use of chemotherapy in this setting, studies have also evaluated novel third-generation aromatase inhibitors as presurgical treatment based on the encouraging results obtained in the metastatic as well as in the adjuvant setting.

To date, the results of four studies in postmenopausal women comparing the efficacy of third-generation aromatase inhibitors with tamoxifen have been reported (Table 2) [68–71]. While the study with exemestane suggests a benefit compared with tamoxifen, the number of patients is too small to allow for statistical conclusions. Notably, the two studies with anastrozole,

**Table 1.** Inhibition of aromatization by aminoglutethimide, second-generation aromatase inhibitors, and third-generation aromatase inhibitors

| Aromatase inhibitor | Dose | Percent inhibition of aromatization (mean) |
| --- | --- | --- |
| Aminoglutethimide class | | |
| Aminoglutethimide [62] | 1000 mg qd | 90.56 |
| Rogletimide [62] | 200 mg b.i.d. | 50.6 |
| Rogletimide [62] | 400 mg b.i.d. | 63.5 |
| Rogletimide [62] | 800 mg b.i.d. | 73.8 |
| Second-generation aromatase inhibitors | | |
| Fadrozole (CGS 16949A) [49] | 1 mg b.i.d. | 82.4 |
| Fadrozole (CGS 16949A) [49] | 2 mg b.i.d. | 92.6 |
| Formestane (4-hydroxyandrostendione) [63] | 250 mg every 14 days | 84.8 |
| Formestane (4-hydroxyandrostendione) [63] | 500 mg every 14 days | 91.9 |
| Formestane (4-hydroxyandrostenedione) [64] | 125 mg qd | 62.3 |
| Formestane (4-hydroxyandrostenedione) [64] | 125 mg b.i.d. | 70.0 |
| Formestane (4-hydroxyandrostenedione) [64] | 250 mg qd | 57.3 |
| Formestane (4-hydroxyandrostenedione) [65] | 500 mg weekly | 91.3 |
| Formestane (4-hydroxyandrostenedione) + aminoglutethimide [65] | 500 mg weekly (4OHA) + 1000 mg qd (AG) | 94.2 |
| Third-generation aromatase inhibitors | | |
| Anastrozole [66] | 1 mg qd | 96.7[a] |
| Anastrozole [66] | 10 mg qd | 98.1[a] |
| Anastrozole [58] | 1 mg qd | 97.3[a] |
| Letrozole [58] | 2.5 mg qd | >99.1[a] |
| Exemestane [67] | 25 mg qd | 97.9[a] |

[a]Geometric mean.
AG, aminoglutethimide.

whether analyzed on their own or combined, did not reveal a significant benefit for anastrozole compared with tamoxifen [68, 69]. In contrast, letrozole was shown to significantly improve the response rate compared with tamoxifen [70].

**Table 2.** Studies of presurgical treatment with aromatase inhibitors compared with tamoxifen or chemotherapy

| Treatment | $n$ | Clinical response (palpation/caliper) | $P$ value versus tamoxifen |
|---|---|---|---|
| Anastrozole versus tamoxifen versus combination [68] | | | |
| Anastrozole | 113 | 37% | 0.87 |
| Tamoxifen | 108 | 36% | |
| Anastrozole + tamoxifen | 109 | 39% | 0.61 |
| Anastrozole versus tamoxifen [69] | | | |
| Anastrozole | 228 | 50.0 | 0.37 |
| Tamoxifen | 223 | 46.2 | |
| Letrozole versus tamoxifen [70] | | | |
| Letrozole | 154 | 55% | <0.001 |
| Tamoxifen | 170 | 36% | |
| Exemestane versus tamoxifen [71] | | | |
| Exemestane | 25 | 76.3% | 0.05 |
| Tamoxifen | 75 | 40.0% | |

## adjuvant therapy

Adjuvant studies on aromatase inhibitors may be separated into four categories (Figure 1): (i) head-to-head comparison, evaluating 5 years of treatment with the novel agent versus 5 years on tamoxifen; (ii) sequential therapy, comparing 2–3 years with either tamoxifen followed by the aromatase inhibitor or the opposite sequence, for a total of 5 years, with 5 years of tamoxifen; (iii) extended therapy, evaluating the benefits of adding extended treatment with an aromatase inhibitor in patients already exposed to tamoxifen treatment of 5 years; and (iv) comparing use of the novel aromatase inhibitor administered sequentially with 5 years of aromatase inhibitor monotherapy. It should be noted that the four-armed Breast International Group (BIG) 1–98 study addresses all these topics except extended therapy.

## head-to-head comparison

The first study evaluating an aromatase inhibitor in the adjuvant setting was not Arimidex, Tamoxifen, Alone or in Combination (ATAC) but a study conducted by the Royal Marsden Hospital, London, comparing aminoglutethimide monotherapy with placebo, each administered for 2 years [72]. Only 354 patients were included,
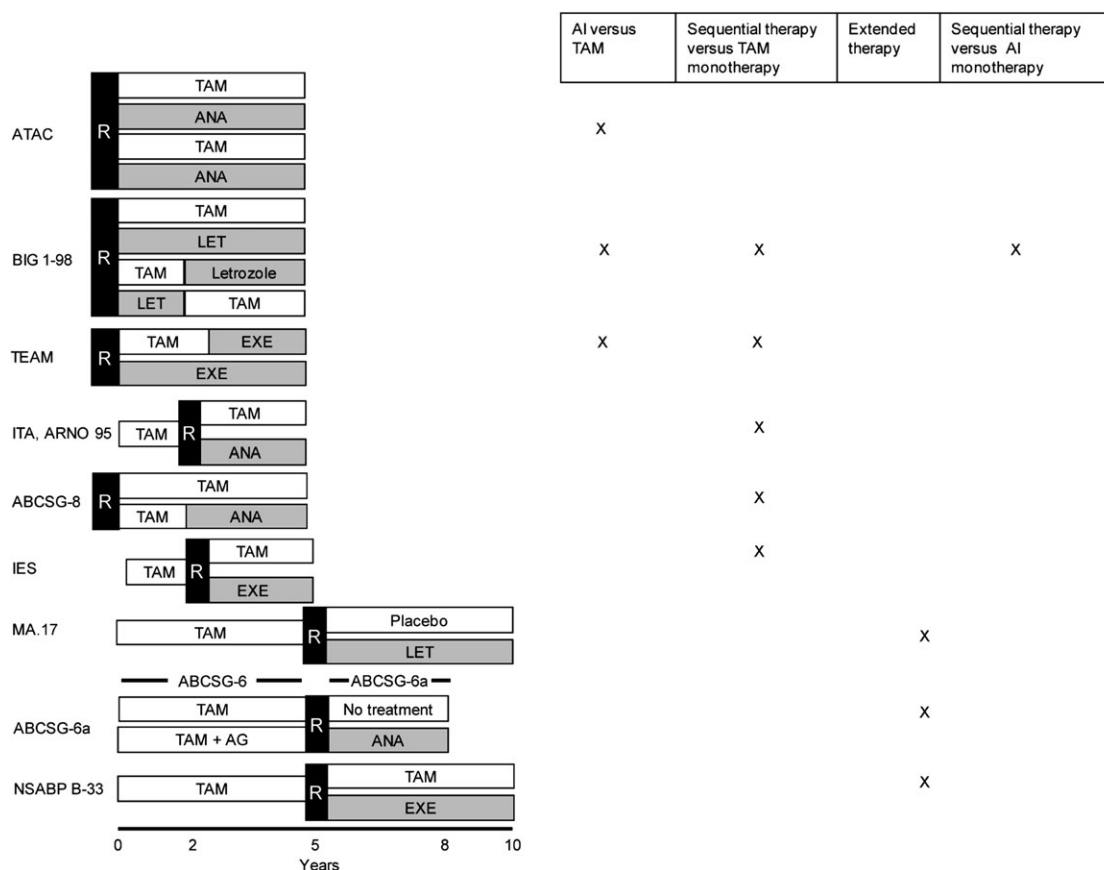


**Figure 1.** Design of adjuvant aromatase inhibitor trials. ABCSG, Austrian Breast and Colorectal Cancer Study Group; AG, aminoglutethimide; ANA, anastrozole; ARNO, Arimidex–Nolvadex; ATAC, Arimidex, Tamoxifen, Alone or in Combination; EXE, exemestane; IES, Intergroup Exemestane Study; ITA, Italian Tamoxifen Anastrozole; LET, letrozole; NSABP, National Surgical Adjuvant Breast and Bowel Project; R, randomization; TAM, tamoxifen; TEAM, Tamoxifen, Exemestane Adjuvant Multicenter.

and no difference in outcome was reported between patients in the two arms.

The ATAC study compared 5 years of tamoxifen monotherapy with anastrozole monotherapy. In addition, a third arm used the two drugs in combination [73]. While anastrozole significantly improved disease-free survival (DFS) compared with tamoxifen monotherapy (Table 3), interestingly, combined therapy provided no benefit compared with tamoxifen monotherapy. Notably, previous studies combining tamoxifen and aminoglutethimide did not improve treatment outcome compared with tamoxifen monotherapy in metastatic disease [87–91].

Results from ATAC at a median 100 months of follow-up [75] are shown in Table 3. Anastrozole significantly improved DFS [hazard ratio (HR) 0.90, $P = 0.25$ in the intent-to-treat (ITT) population; HR 0.85, $P = 0.003$ in the hormone receptor (HR)-positive population]. However, no improvement in overall survival (OS) has been reported to date (HR 1.00, $P = 0.99$ in the ITT population; HR 0.97, $P = 0.7$ in the HR-positive population).

The second study reporting a head-to-head comparison with an aromatase inhibitor was BIG 1–98, comparing letrozole with tamoxifen as monotherapy and as sequential treatments. While the first report at a median of 25.8 months of follow-up compared all patients receiving tamoxifen initially with all patients receiving letrozole initially and censored observations in the sequential treatment arms (C and D) at time of crossover (Figure 1) [76], a second report compared patients allocated with the monotherapy arms (A and B) only at a median follow-up of 51 months [77]. As seen in Table 3, the two different comparisons revealed almost identical results. In the various analyses of BIG 1–98, letrozole improved DFS to a degree resembling that observed with anastrozole. However, in the most recent follow-up in the monotherapy arms (median 76 months), there was also a statistically nonsignificant trend in favor of a survival benefit with letrozole (HR 0.87, $P = 0.08$) [78], in contrast with the lack of improved survival observed with anastrozole in ATAC. It should be noted that the BIG 1–98 ITT analysis was biased against letrozole as 612 patients opted to cross over from tamoxifen to letrozole following the unblinding of the tamoxifen arm in 2005 [78]. An inverse probability of censoring weighted analysis, which corrects for bias due to nonadherence to randomized treatment, showed a significant survival benefit with letrozole compared with tamoxifen (HR 0.83, 95% confidence interval 0.71–0.97) [78]. It is conceivable that the survival benefit observed with letrozole at 76 months' median follow-up is the result of the early reduction in the risk of distant metastases with letrozole versus tamoxifen observed early on at a median follow-up of 25.8 months [76].

The Tamoxifen, Exemestane Adjuvant Multicenter (TEAM) trial, another study comparing tamoxifen with an aromatase inhibitor, was initially designed to compare 5 years of monotherapy with either exemestane or tamoxifen [79]. However, based on favorable results from the Intergroup Exemestane Study (IES), a sequential study with exemestane, the study design for TEAM was amended to compare exemestane monotherapy with sequential therapy comprising 2.5–3 years of tamoxifen followed by exemestane (Figure 1)

[79]. Results from the monotherapy arm analysis from TEAM at 2.75 years of median follow-up showed no significant benefit in DFS with exemestane compared with tamoxifen (Table 3) [79].

## sequential therapy

The first sequential study was a small trial of tamoxifen followed by aminoglutethimide compared with tamoxifen monotherapy in 380 patients [92]. There was no significant difference in event-free survival, although a difference was observed in OS favoring aminoglutethimide.

Sequential trials of third-generation aromatase inhibitors (a total of five) include three [Italian Tamoxifen Anastrozole (ITA), Austrian Breast and Colorectal Cancer Study Group (ABCSG)-8, and Arimidex–Nolvadex (ARNO 95)] evaluating sequential use of 2–3 years of tamoxifen followed by anastrozole for a total of 5 years [80, 81], the IES evaluating sequential treatment with 2–3 years of tamoxifen followed by exemestane for a total of 5 years [82], and the two sequential arms of the BIG 1–98 study evaluating 2 years of tamoxifen followed by 3 years of letrozole or the opposite sequence [78].

For BIG 1–98, results have been reported for the comparison between the monotherapy arms and for the comparison between each of the sequential options and letrozole monotherapy [78], but to date, the results comparing sequential treatment with tamoxifen monotherapy have not been reported. Results from the other sequential studies (ITA, ABCSG-8, ARNO 95, and IES) are summarized in Table 3. These studies demonstrated improved DFS for sequential administration of tamoxifen followed by the aromatase inhibitor compared with tamoxifen monotherapy. However, some differences regarding study design should be mentioned. Although the Austrian and German studies (ABCSG-8 and ARNO 95) were reported together [81], the studies differ in that the Austrian study, similar to BIG 1–98 [76], randomized patients upfront (before beginning any endocrine therapy). In contrast, the German study, similar to the IES [82] and the Italian study [80] randomized patients after 2 years on tamoxifen therapy. However, assuming that the number of relapses was reasonably well balanced in the two arms starting with tamoxifen for 2 years, this difference should have little impact on the HR comparison following switching. It is possible, though, that the German and Italian studies as well as the IES selected for a patient population that was more endocrine responsive.

A second issue relates to OS. While the combined result of the German–Austrian studies so far has revealed no statistical improvement regarding OS [81], a survival benefit was shown when the results from these studies were combined with the results from the Italian trial [93]. This combined analysis, however, has been debated on statistical grounds [94]. In the IES, OS was reported after blocks from ER-unknown tumors were analyzed for ER expression. Following the exclusion of 122 tumors shown actually to be ER negative, a follow-up report at a median of 55.7 months revealed statistically improved survival ($P = 0.04$, when adjusted for potential confounders) in the combined group of ER-positive and (still) ER-unknown tumors [83].

**Table 3.** Studies of third-generation aromatase inhibitors as adjuvant therapy

| Study | Arms | $n$ | Median follow-up (months) | DFS | TTDR/DDFS | OS |
|---|---|---|---|---|---|---|
| ATAC [73] | ANA versus TAM | 6241 | 33.3 | HR 0.78[a], $P = 0.005$ | Not analyzed | Not analyzed |
| ATAC [74] | ANA versus TAM | 6241 | 68 | HR 0.83[a], $P = 0.005$ | HR 0.84[a], $P = 0.06$ | HR 0.97[a], $P = 0.7$ |
| ATAC [75] | ANA versus TAM | 6241 | 100 | HR 0.85[a], $P = 0.003$ | HR 0.84[a], $P = 0.022$ | HR 0.97[a], $P = 0.7$ |
| BIG 1–98 [76] | LET versus TAM | 8010 | 25.8 | HR 0.81, $P = 0.003$ | HR 0.73, $P = 0.001$ | HR 0.86, $P = 0.16$ |
| BIG 1–98 [77] | LET versus TAM | 4922 | 51 | HR 0.82, $P = 0.007$ | HR 0.81, $P = 0.03$ | HR 0.91, $P = 0.35$ |
| BIG 1–98 [78] | LET versus TAM | 4922 | 76 | ITT: HR 0.88, $P = 0.03$; IPCW: HR 0.85, 95% CI 0.76–0.96 | HR 0.85, $P = 0.05$ | ITT: HR 0.87, $P = 0.08$; IPCW: HR 0.83, 95% CI 0.71–0.97 |
| TEAM [79] | EXE versus TAM | 9766 | 33 | HR 0.89, $P = 0.12$ | HR 0.81, $P < 0.03$ | Not analyzed (88 EXE versus 82 TAM) |
| ITA [80] | ANA versus TAM | 448 | 36 | HR 0.35, $P = 0.001$ | HR 0.49, $P = 0.06$ | Not analyzed |
| ABCSG-8/ARNO 95 [81] | ANA versus TAM | 3224 | 28 | HR 0.60[b], $P = 0.0009$ | HR 0.61, $P = 0.0067$ | $P = 0.16$ |
| IES [82] | EXE versus TAM | 4742 | 30.6 | HR 0.68, $P < 0.001$ | Not analyzed | HR 0.88, $P = 0.37$ |
| IES [83] | EXE versus TAM | 4742 | 55.7 | HR 0.76, $P = 0.0001$ | HR 0.83, $P = 0.03$ | HR 0.85, $P = 0.08$ |
| MA.17 [84] | LET versus PLA | 5187 | 30 | HR 0.58, $P < 0.001$ | HR 0.60, $P = 0.002$ | HR 0.82, $P = 0.3$ |
| ABCSG-6a [85] | ANA versus no treatment | 856 | 62.3 | HR 0.62[c], $P = 0.031$ | HR 0.53, $P = 0.034$ | HR 0.89, $P = 0.570$ |
| NSABP B-33 [86] | EXE versus PLA | 1598 | 30 | RR 0.68, $P = 0.07$ | RR 0.69, $P = 0.31$ | $P = $ NS (16 EXE versus 13 PLA) |

[a]Hormone receptor-positive population.
[b]Event-free survival.
[c]Recurrence-free survival.
ABCSG, Austrian Breast and Colorectal Cancer Study Group; ANA, anastrozole; ARNO, Arimidex–Nolvadex; ATAC, Arimidex, Tamoxifen, Alone or in Combination; BIG, Breast International Group; CI, confidence interval; DDFS, distant disease-free survival; DFS, disease-free survival; EXE, exemestane; HR, hazard ratio; IES, Intergroup Exemestane Study; IPCW, inverse probability of censoring weighted; ITA, Italian Tamoxifen Anastrozole; ITT, intent-to-treat; LET, letrozole; NS, not significant; NSABP, National Surgical Adjuvant Breast and Bowel Project; OS, overall survival; PLA, placebo; RR, relative risk; TAM, tamoxifen; TEAM, Tamoxifen, Exemestane Adjuvant Multicenter; TTDR, time to distant recurrence.

## extended therapy

So far, three studies have reported on extended adjuvant therapy with an aromatase inhibitor following the completion of 5 years of tamoxifen (Figure 1): the MA.17 trial evaluating letrozole for 5 years in comparison with placebo [84, 95], the open-labeled Austrian ABCSG-6a trial evaluating anastrozole for 3 years in comparison with no treatment [85], and the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-33 trial evaluating exemestane in comparison with placebo [86]. The results are summarized in Table 3. While the MA.17 and the ABCSG-6a study reported significantly improved DFS and recurrence-free survival, respectively, by adding the aromatase inhibitor after 5 years on tamoxifen, a nonsignificant trend was observed in the NSABP B-33 study. The most likely reason for this disparity was early termination of patient recruitment in NSABP B-33 (only 1598 of a total planned number of 3000 patients were accrued due to the positive results reported from study MA.17). An improvement in OS was noted in MA.17 on longer follow-up (median 30 months) for node-positive but not for node-negative patients [84], most likely because of a smaller number of events among node-negative patients.

## sequential treatment versus aromatase inhibitor monotherapy

So far, the only results published for the comparison of sequential therapy with aromatase inhibitor monotherapy are from BIG 1–98 [78]. While results from the TEAM trial at a median 5.1 years of follow-up showed no significant difference in DFS with sequential tamoxifen followed by exemestane compared with exemestane monotherapy, the results so far have been presented only at a congress [96].

Importantly, the BIG 1–98 study evaluated both tamoxifen followed by letrozole and letrozole followed by tamoxifen. At a median follow-up of 71 months, no significant difference with respect to DFS was recorded between any of the sequential treatment regimens and letrozole monotherapy (Figure 2) [78]. However, while no difference (so far) was seen between letrozole for 2 years followed by tamoxifen and letrozole monotherapy, 2 years of tamoxifen upfront was associated with an increased risk of relapse during that initial time interval. Interestingly, results from the ATAC study [97] as well as from the monotherapy comparison of BIG 1–98 support the conclusion that aromatase inhibition upfront is beneficial. This observation may have a biological rationale. While conventional wisdom considered early relapses (within 2 years
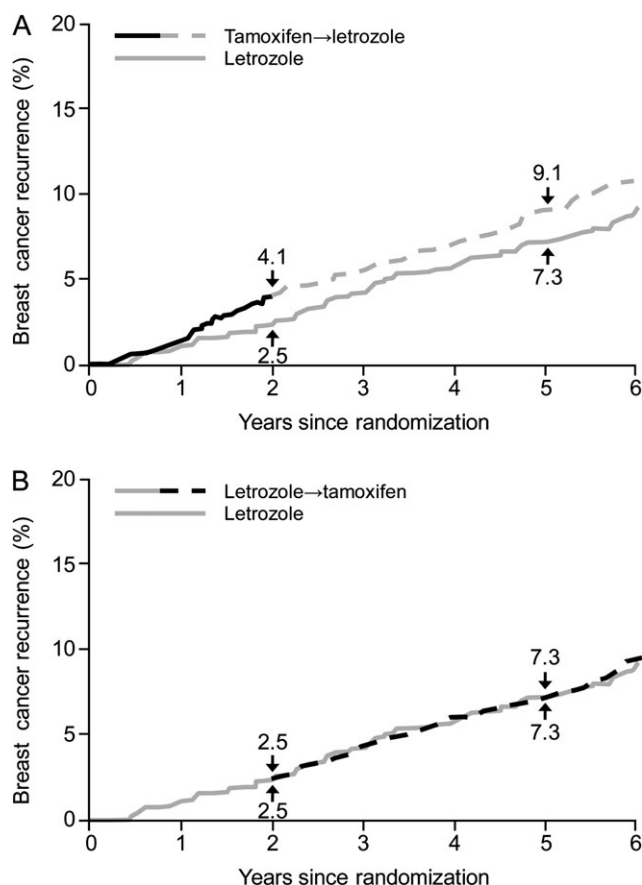
**Figure 2.** Cumulative incidence of breast cancer recurrence in the sequential treatment analysis of the Breast International Group 1–98 trial at a median 71 months of follow-up. A: T - L versus L, B; L - T versus L. Reprinted with permission [78]. Copyright© 2009 Massachusetts Medical Society.

of primary treatment) as an indicator of endocrine resistance [93], these observations were recorded among patients having tamoxifen treatment. Notably, findings from the primary treatment 024 protocol suggested an improved response rate to letrozole among patients with tumor expressing intermediate-to-low expression levels of the ER [98].

In contrast, no difference regarding outcome was seen for sequential treatment with 2 years of letrozole followed by tamoxifen compared with letrozole monotherapy (Figure 2). This finding, however, should be interpreted carefully, considering the limited duration of follow-up.

## meta-analysis of efficacy

Particular attention should be directed at the recent meta-analysis integrating data of the individual trials [99]. Upfront therapy (ATAC and BIG 1–98 combined) showed that use of an aromatase inhibitor caused a significant ($P < 0.00001$) 2.9% absolute reduction in the 5-year relapse rate, with a corresponding nonsignificant 1.1% decrease of breast cancer mortality. It is noteworthy that while no statistically significant difference between anastrozole and letrozole was observed, a trend favoring letrozole was recorded with respect to breast cancer as well as total mortality rate. Similarly, no difference

was recorded regarding the efficacy of anastrozole versus exemestane for sequential treatment (3.1% absolute reduction in relapse rate 3 years after divergence of the aromatase inhibitor and tamoxifen arm, $P < 0.00001$). However, significantly improved survival was recorded with the use of aromatase inhibitors versus tamoxifen (absolute reduction of 0.7%, $P = 0.02$). Therefore, this analysis confirmed the beneficial effect of treatment with third-generation aromatase inhibitors versus tamoxifen either given as monotherapy or applied sequentially. While the analysis confirmed any potential difference between individual compounds (if any) to be minor, longer follow-up is needed to fully address potential effects on long-term survival [99].

## side-effects

The fact that a large number of breast cancer patients are becoming long-term survivors increases the focus on potential side-effects of treatments. With aromatase inhibitors, major concerns relate to detrimental effects on bone and lipid metabolism, which may enhance the risk of osteoporotic fracture rates as well as cardiovascular disease.

Osteoporosis is a major health threat to the aging female population in most countries. The lifetime risk for a hip fracture among western European and USA females is in the range of 15%–20%; for reasons not completely understood, in some countries, such as Sweden, it may exceed 25% [100]. Osteoporotic fractures are associated with a significant excess mortality [101]. Regarding the effects of aromatase inhibitors on bone metabolism, it is well established that all aromatase inhibitors moderately enhance bone loss. While this effect has been shown in comparison with tamoxifen [102, 103], it should be noted that tamoxifen exhibits anabolic effects on bone in postmenopausal women, resulting in increased bone mineral density [104]. However, evaluating the effects of exemestane [105] as well as letrozole [106] versus placebo on bone metabolism has shown a moderate loss in bone density with these aromatase inhibitors. Notably, while ongoing treatment with an aromatase inhibitor is associated with increased bone fracture rate [77, 83, 103] in comparison with tamoxifen, detrimental effects of aromatase inhibitors on bone metabolism are reversible upon terminating the drug [107]. With encouraging results from the ABCSG, which suggest that zoledronic acid may completely prevent aggravated bone loss even among premenopausal women with endocrine-responsive early breast cancer exposed to ovarian ablation and anastrozole in concert [108], and from the Zometa-Femara Adjuvant Synergy Trial [Z-FAST (United States) and ZO-FAST (global)] clinical trial program showing the prevention of bone loss with upfront versus delayed zoledronic acid in postmenopausal women with early breast cancer receiving letrozole [109], detrimental effects on bone metabolism appear to be completely preventable using regular bone mineral density assessment, vitamin D and calcium supplementation, and bisphosphonates.

A second major concern associated with estrogen suppression is the potential for detrimental effects on lipid metabolism as well as homocysteine [110]. As for the latter, recent evidence suggests that plasma homocysteine may not be

a major risk factor with respect to cardiovascular disease [111]. While for decades it was believed that estrogen replacement therapy was protective regarding the risk of cardiovascular events in postmenopausal women, recent evidence has found no reduction in cardiovascular risk with hormone replacement therapy despite confirming an ~10%–15% elevation in high-density lipoprotein cholesterol levels [112–116]. With regard to the effects of aromatase inhibitors on plasma lipid levels, studies conducted on nonfasting samples or on patients with metastatic disease, who often suffer from metabolic disturbances [117], are unreliable [118]. In the two studies evaluating the effects of an aromatase inhibitor versus placebo in early disease, exemestane [105] as well as letrozole [119] had minor effects on plasma lipid levels. Based on the rates of cardiovascular events in the phase 3 trials comparing aromatase inhibitors with tamoxifen or placebo in the adjuvant setting (Table 4), there is no substantial evidence suggesting detrimental effects of aromatase inhibitors with respect to cardiovascular morbidity and mortality in early breast cancer.

A third type of aromatase inhibitor-associated side-effect now receiving more attention is musculoskeletal joint pain and stiffness. While most patients have moderate disturbances, there is evidence that ~20% of the patient population does not adhere to prescribed therapy with aromatase inhibitors [120], and musculoskeletal and joint paint may be responsible for at least 50% of these withdrawals [121]. Recently, Belgian investigators reported synovial deposits detectable by magnetic resonance imaging scans among patients suffering tendon and joint pain while taking aromatase inhibitors [122]. While results from the Anastrozole versus Letrozole, an Investigation of Quality Of Life and Tolerability study, a randomized trial of postmenopausal women who received 12 weeks of letrozole followed by 12 weeks of anastrozole or vice versa, suggest that switching aromatase inhibitors may alleviate joint symptoms in some women, >50% of patients who experienced joint symptoms on one aromatase inhibitor did not experience them on the other aromatase inhibitor [123]; more data are needed to address this topic.

## aromatase inhibition—state of the art and way forward

Recent head-to-head data from BIG 1–98 comparing sequential treatment with monotherapy [78], combined with findings in the ATAC [74] and BIG 1–98 trials that treatment with an aromatase inhibitor reduces relapses compared with tamoxifen during the first 2 years of treatment, advocate using an aromatase inhibitor upfront. While no difference in outcome was recorded between patients receiving letrozole monotherapy, and those receiving letrozole initially then switching to tamoxifen after 2 years, these data need to be interpreted carefully due to the limited duration of follow-up from the time of switching. While previous data revealed no benefit of extending tamoxifen treatment beyond 5 years in the adjuvant setting [124], we do not know the optimal duration of treatment with an aromatase inhibitor or whether switching to tamoxifen or perhaps even estrogen treatment after a certain time period may be beneficial.

Regarding estrogen suppression, similar to what has been recorded with tamoxifen, there is reason to believe that many

**Table 4.** Cardiovascular events in studies of aromatase inhibitors as adjuvant therapy

| Study | Median follow-up (months) | Cardiovascular events |
|---|---|---|
| ATAC [75] | 100 | Myocardial infarction: ANA 34 (annual rate 0.27) versus TAM 33 (annual rate 0.27) on treatment; ANA 26 (annual rate 0.28) versus TAM 28 (annual rate 0.30) off treatment |
| BIG 1–98 [78] | 71[a] | Cardiac events (any grade): LET-containing regimens 6.1%–7.0% versus TAM 5.7% ($P = 0.45$) |
| TEAM [79] | 33 | Myocardial ischemia/infarction: EXE 41 (0.8%) versus 31 (0.6%) TAM ($P =$ NS) |
| | | Cardiac deaths: EXE 18 (0.4%) versus TAM 11 (0.2%) ($P =$ NS) |
| IES [83] | 55.7 | Cardiovascular events: EXE 382 (16.5%) versus TAM 350 (15.0%) ($P = 0.16$) |
| ITA [80] | 36 | Cardiovascular disease events: ANA 16 (7.9%) versus TAM 14 (9.3%) ($P = 0.4$) |
| ABCSG-8/ARNO 95 [81] | 28 | Myocardial infarction: ANA 3 (<1%) versus TAM 2 (<1%) ($P = 1.0$) |
| MA.17 [84] | 30 | Cardiovascular disease events: LET 149 (5.8%) versus PLA 144 (5.6%) ($P = 0.76$) |
| ABCSG6a [85] | 62.3 | Myocardial infarction: ANA 1 (0.3%) versus no further treatment 0 |
| NSABP B-33 [86] | 30 | Not reported |

[a]Sequential treatment analysis.
ABCSG, Austrian Breast and Colorectal Cancer Study Group; ANA, anastrozole; ARNO, Arimidex–Nolvadex; ATAC, Arimidex, Tamoxifen, Alone or in Combination; EXE, exemestane; BIG, Breast International Group; IES, Intergroup Exemestane Study; ITA, Italian Tamoxifen Anastrozole; LET, letrozole; NS, not significant; NSABP, National Surgical Adjuvant Breast and Bowel Project; PLA, placebo; TAM, tamoxifen; TEAM, Tamoxifen, Exemestane Adjuvant Multicenter.

tumors developing acquired resistance to aromatase inhibitors may still be sensitive to endocrine manipulation. Based on experimental evidence, suggesting that long-term estrogen deprivation may sensitize breast cancer cells to estrogen stimulation [125, 126], we implemented treatment with diethylstilbestrol for patients developing resistance to aromatase inhibitors in the metastatic setting [127]. Thirty percent of patients achieved an objective response, suggesting the potential of sequential endocrine manipulation of hormone-sensitive tumor cells [128–130].

An important question is whether one aromatase inhibitor may provide an advantage over the others. Comparing the two nonsteroidal aromatase inhibitors anastrozole and letrozole, there is substantial evidence that letrozole causes more potent *in vivo* aromatase inhibition [58] and plasma [37, 54, 58] and tissue [37] estrogen suppression. While data in the second-line metastatic setting are not straightforward, notably the one randomized study comparing letrozole versus anastrozole showed no significant differences in time to progression, the primary end point. However, letrozole was significantly superior to anastrozole in overall response rate (ORR, secondary end point) and for the two predefined covariates (visceral metastasis and ER status). No differences in ORR were shown between anastrozole and letrozole for ER-positive patients [131].

Considering first-line treatment of metastatic disease, letrozole significantly improved outcome compared with tamoxifen [132], while for anastrozole, the results were conflicting [133–135]. In addition, indirect comparison suggests improved efficacy of letrozole versus anastrozole for presurgical therapy [68–70]. While there is so far no major difference between anastrozole and letrozole monotherapy with respect to improvement of DFS compared with tamoxifen, over 100 months of follow-up, there is no evidence of any improvement in OS for anastrozole compared with tamoxifen [75]. In contrast, a nonsignificant trend ($P = 0.08$) for improved survival is observed for letrozole [78] at a median follow-up of 76 months. In contrast to patients in the ATAC study, who completed assigned treatment without the option of crossing over, patients in BIG 1–98 were subject to early unblinding of the treatment code, resulting in ~40% of patients on tamoxifen monotherapy deciding to switch to letrozole [78]. This further highlights potential differences between anastrozole and letrozole with respect to OS benefits.

A similar comparison with respect to biochemical efficacy is not possible between anastrozole or letrozole on the one hand and exemestane on the other as no head-to-head comparison of aromatase inhibition or estrogen suppression between nonsteroidal and steroidal aromatase inhibitors has been carried out. Notably, there is clear evidence of a lack of cross-resistance between nonsteroidal compounds and exemestane; the evidence and potential mechanisms are discussed in detail elsewhere [136]. Indeed, exemestane was as effective as fulvestrant among patients failing nonsteroidal third-generation aromatase inhibitors [137]. Thus, another question for future studies may be the potential benefit of sequential treatment with steroidal and nonsteroidal aromatase inhibitors in the adjuvant setting.

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