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REVIEW

The discovery and mechanism of action of letrozole

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Abstract Because estrogen contributes to the promotion and progression of breast cancer, a greater understanding of the role of estrogen in breast cancer has led to therapeutic strategies targeting estrogen synthesis, the estrogen receptor, and intracellular signaling pathways. The enzyme aromatase catalyses the final step in estrogen biosynthesis and was identified as an attractive target for selective inhibition. Modern third-generation aromatase inhibitors (AIs) effectively block the production of estrogen without exerting effects on other steroidogenic pathways. The discovery of letrozole (Femara®) achieved the goal of discovering a highly potent and totally selective AI. Letrozole has greater potency than other AIs, including anastrozole, exemestane, formestane, and aminoglutethimide. Moreover, letrozole produces near complete inhibition of aromatase in peripheral tissues and is associated with greater suppression of estrogen than is achieved with other AIs. The potent anti-tumor effects of letrozole were demonstrated in several animal models. Studies with MCF-7Ca xenografts successfully predicted that letrozole would be clinically superior to the previous gold standard tamoxifen and also indicated that it may be more effective than other AIs. An extensive program of randomized clinical trials has demonstrated the clinical benefits of letrozole across the spectrum of hormone-responsive breast cancer in postmenopausal women.

Keywords Aromatase · Breast cancer · Estrogen · Postmenopausal

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Introduction

Studies have consistently shown that lifetime exposure to estrogens increases the risk of breast cancer [1]. The degree of risk is increased by persistently elevated blood concentrations of estrogen [2]; clinical indicators of persistently elevated blood estrogen concentrations, for example, age at menarche, first live birth, menopause, alcohol consumption, and obesity [3–5]; and, although still controversial, exposure to exogenous estrogen, for example, some forms of hormone replacement therapy and oral contraceptives [6–12]. The presence of some of these factors also increases the risk of breast cancer being estrogen receptor (ER)-positive [13]. Studies have shown that higher levels of endogenous estrogen and testosterone (which is converted to estrogen by aromatase) increases breast cancer risk, regardless of predicted breast cancer risk [14–16]. These data indicate that estrogen is an important risk factor even in women considered at high risk of developing the disease, for example, those with a family history of breast cancer.

Estrogen is thought to contribute to the initiation and contributes to the promotion and progression of breast cancer via two complementary mechanisms [1], the carcinogenic effects of estrogen metabolites, notably hydroxyl metabolites [3, 17, 18], and stimulation of ER signaling pathways, including those initiated by activation of epidermal growth factors, notably the mitogen-activated phosphoinositide 3 kinase pathway [19–30]. Greater understanding of the role of estrogen in breast cancer has led to therapeutic strategies targeting estrogen synthesis (aromatase inhibitors [AIs]) [31], the ER (selective ER modulators [SERMs], pure antagonists) [32], and intracellular signaling pathways (signal transduction inhibitors) [33].

Hormone receptor (HR)-positive tumors are defined as those with ER or progesterone receptor (PgR) expression detectable above a pre-set limit [34]. Patients whose ER or PgR expression is below this pre-set limit are considered HR–. Approximately two thirds of breast cancer patients have HR+ tumors [13] and are candidates for treatment strategies designed to counteract the growth effects of estrogen. This review describes the rational development of the potent AI letrozole, which has therapeutic utility in HR+ tumors across the breast cancer continuum.

Mechanism of action of aromatase inhibitors

Aromatase

Aromatase (cytochrome P-450 [CYP] 19) catalyzes the rate-limiting step (conversion of steroidal C-19 androgens to C-18 estrogens) in estrogen biosynthesis [35–37]. Aromatization is the final step in steroid biosynthesis (Fig. 1) [38]; and, therefore, aromatase is an attractive target for selective inhibition [39, 40]. Aromatase is expressed primarily in the ovary and also in central and peripheral tissues, fat, muscle, liver, and breast [41, 42]. With increasing age, as ovarian estrogen production declines [43], the contribution of peripheral production of estrogens increases [44], and in postmenopausal women, peripheral aromatization of androstenedione produced by the adrenal gland (Fig. 1) [38] becomes the main source of endogenous estrogens [45–49]. Of note, normal and malignant breast tissue contributes to the peripheral synthesis of estrogens [14, 50–53]. Thus, expression of aromatase in breast tumors may contribute significantly to the degree of cellular exposure to estrogens [14]; therefore, it is important to target both intra-tumoral and peripheral aromatase [31].

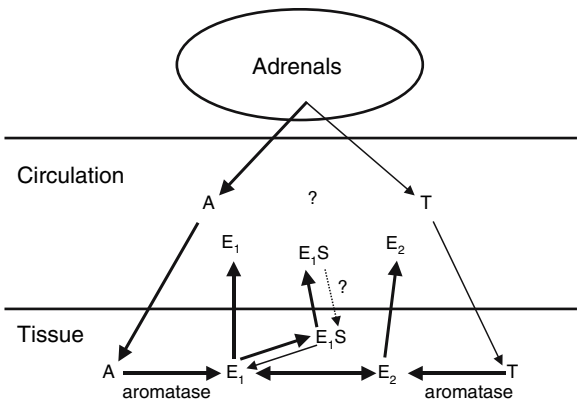

Fig. 1 Aromatization of androgens to estrogens in postmenopausal women. *A* androstenedione, *E1* estrone, *E1S* estrone sulfate, *E2* estradiol, *T* testosterone. Reprinted from [38] with permission from the Society of Endocrinology

The presence of intracellular aromatase activity could explain why estrogen concentrations are 10–20 times higher in peripheral tissue than blood in postmenopausal but not pre-menopausal women [41, 54–58]. Moreover, estrogen concentrations are higher in tumors than in surrounding non-malignant tissue [41, 54–58]. Recent research has increased understanding of how aromatase is regulated by tissue-specific promoters [59] and how genetic variation may affect the pathophysiology of estrogen-dependent disease [60]. Pharmacogenomics may become an increasingly important tool for individualizing hormonal therapy for patients with breast cancer.

Aromatase inhibitors

Modern third-generation AIs effectively block the production of estrogen without exerting effects on other steroidogenic pathways and have been heralded as a "triumph of translational oncology" [61]. The search for potent and selective inhibitors of aromatase started with the first-generation inhibitor aminoglutethimide [62]. However, aminoglutethimide lacked selectivity for aromatase [63] and inhibited biosynthesis of cortisol, aldosterone, and thyroid hormone [64] as well as aromatase; moreover, aminoglutethimide was also found to induce hepatic enzymes (Fig. 2) [65, 66]. Second-generation AIs included the nonsteroidal inhibitor fadrozole and the steroidal

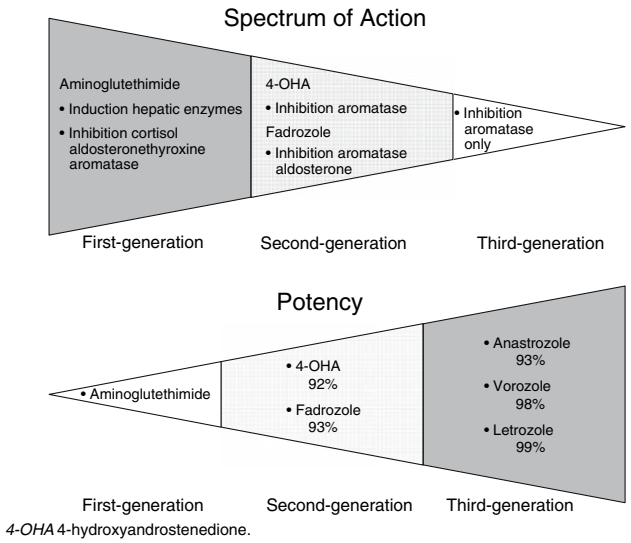

Fig. 2 The development of aromatase inhibitors (AIs) has culminated in agents with high specificity and potency for aromatase. Spectrum of action of first- through third-generation AIs: The third-generation AIs act exclusively on the aromatase enzyme and do not appear to exert additional effects. Potency of AIs determined by degree of inhibition of total body aromatase: *4-OHA* 4-hydroxyandrostenedione. Reprinted from [66] with permission from the Society of Endocrinology

inhibitor formestane (4-hydroxyandrostenedione). Fadrozole was superior to aminoglutethimide in terms of potency, selectivity, and safety [67], but its selectivity was not complete and clinical trials suggested that it was no more effective than tamoxifen [68, 69].

To improve on fadrozole, Novartis synthesized a series of new compounds. Structure-activity relationship studies were then performed to identify the most potent AI from a series of benzyl-azole derivatives of fadrozole [70]. The third-generation AI letrozole (Femara®) was the result of this structure-activity approach to drug design and achieved the research goal of creating a highly potent and totally selective AI [71]. These compounds were also used to design pioneering molecular modeling techniques used to map the active site of aromatase [70, 72]. Other third-generation AIs developed during this period were the nonsteroidal agents vorozole (since discontinued) and anastrozole [73] (Fig. 2) [66] and the steroidal agent exemestane [74]. AIs have been classified as steroidal (type I; for example, exemestane) or nonsteroidal (type II; for example, letrozole and anastrozole) [75]. A comprehensive review of AIs focuses on the pharmacology and clinical development of letrozole [76].

Letrozole pharmacodynamics and pharmacokinetics

Potency

The chemical structure of letrozole (4,4′-[(1H-1,2,4-triazol-1-yl) methylene] bis-benzonitrile) is compared with other AIs in Fig. 3 [77]. The nitrogen-containing structures like the imidazoles and the triazoles bind to the iron in the heme moiety of CYP-450, whereas the cyanobenzyl moiety present in the nonsteroidal AIs such as letrozole partially mimics the steroid backbone of the enzyme's natural substrate androstenedione. Furthermore, the triazole compound letrozole was found to be superior to other derivatives of fadrozole in terms of in vivo inhibition of aromatase [70].

Letrozole is a highly potent inhibitor of aromatase in vitro, in vivo in animals, and in humans. The relative potencies of letrozole, anastrozole, and fadrozole were determined in a variety of model cellular endocrine and tumor systems containing aromatase (hamster ovarian tissue fragments, adipose tissue fibroblasts from normal human breast, the MCF-7Ca human breast cancer cell line transfected with the human aromatase gene, and the JEG-3 human choriocarcinoma cell line) [31]. These studies showed that although letrozole and anastrozole are approximately equipotent in a cell-free aromatase system (human placental microsomes), letrozole is 10–30 times more potent than anastrozole in inhibiting intracellular aromatase in intact rodent cells, normal human adipose fibroblasts, and human cancer cell lines (Fig. 4) [31]. In several other studies, letrozole has consistently demonstrated greater potency compared with anastrozole, exemestane, formestane, and aminoglutethimide (Table 1) [31, 71, 75, 78–82].

The degree of aromatase inhibition can be determined in vivo by measuring uterine weight after treatment with a standard dose of androstenedione in immature female rats [71]. Using this assay, it was found that the in vivo potency of letrozole is more than four orders of magnitude greater than aminoglutethimide (50% effective dose [ED_{50}], 1–3 μg/kg vs. 30 mg/kg, respectively) [71]. It has also been shown that neoadjuvant letrozole profoundly inhibits in situ aromatase activity and reduces endogenous

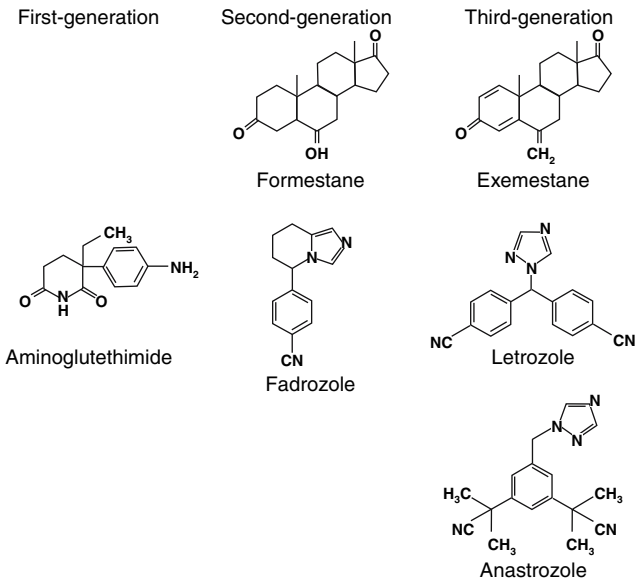

Fig. 3 Comparison of the molecular structures of aromatase inhibitors. Reprinted from [77] with permission from Elsevier

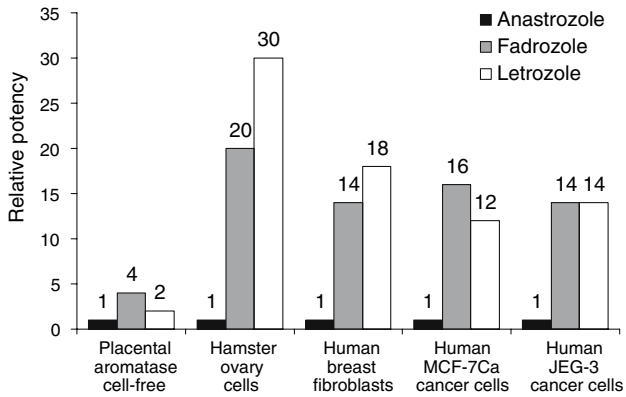

Fig. 4 Relative potencies with which letrozole, anastrozole, and fadrozole inhibit aromatase from non-cellular and intracellular sources. Reprinted from [31] with permission from Elsevier

estrogens within the breast in postmenopausal women with large primary breast cancers [75].

In postmenopausal women, letrozole achieves significantly greater plasma estrogen suppression of estrogens and greater inhibition of in vivo aromatization than anastrozole [83]. In the study, levels of aromatase were detectable in 11 of 12 patients during treatment with anastrozole (mean percentage inhibition in the whole group, 97.3%) but in none of the 12 patients during treatment with letrozole (>99.1% suppression in all patients; Wilcoxon, $P = 0.0022$, comparing the two drug regimens). Suppression of estrone and estrone sulfate was found to be significantly greater during treatment with letrozole compared with anastrozole ($P = 0.019$ and 0.0037, respectively). Another study conducted in 54 postmenopausal women with invasive breast cancer showed that more complete inhibition of aromatase was achieved with 2.5 mg of letrozole than 1 mg of anastrozole, resulting in significantly greater suppression of estradiol ($P < 0.0001$), the most bioactive estrogen [84]. This recent study confirms previous observations showing that letrozole produces near complete inhibition of aromatase in peripheral tissues, associated with greater suppression of estrogen than achieved with other AIs [78, 85–90].

Table 1 Inhibitory concentrations of letrozole, anastrozole, exemestane, fadrozole, 4-hydroxyandrostenedione and aminoglutethimide against the aromatase enzyme derived from various cellular and non-cellular sources. Reprinted from [77] with permission from Elsevier

Aromatase inhibitor	IC_{50} values (nM), (relative potency; letrozole = 1)							
	Human placental microsomes	Particulate fractions of human breast cancer	Rat ovarian microsomes	MCF-7Ca cancer cells	JEG-3 cancer cells	CHO cells	Hamster ovarian tissue	Human breast
Letrozole		2 (1)						0.8 (1)
Anastrozole		8 (0.25)						15 (0.053)
Exemestane		15 (0.13)						5 (0.16)
4-OHA		30 (0.07)						30 (0.027)
AG		20,000 (0.0001)						10,000 (0.0008)
Letrozole	11 (1)			0.07 (1)	0.07 (1)		20 (1)	0.8 (1)
Anastrozole	23 (0.48)			0.82 (0.085)	0.99 (0.071)		600 (0.033)	14 (0.057)
Fadrozole	5 (2.2)			0.05 (1.4)	0.07 (1.0)		30 (0.67)	1 (0.80)
4-OHA	62 (0.18)							
AG	1900 (0.0058)							
Letrozole	1.02 (1)			0.35 (1.0)	0.45 (1)			0.14 (1)
Anastrozole	5.35 (0.19)			3.62 (0.097)	5.66 (0.080)			17.17 (0.0082)
4-OHA				0.59 (0.59)	1.6 (0.28)			0.72 (0.19)
Letrozole			7 (1)					
Anastrozole			25 (0.28)					
Fadrozole			7 (1)					
Letrozole						1.4 (0)		
Anastrozole						27 (0.052)		
4-OHA						60 (0.023)		
AG						5500 (0.00025)		

4-OHA 4-hydroxyandrostenedione, *AG* aminoglutethimide

Values quoted are IC_{50} values representing the concentration needed to achieve 50% inhibition of aromatase activity. The relative potency of each inhibitor compared with letrozole is shown in parentheses

Selectivity

Letrozole is highly selective for aromatase and unlike first- and second-generation AIs does not significantly affect cortisol, aldosterone, or thyroxine [77]. In vitro studies showed that letrozole was more than three orders of magnitude more selective than aminoglutethimide in its effects on progesterone and corticosterone production, and more than 300-fold more selective against aldosterone than fadrozole [71, 78]. In vivo adrenocorticotrophic hormone (ACTH) stimulation tests in rats showed that letrozole had no significant effect on either aldosterone or corticosterone levels, even at a dose 1,000 times greater than that required for inhibition of aromatase [71].

The selectivity of letrozole has been demonstrated in clinical studies in postmenopausal women. These studies showed that letrozole has no effect on the plasma levels of 17α-OH progesterone, thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), or androstenedione and does not affect normal urine electrolyte excretion or thyroid function [86, 91–93]. Of note, the vast majority of patients treated with letrozole have a normal response to synthetic ACTH [86].

Anti-tumor activity in vivo

The potent anti-tumor effects of letrozole have been demonstrated in several animal models [77, 78, 94]. Letrozole induced complete regression of estrogen-dependent, 9,10-dimethylbenz-a-anthracene (DMBA)-induced mammary tumors in adult female rats [95]. The ED_{50} for letrozole was determined to be 10–30 μg/kg/day.

The use of MCF-7 cells transfected with human aromatase gene (MCF-7Ca) and implanted into athymic nude mice has proved to be an effective in vivo model for predicting clinical results with AIs [61, 96, 97]. Using this model, it has been shown that letrozole produces dose-dependent inhibition of tumor growth, resulting in complete inhibition at a daily dose of 10 μg/animal/day [94, 98]. Comparative studies using the MCF-7Ca model have shown that letrozole is more effective at suppressing tumor growth than the pure anti-estrogen fulvestrant and the SERM tamoxifen [99]. While anastrozole was also better than fulvestrant and tamoxifen in suppressing tumor growth, only letrozole was shown to induce tumor regression [99].

Another study, also using the MCF-7Ca model, demonstrated that letrozole potently inhibits mammary tumor growth but does not have the estrogenic effects of tamoxifen, as measured by its uterotrophic effects [100]. The observation that tamoxifen has an agonist effect even when estrogen synthesis is inhibited by letrozole suggests that there may be a degree of antagonism between these compounds [100]. Interestingly, studies in the MCF-7Ca model showed that letrozole is more effective as monotherapy than when combined with tamoxifen [80, 101]. In the study reported by Long et al. [101] tumor volume doubling times were 3–4 weeks in controls, 16 weeks with tamoxifen alone, 18 weeks with tamoxifen plus letrozole, and 34 weeks with letrozole alone. First-line treatment with letrozole was shown to be significantly superior to treatment with tamoxifen alone or with the two drugs combined (at week 16, both $P < 0.001$). Tumors that progressed during treatment with tamoxifen remained sensitive to second-line letrozole therapy, whereas tumors that progressed on letrozole did not respond to second-line treatment with tamoxifen or fulvestrant. In another series of experiments conducted by the same group using the MCF-7Ca model, letrozole was even effective as third-line therapy for a limited period when administered after treatment with tamoxifen and exemestane [102]. The studies showed that although exemestane was more effective than tamoxifen in controlling tumor growth, letrozole as first-line therapy was the most effective treatment overall, both in terms of the degree of tumor suppression and the length of effectiveness of treatment [102].

The potential of letrozole as a chemopreventive agent was investigated in an in vivo model using aromatase-transgenic female mice [103]. The model provided evidence to show that aromatase overexpression is sufficient to induce and maintain early preneoplastic and neoplastic changes that can be completely abrogated by treatment with letrozole. Carcinogenicity studies have also found that letrozole decreases the incidence of spontaneous mammary tumors and granular cell tumors in rats [104].

Pharmacokinetics of letrozole

Clinical pharmacokinetic studies of letrozole have been conducted in healthy volunteers [105–107] and in patients with breast cancer [108, 109]. Following oral administration, letrozole is rapidly and completely absorbed (mean absolute bioavailability of 99.9%) and extensively distributed to tissues. It has a large apparent volume of distribution at steady state (1.87 l/kg [range, 1.47–3.24]), and approximately 60% is bound to plasma proteins, mainly to albumin (55%). The terminal half-life ($T_{1/2}$) of letrozole is 42 h. The terminal $T_{1/2}$ was observed to be longer and area under the curve (AUC) greater in patients with breast cancer than in healthy volunteers, possibly due to reduction in metabolic clearance [109]. The major route of elimination of letrozole is metabolism by CYP-450 isoenzymes (CYP 3A4 and CYP 2A6) into an inactive carbinol metabolite. Systemic exposure to metabolites is,

therefore, low. Steady-state concentrations of letrozole are reached after 2–6 weeks and maintained for long periods with no evidence of drug accumulation.

In marked contrast to the first-generation AI aminoglutethimide, no significant drug interactions have been reported for letrozole; however, when combined with tamoxifen, letrozole plasma concentrations are reduced by between 35% and 40% [110]. Age does not have an effect on the pharmacokinetics of letrozole. Exposure to letrozole, measured by AUC, is increased in renally impaired subjects but remains in the range seen in subjects without impaired function. However, hepatic impairment can markedly increase the $T_{1/2}$ of letrozole, and caution is required in such patients.

Differences in pharmacokinetics, including uptake rates, elimination T1/2, and metabolism and clearance exist between AIs and have been reviewed by Lønning et al. [111]. The clinical significance of such differences is not known.

Clinical development of letrozole

Letrozole entered clinical trials on the basis of its high potency and selectivity for aromatase, the demonstration of unsurpassed anti-tumor effects in models of human breast cancer, and the development of a convenient oral formulation. Daily doses of 0.1–5 mg have been shown to suppress estradiol, estrone, and estrone sulfate plasma concentrations by 75–95% from baseline, while doses >0.5 mg suppress estrogens to below limit of detection [92, 112, 113]. Based on pharmacokinetic and pharmacodynamic studies, the recommended dose of letrozole is one 2.5 mg tablet once daily.

Preclinical models [97, 101] successfully predicted that letrozole would be superior to tamoxifen, the previous gold standard in the treatment of breast cancer. An extensive program of clinical trials has been conducted with letrozole across the spectrum of hormone-responsive breast cancer in postmenopausal women. The first randomized controlled trials demonstrated consistent superiority for letrozole compared with megestrol acetate, aminoglutethimide, and tamoxifen in patients with advanced breast cancer [114–118]. The clinical efficacy of letrozole in advanced breast cancer is described in a review by Dr. Mouridsen in this supplement.

Preclinical MCF-7Ca models have also predicted that letrozole should be clinically more effective than other less potent third-generation AIs [99, 102]. Letrozole (2.5 mg/day) and anastrozole (1 mg/day) were directly compared in a randomized, open-label phase IIIb/IV study involving 713 postmenopausal women with advanced breast cancer previously treated with an anti-estrogen [119]. While there was no difference between the treatment arms in the time to progression, letrozole produced a significantly higher overall response rate than anastrozole (19.1 vs. 12.3%, $P = 0.013$). Letrozole and anastrozole are currently being compared in a large randomized head-to-head trial in early breast cancer (ClinicalTrials.gov identifier NCT00248170) [120]. A review by O'Shaughnessy in this supplement provides the rationale for this trial and a description of its design.

The clinical benefits of letrozole in early breast cancer have already been demonstrated in landmark randomized clinical trials. MA.17 was the first trial to show improved clinical outcomes with extended adjuvant hormone therapy [121]. In this trial, letrozole given after initial adjuvant therapy with tamoxifen significantly improved disease-free survival compared with placebo [121, 122]. Full details of this trial are provided in a review by Dr. Goss in this supplement.

Subsequently, the Breast International Group 1-98 trial provided high-level evidence for the superiority of letrozole over tamoxifen as initial adjuvant therapy [123]. A detailed description of this ongoing trial, which will also help to define the optimal sequence for hormone therapies in hormone-responsive early breast cancer, is provided in a review by Dr. Thürlimann in this supplement. Letrozole has also demonstrated superior efficacy compared with tamoxifen when used as neoadjuvant therapy [124]. This treatment setting is particularly interesting in terms of drug development because the effects of hormone therapy on breast tumors can be detected early and may be predictive of long-term outcome [125].

Conclusions

Letrozole is a highly potent and selective AI that inhibits the enzyme activity of intracellular aromatase at the major sites where it is found, resulting in almost complete suppression of whole body aromatization. By effectively blocking estrogen synthesis, letrozole inhibits the growth or induces the regression of hormone-responsive breast tumors in vivo. Estrogen is implicated as a major risk factor in the majority of breast cancers; therefore, use of the most potent AI is a logical treatment strategy.

Studies conducted using in vitro and in vivo models have demonstrated that letrozole is the most potent of the third-generation AIs. Preclinical data obtained from MCF-7Ca xenograft models suggest that the greater potency of letrozole compared with anastrozole and exemestane may translate into clinically meaningful differences in postmenopausal women with hormone-responsive breast cancer. These models accurately predicted that letrozole would be more effective than tamoxifen in the clinical

setting. The superiority of letrozole over tamoxifen has been consistently demonstrated in advanced and early breast cancer [118, 123]. Outstanding clinical questions, including what is the most effective AI and what is the optimal sequence for adjuvant hormonal therapy, will be answered by the results of ongoing trials involving letrozole.

In conclusion, experimental data indicating that letrozole efficiently inhibits aromatase activity have been confirmed clinically, leading to approved indications across the spectrum of breast cancer. The broad range of indications for letrozole in unique clinical settings is reshaping the management of hormone-sensitive breast cancer.

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