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BREAST CANCER (B OVERMOYER, SECTION EDITOR)

Optimizing the Use of Neoadjuvant Endocrine Therapy

Laila S. Agrawal[1] · **Ingrid A. Mayer**[1,2]

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Abstract Nowadays, neoadjuvant endocrine therapy is a clinically acceptable (and sometimes preferred) strategy in patients with operable estrogen receptor-positive (ER+) breast cancer. Despite the overall effectiveness of endocrine therapy in breast cancer in all settings, de novo (primary) and acquired (secondary) endocrine therapy resistance remains a major clinical problem. Neoadjuvant endocrine therapy trials for breast cancer are not only a great opportunity to determine which ER+ breast cancers can be treated without chemotherapy, but also a great strategy to develop insights into the biologic basis for the efficacy of estrogen-receptor-targeting agents, alone or in combination, in an effort to counteract resistance to endocrine therapy and discover actionable molecular targets that can be the focus of future drug discovery efforts and/or translational/clinical investigation in ER+ breast cancers.

Keywords Breast cancer · Neoadjuvant endocrine therapy · Neoadjuvant trials · Endocrine therapy resistance · Growth factor signaling pathways · PI3K/AKT/mTOR pathway · Targeted inhibitors

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✉ Ingrid A. Mayer
ingrid.mayer@vanderbilt.edu

[1] Department of Medicine, Breast Cancer Research Program, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN, USA

[2] Division of Hematology/Oncology, Department of Medicine, Vanderbilt University School of Medicine, 2220 Pierce Avenue, 777 PRB, Nashville, TN 37232-6307, USA

Introduction

While neoadjuvant endocrine therapy was initially studied in elderly patients who were not considered candidates for chemotherapy or surgery [1, 2], several trials have established the role of neoadjuvant endocrine therapy in a broader patient population [3–5, 6••]. Nowadays, neoadjuvant endocrine therapy is a clinically acceptable strategy, and it has been well established that while longer treatment duration (6–12 months) could be warranted [7•], overall clinical results are comparable to chemotherapy [8].

Despite the overall effectiveness of endocrine therapy in breast cancer in all settings, de novo (primary) and acquired (secondary) endocrine therapy resistance remains a major clinical problem, accounting for metastatic recurrence and consequent death for some women with estrogen receptor-positive (ER+) breast cancer [9–12]. Neoadjuvant endocrine therapy may provide a novel framework for the study of endocrine therapy and targeted agent combinations against endocrine resistance, which ultimately could help reduce mortality for patients with ER+ breast cancers.

This review will discuss neoadjuvant endocrine therapy trials and endpoints, the rationale of using targeted combinations in the neoadjuvant setting, and completed and ongoing clinical trials of neoadjuvant endocrine therapy with novel targeted therapies.

Neoadjuvant Endocrine Therapy

The significance of neoadjuvant endocrine treatment was first emphasized by the results of the P024 trial, a double-blind randomized phase III neoadjuvant endocrine study that compared 4 months of the aromatase inhibitor letrozole with tamoxifen as presurgical treatment for women with hormone

receptor-positive tumors who were ineligible for breast-conserving surgery. This trial demonstrated statistically significant improvement in clinical response rates (55 vs. 36 %) and rates of breast-conserving surgery (45 vs. 35 %) in favor of letrozole [3]. Letrozole also outperformed tamoxifen in terms of inhibition of proliferation measured by Ki67 of tumor sections [13, 14]. The advantage of letrozole appeared to be particularly evident in a subpopulation of tumors with ER-positive and epidermal growth factor receptor (EGFR)- and/or human epidermal growth factor receptor-2 (HER2)-positive tumors, indicating that the comparison of endocrine agents in the presurgical setting could provide insights into the molecular basis for differences in efficacy between endocrine agents [15]. Nevertheless, further investigation on the impact of EGFR and/or HER2 gene amplification on neoadjuvant endocrine therapy responsiveness suggested that ER/progesterone receptor (PR)-positive and HER2 FISH-positive tumors showed less Ki67 suppression after both letrozole and tamoxifen treatment when compared to ER/PR-positive and HER2-negative tumors, despite similar short-term clinical efficacy [16]. This continued proliferation despite letrozole treatment could imply the therapeutic resistance that may manifest later in the clinical course of the disease.

Immediate preoperative anastrozole, tamoxifen, or combined with tamoxifen (IMPACT) was a trial of 330 patients treated with neoadjuvant anastrozole, tamoxifen, or the combination for 12 weeks and demonstrated similar clinical response rates and a trend towards improved breast-conserving surgery with the aromatase inhibitor (AI) arm (44 vs. 31 % for tamoxifen vs. 24 % for combination) [5, 17, 18]. Interestingly, suppression of the proliferation marker Ki67 after 2 and 12 weeks was significantly greater with anastrozole than with tamoxifen but similar between tamoxifen and the combination. There was no significant correlation between a fall in Ki67 and clinical tumor response, although more patients in the anastrozole arm were eligible for breast-conserving surgery.

The PROACT trial randomized 451 patients to neoadjuvant anastrozole or tamoxifen for 3 months and showed a trend towards improvements in breast-conserving surgery rate in the group receiving the AI (43 vs. 30.8 %) [4]. A meta-analysis found that neoadjuvant aromatase inhibitors had better rates of clinical response, radiographic response, and breast-conserving surgery compared to tamoxifen [19]. A randomized study Z1031 compared neoadjuvant exemestane, anastrozole, and letrozole and found similar surgical and biomarker outcomes between the three aromatase inhibitors, suggesting biologic equivalence [6••].

Neoadjuvant endocrine therapy has also been compared to neoadjuvant chemotherapy. A study of 239 postmenopausal women with ER- and/or progesterone receptor (PR)-positive breast cancer randomized to chemotherapy with doxorubicin and paclitaxel (every 3 weeks for 4 cycles) or exemestane or anastrozole for 3 months demonstrated similar clinical response rates, pathologic complete response (pCR) rates, and disease progression and a breast-conserving surgery rate of 33 % in the endocrine therapy group compared to 24 % in the chemotherapy group [8]. These studies together establish neoadjuvant endocrine therapy with aromatase inhibitors as a clinically acceptable strategy in postmenopausal women and provide a framework for the study of combinations with targeted agents against endocrine resistance.

Rational Endpoints for Neoadjuvant Endocrine Therapy Trials

A variety of endpoints have been used in neoadjuvant studies, such as clinical response rate, breast-conserving surgery rate, the proliferation index Ki67, the preoperative endocrine prognostic index (PEPI) score, and rates of pathologic complete response (pCR).

Expression of the proliferation antigen Ki67 after neoadjuvant endocrine therapy can be used to predict outcomes. Higher expression of Ki67 after 2 weeks of neoadjuvant endocrine therapy was significantly associated with a lower recurrence free survival [20]. In the IMPACT trial, suppression of the proliferation marker Ki67 after 2 and 12 weeks was significantly greater with anastrozole than with tamoxifen but similar between tamoxifen and the combination [5, 17, 18]. In this neoadjuvant trial, there was no correlation between a fall in Ki67 and clinical tumor response. However, in the Arimidex, tamoxifen, alone, or in combination (ATAC) phase III randomized adjuvant trial (anastrozole vs. tamoxifen vs. the combination in over 9000 postmenopausal women with early ER-positive cancers) which was conducted at about the same time as IMPACT, there were fewer recurrences and fewer new primary breast cancers as well as a statistically better disease-free survival in the anastrozole arm compared to the other two [21]. One could argue that had the results of IMPACT been known before ATAC, the data in the former trial could have streamlined ATAC, providing a justification to eliminating the combination arm. The potential elimination of this arm, whose "molecular rationale" was never robust, would have spared enrollment of over 3000 patients without masking the superiority of anastrozole over tamoxifen. Such a randomized two-arm study would have required far fewer patients and would have been completed in a shorter time than 33 months. The similarities in the direction of the outcomes of the ATAC and IMPACT trials suggest the possibility that neoadjuvant studies with cellular and molecular endpoints in addition to clinical endpoints can "predict" the outcome of larger clinical studies in the metastatic setting and thus provide a novel platform for the prioritization of new drugs and/or combinations.

The PEPI score was developed from a multi-variable analysis of data from the P024 trial comparing neoadjuvant letrozole and tamoxifen for 4 months prior to surgery. The four factors found to have prognostic value for relapse and death after relapse were pathologic tumor size, node status, the natural logarithm of the Ki67 value, and the ER status of the final surgical specimen. The PEPI score was then validated in the independent dataset from the IMPACT trial [22, 23]. Patients with T1 and N0 tumors with a PEPI score of 0 (residual tumor with Ki67 index of 2.7 %) may be candidates for avoidance of chemotherapy [23].

Molecular signatures such as the 21-gene recurrence score, PAM50, and the Netherlands Cancer Institute (NKI) 70 gene have been investigated as predictive markers of response and could be useful as a research tool in the post-neoadjuvant setting [6••, 24, 25].

Certain targeted agents such as phosphatidylinositol 3-kinase (PI3K) inhibitors or cycle-dependent kinase 4/6 (CDK4/6) inhibitors can lead to increased cell death in vitro. The combination of some of the newer targeted agents and endocrine therapy could lead to an increased pathologic response in vivo by eliciting tumor apoptosis and killing breast cancer cells before the development of resistance to endocrine therapy. It is possible that in combination with targeted agents that promote cell death, pCR could become a meaningful clinical trial endpoint in neoadjuvant trials, potentially being used as a "proving ground" for novel combinations.

Counteracting Endocrine Therapy Resistance

The major downfall of endocrine therapy is the development of de novo or acquired resistance. Multiple mechanisms contribute to endocrine therapy resistance [26, 27], including deregulation of the ER pathway [28] (rare loss of ER by tumors, selection of cells with ER mutations, alterations in the intracellular pharmacology and/or binding of antiestrogens to breast cancer cells, and perturbation of the interaction ER and co-activators and co-repressors of transcription [9–12]) and development of ligand-independent ER-mediated transcription, with increased growth factor receptor signaling and activation of downstream signaling pathways, including growth factor receptor tyrosine kinases, human epidermal growth factor receptor-2 (HER2) [29], the PI3/AKT/mammalian target of rapamycin (mTOR) [30], mitogen-activated kinase (MAPK)/ERK, fibroblast growth factor and receptor (FGFR), insulin-like growth factor-1 receptor, and finally, epigenetic modification including histone modification [26, 27, 31]. These pathways all represent potential targets to overcome endocrine resistance.

ErbB Pathway

Preclinical models and some clinical observations suggest that ER+ breast cancers initially inhibited by a selective estrogen-receptor modulator (SERM) can use autocrine ErbB signaling in order to escape SERM action [32–35]. This mechanism involves "cross talk" between growth factor signaling pathways and the ER. Ligand-independent activation of ER by growth factor signaling could then contribute to resistance to estrogen deprivation by rendering the cells exquisitely sensitive to very low estrogen levels [36–38]. EGFR and/or HER2 signaling becomes important for the tumor cell at the time of escape from hormone deprivation. Both EGFR and HER2 inhibitors have been shown to enhance the antitumor effect of antiestrogens or reverse antiestrogen resistance in ErbB receptor overexpressing and ER+ breast cancer cells in the preclinical setting [37, 39, 40]. MCF-7 human breast cancer cells transfected with aromatase and selected for resistance to letrozole overexpress HER2 and activated MAP kinase; the emergence of this resistance was shown to be abrogated by treatment with gefitinib (an EGFR inhibitor) or inhibitors of MAPK [41, 42]. Several data suggest a causal association between overexpression and/or aberrant activity of the HER2 signaling pathway and antiestrogen resistance in human breast cancer [43]. Patients with tumors that overexpress HER2 also exhibit statistically lower responses and or shorter duration of response to antiestrogen therapy [43, 44]. Small molecule inhibitors of EGFR and HER2, such as AG1478, enhance tamoxifen action against HER2-overexpressing and tamoxifen-resistant human breast cancer cells both in vitro and in vivo [45]. Taken together, these data implicate the EGFR/HER2 signaling network as a robust molecular target in antiestrogen-resistant human breast carcinoma.

Two neoadjuvant studies have evaluated the role of gefitinib, a tyrosine kinase inhibitor against EGFR, with mixed results. First, a double-blind, placebo-controlled neoadjuvant study randomized 56 postmenopausal women with ER-positive and EGFR-positive breast cancer to treatment with gefitinib (250 mg orally once a day) and the aromatase inhibitor anastrozole (1 mg orally daily) or to gefitinib and placebo for 4–6 weeks prior to surgery. The combination of aromatase inhibitor and gefitinib resulted in a greater reduction in pretreatment values of Ki67 compared to gefitinib alone (mean % reduction 98.0 [95 % CI 96.1–98.9] vs 92.4 [85.1–96.1]; difference between groups 5.6 % [5.1 –6.0], p=0.0054). A partial response assessed by ultrasound was achieved in 14 of 28 patients in the combination group and in 12 of 22 patients treated with gefitinib. Tumor size was reduced by 30–99 % (partial response) in 14 of 28 patients assigned with gefitinib and anastrozole and in 12 of 22 assigned with gefitinib, as assessed by ultrasonography [46]. The second, a phase II study which randomized women with stages I to IIIB ER+ breast cancer to anastrozole 1 mg orally daily with gefitinib

250 mg orally daily (given for 16 weeks or starting at week 3 of 16 weeks) or placebo, did not show benefit to the combination. There was no significant difference in the change in Ki67 from baseline to 2 or 16 weeks, and there was a trend against gefitinib in the clinical response (48 vs. 61 % for anastrozole alone) [47].

PI3K Pathway

Studies in cell lines and human xenografts have shown that growth factor receptor signaling pathways, in particular those that converge on phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK/ERK), can mediate resistance to all forms of endocrine therapy [9–12]. Cross talk between the PI3K and ER pathways has been suggested as a mechanism of endocrine resistance [28]. Molecular alterations involving the PI3K pathway are considered the most frequent in breast cancer, encompassing together over 30 % of invasive tumors [48, 49]. These alterations often result in PI3K pathway activation, which has been shown to induce ER phosphorylation at Ser_{167} and estrogen-independent transcriptional activity [50, 51]. Several studies have shown that hyperactivation of PI3K signaling promotes resistance to endocrine therapy [52–54].

In endocrine therapy-resistant breast cancer cells, hyperactivation of the PI3K pathway, variable changes in ER levels and E2 sensitivity, and PI3K-dependent and estrogen (ligand)-independent growth can be abrogated by PI3K inhibitors, as well as inhibitors of kinases upstream (IGF-IR/InsR/ErbBs) and downstream (mTOR) of PI3K. Additionally, inhibition of PI3K prevents the emergence of hormone-independent cells, which suggests that early intervention with antiestrogens and PI3K inhibitors could limit the escape from endocrine therapy in patients with ER+ breast cancer [54]. These data suggest that patients with hormone receptor-positive tumors exhibiting a high degree of PI3K signaling, and patients who relapse on endocrine therapy, may benefit from therapeutics targeting both the ER and the PI3K pathways.

Clinically, several studies combining PI3K pathway inhibitors with endocrine therapy have been conducted in the metastatic setting. A number of studies have evaluated the addition of mTOR inhibitors to endocrine therapy in the metastatic setting, most notably, Breast Cancer Trials of Oral Everolimus-2 (BOLERO-2), a phase III randomized trial which showed that the addition of everolimus to exemestane in patients with ER+ metastatic breast cancer refractory to aromatase inhibitors significantly improved progression free survival, but not overall survival [55••]. One phase Ib study of the pan-PI3K inhibitor buparlisib with letrozole, in patients with ER+ metastatic breast cancer refractory to endocrine therapies, reported that the combination was safe, and several patients responded for over 12 months [56].

In the neoadjuvant setting, a randomized phase II study of 270 postmenopausal women with ER-positive operable breast cancer showed that the combination of letrozole (2.5 mg orally daily) and the mTOR inhibitor everolimus (10 mg orally daily) for 4 months compared to letrozole and placebo had a statistically significant improved response rate by clinical palpation (68.1 vs. 59.1 %) and antiproliferative response (reduction in Ki67 expression to the natural logarithm of percentage positive Ki67 of less than 1 at day 15) in 57 % of the combination arm compared to 30 % in the letrozole alone arm [57]. Similar to the IMPACT/ATAC analogy, this neoadjuvant trial also predicted the results of the BOLERO-2 trial.

Ongoing Neoadjuvant Studies

There are several ongoing clinical trials evaluating the combination of targeted agents in combination with endocrine therapy in the neoadjuvant setting (Table 1).

PI3K Pathway Inhibitors

Two ongoing phase II randomized, double-blinded, and placebo-controlled clinical trials are evaluating the addition of PI3K inhibition to letrozole for postmenopausal women with ER+/HER2-negative operable breast cancer in the neoadjuvant setting: NCT01923168—letrozole with or without alpelisib (an α-specific PI3K inhibitor) or buparlisib (a pan-PI3K inhibitor), for 24 weeks and LORELEI (NCT02273973), a parallel cohort study of letrozole with or without GDC-0032 (a β-sparing PI3K inhibitor), for 16 weeks. Results will not only determine the clinical benefit added from PI3K inhibition, but will also elucidate if a more specific pathway inhibitor performs better that the other in the two patient populations being evaluated (patients with *PIK3CA* mutation or not). Furthermore, information derived from the surgical outcome (clinical response, pCR and PEPI score) will tease out tumors with primary endocrine therapy

Table 1 Phase II neoadjuvant studies of endocrine therapy combined with the targeted agents

Targeted agent	Targeted agent (endocrine agent)	ClinicalTrials.gov ID
PI3K inhibitor	BYL719 or buparlisib (letrozole)	NCT01923168
	GDC-0032 (letrozole)	NCT02273973
Akt inhibitor	MK-2206 (anastrozole)	NCT01776008
Cdk 4/6 inhibitor	PD0332991 (anastrozole)	NCT01723774
	Palbociclib (letrozole)	NCT02296801
HER2 therapy (in HER2+ patients)	Lapatinib (letrozole)	NCT01275859
	Trastuzumab (letrozole)	NCT02214004

resistance or not (i.e., delineating the ones that will still need adjuvant chemotherapy).

The Akt inhibitor MK-2206 is being evaluated in a phase II study in combination with anastrozole for postmenopausal women or anastrozole and goserelin for premenopausal women with clinical stage II or III *PIK3CA*-mutated ER+/HER2-negative (NCT01776008).

Anti-HER2 Therapy

The combination of neoadjuvant letrozole and lapatinib is being studied in postmenopausal women with ER+/HER2-positive breast cancer (NCT01275859).

Cycle-Dependent Kinase 4/6 Inhibitors

Two neoadjuvant phase II trials are evaluating palbociclib, a CDK4/6 inhibitor, in combination with endocrine therapy (anastrozole for 16 weeks—NCT01723774; and letrozole—NCT02296801) in postmenopausal women with ER+ primary breast cancer. The primary endpoint is measurement and change of Ki76 at baseline and 14 weeks.

Conclusion

The unfortunate problem with all antiestrogen therapy is that breast cancer cells become resistant to their action over time or have de novo resistance. Increasing preclinical evidence suggests that bidirectional cross talk between the estrogen receptor (ER) and growth factor receptors and downstream kinases mediate endocrine resistance [52, 58–60]. Numerous targeted inhibitors to these pathways are clinically available or in development. Therefore, combining two or more of these targeted agents with endocrine therapy may be required for a more optimal approach to ER+ breast cancer treatment, since combination of "complementary" pathway inhibitors would potentially maximize efficacy and would minimize therapeutic resistance. However, there are still no clinical tools to determine which patients are most likely to benefit or, alternatively, be primarily resistant to novel agents or drug combinations. The study of biomarkers of drug exposure and sensitivity in metastatic tumors, although feasible, is not easy due to the inherent difficulty of obtaining sequential tumor samples only for research purposes.

Neoadjuvant endocrine therapy studies for breast cancer are not only a great opportunity to determine which ER+ breast cancers can be treated without chemotherapy, but also a great strategy to develop insights into the biologic basis for the efficacy of estrogen-receptor-targeting agents, alone or in combination (i.e., more effective therapies for endocrine therapy-resistant ER+ breast cancers). By allowing the collection of both diagnostic and surgical tumor material, these studies have the added benefit of providing paired pre- and post-therapy tumor tissues with pharmacodynamic endpoints in 100 % of subjects enrolled. This would allow a much better selection of patients for clinical trials, patients to be classified for endocrine therapy responsiveness (therefore guiding future choices of therapy), and a better insight on the target dependency in the ER+ breast cancer cell.

In summary, the neoadjuvant endocrine therapy setting for ER+ breast cancer is a great opportunity to develop insights into the biologic basis for the efficacy of ER-targeting agents, alone or in combination. Additionally, the comprehensive molecular analysis of cancers remaining in the breast after therapy provides a unique opportunity to discover, in unbiased fashion, the molecular mechanisms of resistance to estrogen deprivation ± a targeted inhibitor. These mechanisms, in turn, may represent actionable molecular targets that can be the focus of future drug discovery efforts and/or translational/clinical investigation in ER+ breast cancers.

Compliance with Ethics Guidelines

Conflict of Interest Laila S. Agrawal declares that she has no conflict of interest.

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- •• Of major importance

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