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Optimizing the Use of Neoadjuvant Endocrine Therapy

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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

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 [3–6]. 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 ER+ breast cancer [9–12]. Neoadjuvant endocrine therapy may provide a novel framework for the study of endocrine therapy and targeted agent combinations against

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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 four months of the aromatase inhibitor letrozole with tamoxifen as pre-surgical 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 EGFR and/or HER2-positive tumors, indicating that the comparison of endocrine agents in the pre-surgical 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/PR positive, HER2 FISH positive tumors showed less Ki67 suppression after both letrozole and tamoxifen when compared to ER/PR positive, HER2 negative tumors, despite similar clinical efficacy short-term [16]. This continued proliferation despite letrozole treatment could imply therapeutic resistance that may manifest later in the clinical course of the disease.

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 three 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 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 three 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 pre-operative 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 two 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 ATAC phase III randomized adjuvant trial (anastrozole vs. tamoxifen vs. the combination in over 9,000 post-menopausal 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 3,000 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 four months prior to surgery. The four factors found to have prognostic value for relapse and death after relapse were pathologic tumor size, node status, and 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, 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 PI3K inhibitors or 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 SERM can use autocrine ErbB signaling in order to escape SERM action[32–35]. This mechanism involves “crosstalk” 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 become important for the tumor cell at the time of escape from hormone deprivation. Both EGFR and HER2 inhibitors have been shown to enhance the anti-tumor effect of anti-estrogens or reverse anti-estrogen resistance in ErbB receptor overexpressing, ER+ breast cancer cells in the pre-clinical 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, 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 (250mg orally once a day) and the aromatase inhibitor anastrozole (1mg 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 pre-treatment 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 gefitinib and anastrozole and in 12 of 22 assigned gefitinib, as assessed by ultrasonography[46]. The second, a phase II study which randomized women with Stage I to IIIB ER+ breast cancer to anastrozole 1mg orally daily with gefitinib 250mg 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 weeks or 16 weeks and there was a trend against gefitinib in 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]. Crosstalk 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 Ser₁₆₇ 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, 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 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 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 (2.5mg orally daily) and the mTOR inhibitor everolimus (10mg orally daily) for four months compared to letrozole and placebo had a statistically significant improved response rate by clinical palpation (68.1% vs. 59.1%), anti-proliferative 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.

PI3K Pathway Inhibitors

Two ongoing phase II randomized, double-blinded, 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 than 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 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 post-menopausal women or anastrozole and goserelin for pre-menopausal women with clinical stage 2 or 3 *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 (Cdk) 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 post-menopausal women with ER+ primary breast cancer. The primary end-point is measurement and change of Ki76 at baseline and 14 weeks.

Conclusion

The unfortunate problem with all anti-estrogen therapy is that breast cancer cells become resistant to their action over time or have de novo resistance. Increasing pre-clinical evidence suggests that bidirectional crosstalk 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, allow 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 $\pm$ 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.

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References

1. Horobin JM, Preece PE, Dewar JA, et al. Long-term follow-up of elderly patients with locoregional breast cancer treated with tamoxifen only. *Br J Surg.* 1991; 78:213–217. [PubMed: 2015478]
2. Bergman L, van Dongen JA, van Ooijen B, van Leeuwen FE. Should tamoxifen be a primary treatment choice for elderly breast cancer patients with locoregional disease? *Breast Cancer Res Treat.* 1995; 34:77–83. [PubMed: 7749163]
3. Eiermann W, Paepke S, Appfelstaedt J, et al. Preoperative treatment of postmenopausal breast cancer patients with letrozole: A randomized double-blind multicenter study. *Ann Oncol.* 2001; 12:1527–1532. [PubMed: 11822750]
4. Cataliotti L, Buzdar AU, Noguchi S, et al. Comparison of anastrozole versus tamoxifen as preoperative therapy in postmenopausal women with hormone receptor-positive breast cancer: the Pre-Operative "Arimidex" Compared to Tamoxifen (PROACT) trial. *Cancer.* 2006; 106:2095–2103. [PubMed: 16598749]
5. Smith IE, Dowsett M, Ebbs SR, et al. Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) multicenter double-blind randomized trial. *J Clin Oncol.* 2005; 23:5108–5116. [PubMed: 15998903]
- ***6. Ellis MJ, Suman VJ, Hoog J, et al. Randomized phase II neoadjuvant comparison between letrozole, anastrozole, and exemestane for postmenopausal women with estrogen receptor-rich stage 2 to 3 breast cancer: clinical and biomarker outcomes and predictive value of the baseline PAM50-based intrinsic subtype--ACOSOG Z1031. *J Clin Oncol.* 2011; 29:2342–2349. Neoadjuvant letrozole, anastrozole, and exemestane had surgical outcomes, PEPI score, and Ki67 suppression and therefore are likely clinically equivalent. Post-treatment increase in Ki67 may identify treatment resistant cells. [PubMed: 21555689]
- **7. Allevi G, Strina C, Andreis D, et al. Increased pathological complete response rate after a long-term neoadjuvant letrozole treatment in postmenopausal oestrogen and/or progesterone receptor-positive breast cancer. *Br J Cancer.* 2013; 108:1587–1592. Compared to a shorter duration of 4 or 8 months, a longer duration of neoadjuvant letrozole (12 months) resulted in higher clinical response rates and improved rates of pathologic complete response. [PubMed: 23579222]
8. Semiglazov VF, Semiglazov VV, Dashyan GA, et al. Phase 2 randomized trial of primary endocrine therapy versus chemotherapy in postmenopausal patients with estrogen receptor-positive breast cancer. *Cancer.* 2007; 110:244–254. [PubMed: 17538978]
9. Ali S, Coombes RC. Endocrine-responsive breast cancer and strategies for combating resistance. *Nat Rev Cancer.* 2002; 2:101–112. [PubMed: 12635173]
10. Clarke R, Skaar T, Leonessa F, et al. Acquisition of an antiestrogen-resistant phenotype in breast cancer: role of cellular and molecular mechanisms. *Cancer Treat Res.* 1996; 87:263–283. [PubMed: 8886457]
11. Lu Q, Yue W, Wang J, et al. The effects of aromatase inhibitors and antiestrogens in the nude mouse model. *Breast Cancer Res Treat.* 1998; 50:63–71. [PubMed: 9802621]
12. Wiebe VJ, Osborne CK, Fuqua SA, DeGregorio MW. Tamoxifen resistance in breast cancer. *Crit Rev Oncol Hematol.* 1993; 14:173–188. [PubMed: 8397846]
13. Ellis MJ, Coop A, Singh B, et al. Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor- positive primary breast cancer: evidence from a phase III randomized trial. *J Clin Oncol.* 2001; 19:3808–3816. [PubMed: 11559718]
14. Ellis MJ, Coop A, Singh B, et al. Letrozole inhibits tumor proliferation more effectively than tamoxifen independent of HER1/2 expression status. *Cancer Res.* 2003; 63:6523–6531. [PubMed: 14559846]

15. Ellis MJ. Neoadjuvant endocrine therapy for breast cancer: more questions than answers. *J Clin Oncol.* 2005; 23:4842–4844. [PubMed: 15998901]
16. Ellis MJ, Tao Y, Young O, et al. Estrogen-independent proliferation is present in estrogen-receptor HER2-positive primary breast cancer after neoadjuvant letrozole. *J Clin Oncol.* 2006; 24:3019–3025. [PubMed: 16754938]
17. Dowsett M, et al. Greater Ki67 response after 2 weeks neoadjuvant treatment with anastrozole (A) than with tamoxifen (T) or anastrozole plus tamoxifen (C) in the IMPACT trial: a potential predictor of relapse-free survival. *Breast Cancer Res Treat.* 2003; 82(Suppl. 1):S6.
18. Dowsett M, Ebbs SR, Dixon JM, et al. Biomarker changes during neoadjuvant anastrozole, tamoxifen, or the combination: influence of hormonal status and HER-2 in breast cancer—a study from the IMPACT trialists. *J Clin Oncol.* 2005; 23:2477–2492. [PubMed: 15767642]
19. Seo JH, Kim YH, Kim JS. Meta-analysis of pre-operative aromatase inhibitor versus tamoxifen in postmenopausal woman with hormone receptor-positive breast cancer. *Cancer Chemother Pharmacol.* 2009; 63:261–266. [PubMed: 18365197]
20. Dowsett M, Smith IE, Ebbs SR, et al. Prognostic value of Ki67 expression after short-term presurgical endocrine therapy for primary breast cancer. *J Natl Cancer Inst.* 2007; 99:167–170. [PubMed: 17228000]
21. Howell A, Cuzick J, Baum M, et al. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. *Lancet.* 2005; 365:60–62. [PubMed: 15639680]
22. Ellis MJ, Tao Y, Luo J, et al. Outcome prediction for estrogen receptor-positive breast cancer based on postneoadjuvant endocrine therapy tumor characteristics. *J Natl Cancer Inst.* 2008; 100:1380–1388. [PubMed: 18812550]
23. Chia YH, Ellis MJ, Ma CX. Neoadjuvant endocrine therapy in primary breast cancer: indications and use as a research tool. *Br J Cancer.* 2010; 103:759–764. [PubMed: 20700118]
24. van de Vijver MJ, He YD, van't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med.* 2002; 347:1999–2009. [PubMed: 12490681]
25. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med.* 2004; 351:2817–2826. [PubMed: 15591335]
26. Zhao M, Ramaswamy B. Mechanisms and therapeutic advances in the management of endocrine-resistant breast cancer. *World J Clin Oncol.* 2014; 5:248–262. [PubMed: 25114842]
27. Johnston SR. Integration of endocrine therapy with targeted agents. *Breast Cancer Res.* 2008; 10(Suppl 4):S20. [PubMed: 19128434]
28. Musgrove EA, Sutherland RL. Biological determinants of endocrine resistance in breast cancer. *Nat Rev Cancer.* 2009; 9:631–643. [PubMed: 19701242]
29. Arpino G, Wiechmann L, Osborne CK, Schiff R. Crosstalk between the estrogen receptor and the HER tyrosine kinase receptor family: molecular mechanism and clinical implications for endocrine therapy resistance. *Endocr Rev.* 2008; 29:217–233. [PubMed: 18216219]
30. Miller TW, Balko JM, Arteaga CL. Phosphatidylinositol 3-kinase and antiestrogen resistance in breast cancer. *J Clin Oncol.* 2011; 29:4452–4461. [PubMed: 22010023]
31. Fedele P, Calvani N, Marino A, et al. Targeted agents to reverse resistance to endocrine therapy in metastatic breast cancer: where are we now and where are we going? *Crit Rev Oncol Hematol.* 2012; 84:243–251. [PubMed: 22494933]
32. McClelland RA, Barrow D, Madden TA, et al. Enhanced epidermal growth factor receptor signaling in MCF7 breast cancer cells after long-term culture in the presence of the pure antiestrogen ICI 182,780 (Faslodex). *Endocrinology.* 2001; 142:2776–2788. [PubMed: 11415996]
33. Robertson JFR, Gutteridge E, Cheung KL, et al. Gefitinib (ZD1839) is active in acquired tamoxifen-resistant oestrogen receptor positive and ER-negative breast cancer: results from a phase II study. *Proc. Am. Soc. Clin. Oncol.* 2003; 22:A23.
34. Nicholson RI, Hucheson IR, Harper ME, et al. Modulation of epidermal growth factor receptor in endocrine-resistant, oestrogen receptor-positive breast cancer. *Endocr Relat Cancer.* 2001; 8:175–182. [PubMed: 11566608]

35. Yue W, Wang JP, Conaway MR, et al. Adaptive hypersensitivity following long-term estrogen deprivation: involvement of multiple signal pathways. *J Steroid Biochem Mol Biol.* 2003; 86:265–274. [PubMed: 14623520]
36. Lavinsky RM, Jepsen K, Heinzel T, et al. Diverse signaling pathways modulate nuclear receptor recruitment of N-CoR and SMRT complexes. *Proc Natl Acad Sci U S A.* 1998; 95:2920–2925. [PubMed: 9501191]
37. Shou J, Massarweh S, Osborne CK, et al. Mechanisms of tamoxifen resistance: increased estrogen receptor-HER2/neu cross-talk in ER/HER2-positive breast cancer. *J Natl Cancer Inst.* 2004; 96:926–935. [PubMed: 15199112]
38. Shin I, Miller T, Arteaga CL. ErbB receptor signaling and therapeutic resistance to aromatase inhibitors. *Clin Cancer Res.* 2006; 12:1008s–1012s. [PubMed: 16467117]
39. Kunisue H, Kurebayashi J, Otsuki T, et al. Anti-HER2 antibody enhances the growth inhibitory effect of anti-oestrogen on breast cancer cells expressing both oestrogen receptors and HER2. *Br J Cancer.* 2000; 82:46–51. [PubMed: 10638965]
40. Kurokawa H, Lenferink AE, Simpson JF, et al. Inhibition of HER2/neu (erbB-2) and mitogen-activated protein kinases enhances tamoxifen action against HER2-overexpressing, tamoxifen-resistant breast cancer cells. *Cancer Res.* 2000; 60:5887–5894. [PubMed: 11059787]
41. Jelovac D, Sabnis G, Long BJ, et al. Activation of mitogen-activated protein kinase in xenografts and cells during prolonged treatment with aromatase inhibitor letrozole. *Cancer Res.* 2005; 65:5380–5389. [PubMed: 15958587]
42. Sabnis GJ, Jelovac D, Long B, Brodie A. The role of growth factor receptor pathways in human breast cancer cells adapted to long-term estrogen deprivation. *Cancer Res.* 2005; 65:3903–3910. [PubMed: 15867390]
43. Ropero S, Menendez JA, Vazquez-Martin A, et al. Trastuzumab plus tamoxifen: anti-proliferative and molecular interactions in breast carcinoma. *Breast Cancer Res Treat.* 2004; 86:125–137. [PubMed: 15319565]
44. Slamon DJ, Godolphin W, Jones LA, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science.* 1989; 244:707–712. [PubMed: 2470152]
45. Kurokawa H, Arteaga CL. Inhibition of erbB receptor (HER) tyrosine kinases as a strategy to abrogate antiestrogen resistance in human breast cancer. *Clin Cancer Res.* 2001; 7:4436s–4442s. discussion 4411s–4412s. [PubMed: 11916237]
46. Polychronis A, Sinnott HD, Hadjiminas D, et al. Preoperative gefitinib versus gefitinib and anastrozole in postmenopausal patients with oestrogen-receptor positive and epidermal-growth-factor-receptor-positive primary breast cancer: a double-blind placebo-controlled phase II randomised trial. *Lancet Oncol.* 2005; 6:383–391. [PubMed: 15925816]
47. Smith IE, Walsh G, Skene A, et al. A phase II placebo-controlled trial of neoadjuvant anastrozole alone or with gefitinib in early breast cancer. *J Clin Oncol.* 2007; 25:3816–3822. [PubMed: 17679728]
48. Saal LH, Holm K, Maurer M, et al. PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. *Cancer Res.* 2005; 65:2554–2559. [PubMed: 15805248]
49. Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, et al. An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. *Cancer Res.* 2008; 68:6084–6091. [PubMed: 18676830]
50. Campbell RA, Bhat-Nakshatri P, Patel NM, et al. Phosphatidylinositol 3-kinase/AKT-mediated activation of estrogen receptor alpha: a new model for anti-estrogen resistance. *J Biol Chem.* 2001; 276:9817–9824. [PubMed: 11139588]
51. Yamnik RL, Digilova A, Davis DC, et al. S6 kinase 1 regulates estrogen receptor alpha in control of breast cancer cell proliferation. *J Biol Chem.* 2009; 284:6361–6369. [PubMed: 19112174]
52. Crowder RJ, Phommaly C, Tao Y, et al. PIK3CA and PIK3CB inhibition produce synthetic lethality when combined with estrogen deprivation in estrogen receptor-positive breast cancer. *Cancer Res.* 2009; 69:3955–3962. [PubMed: 19366795]

53. Creighton CJ, Fu X, Hennessy BT, et al. Proteomic and transcriptomic profiling reveals a link between the PI3K pathway and lower estrogen receptor (ER) levels and activity in ER+ breast cancer. *Breast Cancer Res.* 12:R40. [PubMed: 20569503]
54. Miller TW, Hennessy BT, Gonzalez-Angulo AM, et al. Hyperactivation of phosphatidylinositol-3 kinase promotes escape from hormone dependence in estrogen receptor-positive human breast cancer. *J Clin Invest.* 120:2406–2413.
- ***55. Piccart M, Hortobagyi GN, Campone M, et al. Everolimus plus exemestane for hormone-receptor-positive, human epidermal growth factor receptor-2-negative advanced breast cancer: overall survival results from BOLERO-2 dagger. *Ann Oncol.* 2014; 25:2357–2362. The addition of everolimus to exemestane in hormone receptor positive, HER2-negative advanced breast cancer improved progression free survival by 4.6 months, but did not result in a statistically significant improvement in overall survival. [PubMed: 25231953]
56. Mayer IA, Abramson VG, Isakoff SJ, et al. Stand up to cancer phase Ib study of pan-phosphoinositide-3-kinase inhibitor buparlisib with letrozole in estrogen receptor-positive/human epidermal growth factor receptor 2-negative metastatic breast cancer. *J Clin Oncol.* 2014; 32:1202–1209. [PubMed: 24663045]
57. Baselga J, Semiglazov V, van Dam P, et al. Phase II randomized study of neoadjuvant everolimus plus letrozole compared with placebo plus letrozole in patients with estrogen receptor-positive breast cancer. *J Clin Oncol.* 2009; 27:2630–2637. [PubMed: 19380449]
58. Sabnis G, Goloubeva O, Jelovac D, et al. Inhibition of the phosphatidylinositol 3-kinase/Akt pathway improves response of long-term estrogen-deprived breast cancer xenografts to antiestrogens. *Clin Cancer Res.* 2007; 13:2751–2757. [PubMed: 17473209]
59. Miller TW, Hennessy BT, Gonzalez-Angulo AM, et al. Hyperactivation of phosphatidylinositol-3 kinase promotes escape from hormone dependence in estrogen receptor-positive human breast cancer. *J Clin Invest.* 2010; 120:2406–2413. [PubMed: 20530877]
60. Fox EM, Arteaga CL, Miller TW. Abrogating endocrine resistance by targeting ERalpha and PI3K in breast cancer. *Front Oncol.* 2012; 2:145. [PubMed: 23087906]

Table 1

Phase II neoadjuvant studies of endocrine therapy combined with 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