A Review of Novel Approaches to Enhance Efficacy of Medical Treatment of Hormone Receptor Positive, Her2 Negative Breast Cancer

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Abstract

The purpose of this project is to review approaches to improve the medical treatment of breast cancer. This review will focus on the current literature that addresses (1) the current strategy for the sequence of adjuvant chemohormonal therapy in non-metastatic hormone receptor positive (HR+), human epidermal growth factor 2 (HER2) negative breast cancer, (2) progesterone therapy in the neoadjuvant setting, (3) high dose estrogens as salvage therapy in hormone therapy-refractory patients, (4) the concurrent use of mTOR or CDK4/6 inhibitors with hormone therapy, and (5) the use of efflux pump reversal agents in chemotherapy-refractory patients. Based on the presented evidence, discussions will focus on limitations of the current data and the role of novel therapies within the framework of current standard of care.

Introduction

In the United States, there were approximately 232,000 new cases of invasive female breast cancer diagnosed in 2015 with approximately 40,000 deaths due to the disease. Based on these statistics, roughly 1 in 8 women in the United States will develop breast cancer in her lifetime. Fortunately, increasingly specific and effective treatment regimens have brought about a 36% decline in mortality due to breast cancer from 1989 to 2012. [1] Current approaches to treatment are classically based on the TNM (tumor size, nodes, metastasis) staging system in conjunction with biomarkers such as estrogen (ER), progesterone receptors (PR), and HER2. In addition, other markers such as Ki-67 and tumor gene expression profiles help to quantify the risk of relapse and prognosis and contribute to the development of specific treatment and follow up plans. [2]

A large percentage of breast tumors are positive for ER. For this population of breast tumors, estrogen stimulates tumor growth. Therefore over the past 40 years, targeting estrogen has been a standard adjuvant treatment for ER-positive (ER+) breast tumors. In premenopausal women, tamoxifen (a selective estrogen receptor modulator or SERM), and in postmenopausal women, aromatase inhibitors (AI), are the major therapeutic options for these tumors in the adjuvant setting. [3] The mechanism of action of anti-hormonal therapies is primarily based on inhibiting the interaction of estrogen with ER – AIs act by suppressing the peripheral conversion of androstenedione into estrogen while SERMs primarily act by saturating nuclear ERs and blocking their interaction with estrogen. In addition, SERMs also promote growth arrest via cytostatic mechanisms and promote apoptosis. [4, 5] In addition to endocrine therapy, chemotherapy is also an established treatment regimen for some subsets of breast cancer. Ten and fifteen year survival studies show that chemotherapy alone and in combination with hormonal therapy causes a significant reduction in the risk of mortality due to breast cancer. [6] However since chemotherapeutic drugs are more effective in rapidly dividing cells and SERMs promote growth arrest, it is conceivable that chemotherapy and tamoxifen may potentially be antagonistic if given concurrently. [7, 8]

Given the significance of the hormonal environment on HR+ breast cancer, even small hormonal changes can cause fluctuations in tumor cell viability. For instance, changes in estrogen concentrations can cause markedly differing effects. Physiologic concentrations are conducive to tumor growth while higher doses, ironically, can lead to tumor cell apoptosis and regression. [9] In addition, the hormonal environment as dictated by the menstrual cycle phase in the perioperative period has been theorized to play a role in tumor metastatic potential. [10] Despite improving therapy, initial and acquired resistance to both chemotherapy (multi-drug resistance or MDR) and hormonal manipulations remains a problem in patients with advanced disease. The MDR phenotype is multifactorial in etiology but classically is

associated with the overexpression of efflux pumps, notably the ATP-binding cassette (ABC) transporters. [11] On the other hand, the mechanism of resistance for endocrine therapy is less well studied though it is likely that cross-talk between the steroid hormone-directed pathways and growth factor-directed pathways may play a significant role in this process, presenting potentially novel upstream and downstream targets of inhibition. [12]

Sequential or concurrent administration of adjuvant chemohormonal therapy in node-positive breast cancer

Efforts to elucidate the most effective sequence of administration of chemohormonal therapy, whether given sequentially or concurrently, began in the 1970s. However, most studies at the time did not properly address the issue due to questions surrounding the benefits of hormone deprivation therapy itself. Instead, most studies compared any combinatorial sequence of chemohormonal therapy versus either chemotherapy or hormonal therapy alone followed by crossover at time of progression, often showing no difference in objective response rates (ORR) and overall survival (OS). In addition, HR-status was unknown in a vast majority of patients and even those patients who were HR-negative were routinely included in trials. [8] For instance, a study conducted by the Australian and New Zealand Breast Cancer Trials Group failed to show any significant differences in both ORR and OS in 339 postmenopausal women with advanced breast cancer. [13] ER-status was known for only 25% of the patients involved in the trial. Regardless of treatment group, patients randomized to any of the three treatment cohorts of concurrent tamoxifen and doxorubicin/cyclophosphamide (AC), initial AC followed by crossover to tamoxifen, or initial tamoxifen followed by crossover to AC, had an average ORR of approximately 45%.

Studies in the 1980s continued to examine the timing of administration of chemohormonal therapy. An Italian study led by Sertoli et al randomized 431 pre- and postmenopausal women with node-positive breast cancer into groups receiving tamoxifen concurrently or sequentially following adjuvant chemotherapy. HR-status was known in only a subset of enrolled patients – positive in 53%, negative in 23%, and unknown in 24%. Although initial analyses suggested that concurrent administration of chemotherapy and tamoxifen was superior to sequential administration [14-16], long-term analyses revealed that the 10-year OS was nearly equivalent for both arms of the study (concurrent – 66%, sequential – 65%; $p = 0.86$). [17] In 1989, Albain et al, as part of the Southwest Oncology Group (SWOG-8814) and North American Intergroup-0100 (INT-0100), conducted a phase III trial to both further elucidate the effects of adding chemotherapy to adjuvant tamoxifen and to find the optimal sequence of adjuvant chemohormonal therapy. A group of 1,558 postmenopausal women with HR+, node-positive breast cancer were randomized into 3 treatment groups: (1) tamoxifen alone, (2) cyclophosphamide, doxorubicin, and fluorouracil (CAF) followed by tamoxifen (CAF-T), or (3) CAF and tamoxifen concurrently (CAFT). In contrast to the earlier Italian study, this study had a much larger sample size and only included HR+ patients. Interim analyses presented in 2002 showed a statistically significant benefit in disease-free survival (DFS) of the sequential arm of the study as compared to the concurrent arm. [18] These findings led to a change in clinical practice following the St Gallen consensus recommendation that endocrine therapy-responsive intermediate and high-risk breast cancer patients should receive hormonal treatment in a sequential manner following chemotherapy. [19] However as with the previous study, long-term follow-up did not completely confirm the findings. Although the adjusted hazard ratios for DFS (hazard ratio 0.84, 95% CI = 0.70 – 1.01; $p = 0.061$) and OS (hazard ratio 0.90, 95% CI = 0.73 – 1.10; $p = 0.30$) favored the sequential over the concurrent regimen, the findings did not reach statistical significance. [20] Another prospective study also attempted to unsuccessfully clarify the issue. The study, conducted only in postmenopausal women by the Spanish Breast Cancer Research Group (GEICAM), randomized 474 node-positive patients to receive either epirubicin and cyclophosphamide (EC) concurrently or sequentially with tamoxifen. Patients were eligible for the study regardless of HR-status (17% ER-negative, 13% unknown). Similar to the previous studies, the trial did not find a statistically significant difference in DFS between the treatment groups. [21]

Although the available evidence has pointed to the superiority of sequential chemohormonal therapy in the adjuvant setting, results have been mixed. In addition, both the SWOG-8814 and the GEICAM trial were conducted only in postmenopausal patients, leaving the possibility that the optimal

timing may differ in premenopausal women. Thus, a retrospective study examined the timing of therapy in both pre- and postmenopausal women. Patient data was selected from two previously randomized clinical trials and were chosen based on HR+ and tamoxifen administration with chemotherapy (anthracycline-based). For the entire patient group, there was no statistically significant difference in 10-year OS for either treatment regimen (concurrent – 83%, sequential – 80%). [22] Interestingly, subgroup analyses combining the two treatment arms showed a statistically significant increasing hazard of death with younger age. The 40 years of age or younger group was found to have a 2.12-fold increased risk of death as compared to older patients whose risk decreased with age. Given the increased hazard of death in the younger patient subset, the potential utility of administering chemohormonal therapy concurrently in younger patients to avoid any treatment delays could be studied.

Neoadjuvant progesterone in pre- and postmenopausal women with operable breast cancer (OBC)

In patients with OBC, the hormonal environment during resection of the primary tumor has occasionally been implicated in long-term prognosis. The initial study to demonstrate this potential effect was a retrospective analysis of 44 patients in 1989 by Hrushesky et al. Premenopausal women who had their primary tumors removed during the perimenstrual period (0-6 and 21-30 days following last menstrual period or LMP) were found to have a 4 to 5-fold increased risk of relapse as compared to women who had their tumors removed mid-cycle, lending credence to the theory that unopposed estrogen during the perioperative period may be beneficial to survival. [23] However two years later, Badwe et al performed a similar retrospective study, albeit with a larger patient cohort (249 patients). Interestingly, ten year OS significantly favored the patients who had their tumors resected during the perimenstrual period (0-2 or 13-32 days after LMP) with a 10-year statistically significant OS difference of 30% between the groups. [24] This result contradicted the results of the earlier study in that unopposed estrogen in the perioperative period was now correlated with worsening survival. However, the cutoff periods of the menstrual cycle varied between studies and there were some obvious shortcomings of determining the hormonal environment based on LMP. Nevertheless, subsequent studies by other groups that were performed with measurements of plasma hormone levels prior to surgical resection did not show a difference in DFS or OS. [25, 26]

However in 2011, Badwe et al completed a prospective study of 976 patients with OBC who were randomized to receive an injection of depot progesterone prior to operation. Patients were not excluded based on menopausal or HR-status. For the study group as a whole, there was no difference in 5-year DFS and OS between the groups but in a subset analysis of 471 node-positive patients, there was a statistically significant increase in 5-year DFS and OS in favor of the progesterone administration group. The increased mortality seen in node-positive patients did not differ based on HR or menopausal status, suggesting an indirect rather than direct effect of progesterone on tumor metastatic potential. [27] *In vivo* rat models have also revealed increased tumor metastatic potential during times of elevated estradiol and low progesterone. [28]

Mechanistically, estrogen may indirectly influence the cytotoxic effect of NK cells by increasing their susceptibility to the adrenergic response in the surgical setting, thereby decreasing their effective activity and increasing tumor metastatic potential. [10] However, data has been limited and further studies are needed to address this hypothesis.

High-dose estrogens (HDE) as salvage therapy following multiple endocrine regimens in postmenopausal women with advanced breast cancer

In postmenopausal women with HR+ advanced breast cancer, both tamoxifen and HDE have been utilized successfully in the past as initial hormonal therapy without a significant difference in ORR or OS. [29] However given the superior side effect profile of tamoxifen, estrogen deprivation therapy became the preferred modality of treatment. The mechanism by which HDE may suppress tumor growth is unknown. *In vitro* models with LTED (long term estradiol-deprived) cells, which were derived by growing MCF-7 breast tumor cells for 6 month to 2 years in an estrogen-depleted environment, found that

treatment of LTED cells with estradiol at 0.1 nM resulted in a 60% statistically significant reduction in growth and a 7-fold increase in apoptosis, likely via the Fas pathway, as compared to MCF-7 cells, which were stimulated by the equivalent concentration of estradiol. [30]

In 2001, Lonning et al studied the effects of HDE in the treatment of postmenopausal patients with advanced breast cancer who had been heavily pre-treated with chemoendocrine therapy and had continued to progress despite hormone deprivation. The prospective Norwegian trial showed an ORR of 31% with a median duration of response of 50 weeks. Although it involved a small cohort of 32 patients, it showed that HDE may have an antitumor effect in patients who had previously been exposed to multiple endocrine treatment regimens. [31] These results were re-iterated by two retrospective studies of small cohorts of patients who had been refractory to multiple lines of chemoendocrine therapy at the time as well. [32, 33] The ORR of the patients for both studies was 25%, which was similar to that detailed in the original study by Lonning et al. A recent prospective trial of 18 patients came to similar conclusions albeit with a higher ORR of 50%. [34] Although the patient cohorts were small, these trials highlight the potential of including HDE therapy in sequence with estrogen deprivation therapy in the future.

Targeted therapy concurrently with endocrine therapy in patients with advanced breast cancer

mTOR Inhibitors

Although hormonal therapy has shown mortality benefit, many patients eventually develop resistance to hormone therapy, either via acquired or *de novo* processes. One mechanism that has been hypothesized to contribute to the development of resistance is a circumvention of the steroid hormone pathway via growth factor-mediated pathways, allowing tumor growth despite adequate steroid hormone ligand blockade. This model of resistance involves cross-talk of the steroid hormone pathway and the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway. [35-38] *In vitro*, MCF-7 breast cancer cells grown in LTED medium have shown hyperactivation of the mTOR pathway. In this setting, upstream, downstream, and direct inhibition of PI3K signaling has been shown to inhibit the LTED tumor cell line. [39] In addition, AKT1 cells, which are MCF-7 cells with constitutively active AKT, have displayed growth inhibition when treated concurrently with dual inhibition of mTOR and steroid hormone pathways and a restoration of sensitivity to hormone deprivation therapy. [40, 41] Thus, agents that inhibit growth factor signaling pathways may be clinically beneficial in patients with breast cancer refractory to hormonal therapy.

The BOLERO-2 trial sought to apply this data to women with advanced breast cancer who had a recurrence or progression on previous hormonal therapy or other therapy to treat advanced disease. The trial enrolled a total of 724 postmenopausal women with HR+, HER2-negative disease. Patients were randomized to either a concurrent combination of everolimus (mTOR inhibitor) and exemestane (AI) or placebo and exemestane. On interim analysis, the median progression-free survival (PFS) of the combination of everolimus and exemestane was 6.9 months, which was statistically significantly improved as compared to 2.8 months for the placebo and exemestane group (HR 0.43; 95% CI 0.35-0.54; $p < 0.001$). [42] These results were confirmed on final analysis. [43] In agreement with the results of the BOLERO-2 trial were those of the TAMRAD trial (Tamoxifen Plus Everolimus), a European phase II trial that compared the effect of concurrent everolimus and tamoxifen versus tamoxifen alone in postmenopausal women. Patients were HR+, HER2-negative and resistant to aromatase inhibitors (AIs). The clinical benefit rate (CBR) was 61% in the combination group versus 42% in the tamoxifen alone group with an increased time to progression (TTP) of 8.6 months for the combination group versus 4.5 months for the tamoxifen alone group, both of which were statistically significant. [44] Given the benefit in this subset of patients, everolimus is currently approved for concurrent combinatorial use with exemestane in HR+, HER2-negative postmenopausal women with advanced disease who are recurring or progressing following the prior use of non-steroidal AIs.

Cyclin D Kinase Inhibitors

In addition to mTOR, cyclin D kinases (CDK) lie further downstream of the PI3K/AKT/mTOR pathway and serve as the convergence point of different mitogenic signaling pathways, including steroid hormone and WNT B-catenin pathways, making them potentially amenable to inhibition. CDK 4 and 6 are known to promote cell division by phosphorylating the retinoblastoma protein (Rb), a potent tumor suppressor, and inactivating it. [45, 46] Since Rb, unlike p53, is not frequently mutated in most malignancies, its normal function of suppressing cell division is potentially salvageable through upstream inhibition of its inactivation pathway.

The PALOMA-1/TRIO-18 trial was a phase II trial completed in 2014 that compared the effects of the CDK 4/6 inhibitor, palbociclib, in concurrent combination with letrozole (AI) versus letrozole alone in postmenopausal women with ER+, HER2-negative advanced breast cancer. Patients were required to have received no prior systemic treatment for their advanced disease. At interim follow-up, the median PFS was significantly improved for the concurrent combination group of palbociclib and letrozole as compared to letrozole alone – 20.2 months versus 10.2 months, respectively (hazard ratio 0.488, 95% CI 0.319-0.748; p = 0.0004). [47] Although patients were initially enrolled into 2 separate cohorts – the first for patients with baseline ER+, HER2-negative disease and the second for those with additional cyclin D1 (CCND1) amplification, loss of p16, or both – an interim analysis showed meaningful combination drug effectiveness in cohort 1 and as a result, enrollment in cohort 2 was stopped and the results of both cohorts were combined. Following the completion of the PALOMA-1 trial, interim results for another similar trial, the PALOMA-3 phase III trial, were reported. This study compared the effects of a concurrent combination of palbociclib and fulvestrant, a selective estrogen receptor degrader (SERD), versus placebo and fulvestrant in both pre- and postmenopausal women with ER+, HER2-negative advanced breast cancer who were refractory to prior endocrine therapy. Although women were not excluded based on their menopausal status, both premenopausal and perimenopausal patients received goserelin for the duration of the study treatment. At the time of interim analysis at a median observation period of 5.6 months, there was a significantly increased median PFS for the palbociclib and fulvestrant combination group as compared to the placebo and fulvestrant group – 9.2 months versus 3.8 months, respectively (hazard ratio 0.42; 95% CI, 0.32 to 0.56, p < 0.001). [48] Given these results, palbociclib has been approved for use in women with HR+, HER2-negative advanced breast cancer both in combination with letrozole as initial therapy and in combination with fulvestrant for those with disease progression following prior hormonal therapy.

Sequential use of efflux pump reversal agents with chemotherapy in MDR patients with advanced or recurrent breast cancer

P-glycoprotein (Pgp), a membrane protein encoded by the MDR1 (ABCB1) gene, is a member of the ABC superfamily of transport proteins. This family of transport proteins also includes lesser-studied efflux pumps such as multidrug resistance-associated proteins (MRP or ABCC) and breast cancer resistance protein (BCRP, ABCG2, MXR, or ABCP). MDR1 has been implicated in the development of the MDR phenotype. Although it is likely that other mechanisms of resistance also contribute to this phenomenon, transport proteins are known to play an important role in the development of MDR. [11, 49] Many studies in the 1980s and 1990s explored the correlation of MDR1 expression with response to chemotherapy but the large amount of interstudy variability limited conclusions. However, a meta-analysis of studies from 1989 to 1996 found that tumors with MDR1 expression prior to or following chemotherapy were 3.21 times more likely to have a less than partial response (PR) than in tumors without MDR1 expression. The relative risk increased to 3.87 and 4.19 when studies were limited to those only measuring MDR1 expression following chemotherapy with MDR1 substrates and all cytotoxic agents, respectively. [50] In support of the notion that MDR1/Pgp expression following anthracycline-based chemotherapy had prognostic significance, pooled results in 6 studies with anthracycline-containing chemotherapy indicated an ORR of 93% (56/60 patients) in those with Pgp-negative tumors and 52% (45/82 patients) in those with Pgp-positive tumors. [51] In addition, there may also be some prognostic value of MDR1 hyper-expression prior to treatment. A retrospective study correlated MDR1 RNA hyper-expression prior to treatment with decreased chemotherapeutic efficacy and predicted a poor prognosis.

Notably, in the subset of patients with MDR1-high tumors, 0 out of 6 patients responded to anthracycline-based chemotherapy (FAC/FEC) as compared to 19 out of 25 patients with MDR1-low tumors ($p<0.001$). [52]

As compared to MDR1, studies regarding MRP1 and its prognostic significance have been fewer and more ambiguous. MRP1 RNA hyper-expression prior to anthracycline-based chemotherapy has been shown to be predictive of decreased PFS, but the subgroup was limited by a small sample size of only 17 patients. [52] Additional retrospective studies have associated increased MRP1 protein expression prior to CMF-based chemotherapy with a poorer relapse-free survival (RFS) and OS [53, 54] while another study showed no difference. [55]

Given the potential prognostic implications, the search for an agent that could potentially reverse the MDR1 efflux pump ensued. Verapamil, a calcium channel blocker, has been studied most extensively as a potential MDR1 reversing agent in breast cancer but results were mixed. In 2003, Leonessa and Clarke pooled the results of four different cross-over trials conducted between 1995 and 1998 in which verapamil was added concurrently to anthracycline-based chemotherapy in chemotherapy-refractory patients with advanced breast cancer. Verapamil was found to re-sensitize a small but consistent percentage (13 of 84 or 15%) of patients to anthracycline-based chemotherapy. [51] Prospective randomized trials have also attempted to elucidate the role of verapamil as a MDR1 inhibitor. One study found that in anthracycline-resistant patients with MBC, oral verapamil added to a partially noncross-resistant chemotherapy regimen of VF (vindesine and 5-fluorouracil) increased OS (median OS – 323 versus 209 days, $p = 0.036$). [56] On the other hand, another study found no difference in ORR and OS in a trial of 51 patients with MBC randomized to receive either epirubicin with or without oral verapamil 480 mg per day during each cycle. [57] However in this trial, verapamil was given with first-line chemotherapy instead of only in treatment-refractory patients. Unfortunately, verapamil and other first (such as cyclosporine A, quinidine, and quinine) and second generation (such as PSC-833 and VX-710) Pgp inhibitors have been found to cause unacceptable side effects at the required doses for MDR1 inhibition or to affect the pharmacokinetics of chemotherapeutic agents and CYP3A enzyme activity, causing some chemotherapeutic toxicity exacerbations as well. [58-61] Third generation inhibitors (such as tariquidar) are currently in development and have higher affinity for Pgp while not interfering with chemotherapeutic pharmacokinetics. [62, 63] Although many of these third generation inhibitors have been found to be ineffective, some agents, such as dofequidar fumarate, may have some efficacy. In a phase III trial, dofequidar fumarate was combined with CAF therapy in patients with either advanced or recurrent breast cancer. Though not statistically significant, the ORR was increased for the dofequidar and CAF group as compared to the placebo and CAF group (53.1% versus 42.6%, respectively, $p = 0.077$). [64] The pharmacokinetics of doxorubicin was not found to be significantly different between the groups up to 6 hours post-administration but notably, there were statistically significant increased cases of neutropenia ($p = 0.006$) and leukopenia ($p = 0.005$) reported in the experimental arm.

Hormone therapy with tamoxifen has also been implicated in the potential reversal of the MDR1 efflux pump. One *in vitro* study found that in both a leukemia and a lymphoma cell line expressing the MDR phenotype, treatment with a combination of tamoxifen and daunorubicin resulted in increased intracellular concentrations of daunorubicin and marked cell growth inhibition as compared to tamoxifen or daunorubicin alone. [65] A similar effect was also found to be present in breast cancer cell lines expressing the MDR1 efflux pump (CL10.3 and MCF-7^{ADR}). In this study, tamoxifen administered in combination with doxorubicin or vinblastine was found to increase the cytotoxicity of the chemotherapeutic agents, increase the intracellular accumulation of vinblastine, and inhibit the efflux of azidopine, a Pgp substrate. [66] Notably, these effects were present in both the CL10.3 and MCF-7^{ADR} cell lines, the first of which expresses ER while the latter does not, leading to the conclusion that ER may not be necessary for tamoxifen's role in MDR1 inhibition. Unfortunately, studies *in vivo* have not found tamoxifen to play a significant role in MDR reversal in solid tumors. A phase I trial performed in combination with vinblastine in 53 patients with a variety of advanced refractory malignancies found that it was possible to achieve steady state concentrations of tamoxifen in the MDR1-inhibiting range (4 to 6 uM) without dose-limiting toxic effects of tamoxifen, which were mostly cerebellar at higher concentrations. However of the 53 patients, only 3 partial responses were seen (1 of 10 in renal cell carcinoma; 2 of 11 in breast cancer). [67] Two other studies with tamoxifen, one completed in patients with refractory advanced

colorectal cancer and the other in refractory metastatic renal cell carcinoma, were unsuccessful in re-sensitizing tumors to chemotherapy. [68, 69]

Given the relative failures of MDR1-inhibiting agents to modulate chemoresistance in refractory breast and other solid tumors, interest in developing novel agents has diminished. However, small trial successes of MDR1-inhibition in some blood and solid malignancies have continued the hope of development of more effective agents. [70-72]

Discussion

The heterogeneity of breast cancer has posed a complex challenge to achieving successful treatment and remission in all patient subgroups. In terms of treatment and mortality, the importance of the discovery of HR-status and HER2 over-expression cannot be understated. Even so, breast cancer mortality remains high and the exploration of novel biomarkers and treatment mechanisms is essential.

Given the discovery of these tumor subgroups, the results of studies that were subgroup agnostic but dictate current treatment modalities should be re-examined. For instance in the adjuvant setting for HR+ and node-positive breast cancer, hormonal therapy is given sequentially following chemotherapy. This treatment protocol is driven largely by the results of the large scale SWOG-8814/INT-0100, which examined a study cohort comprised of postmenopausal women receiving either tamoxifen concurrently or sequentially following chemotherapy. Although at the time HER2 status was not routinely assessed, we now understand that the lack of adjustment for HER2-positivity could critically sway the results of a study in either direction. In 2010, an analysis of a small subset of 367 tumor specimens from this study found that 11.7% (43 of 367) were HER2-positive. [73] In addition, the study cohort was comprised solely of postmenopausal women and was conducted at a time when tamoxifen was considered the standard of care for postmenopausal women with HR+ breast cancer. As AIs are now the preferred therapy in this patient population and the mechanism of action of AIs is significantly different from that of SERMs, the results from the SWOG-8814/INT-0100 should not be extrapolated to encompass current therapy with AIs. A new Italian study (GIM10-CONSENT) is currently recruiting postmenopausal patients to further investigate this question. [74]

The benefits of endocrine therapy are not limited to hormone deprivation therapy as there may be some benefit with hormone additive therapy as well. According to the study by Badwe et al in 2011, depot progesterone injection in the neoadjuvant perioperative setting may potentially minimize tumor metastatic potential in node-positive patients by decreasing the susceptibility of NK cells to the adrenergic response. Since this finding has not been confirmed by other studies, a larger clinical trial led by Badwe et al is currently ongoing. [75] This result potentially lends credence to the idea that menstrual cycle phase during the perioperative period could play a role in tumor metastatic potential as well but studies correlating menstrual cycle phase with outcome data have varied with some important methodological differences and shortcomings. The obvious difference between the two initial studies mentioned (Hrushesky 1989 and Badwe 1991) was a difference in cutoff period of the follicular and luteal phases of the menstrual cycle. Hrushesky et al proposed that the ideal time of the follicular phase (elevated unopposed levels of estrogen) was 7-20 days following LMP while Badwe et al analyzed their results based on this same period being 3-12 days following LMP. When Badwe et al re-analyzed their data based on the parameters of the original Hrushesky study, the difference in 10-year OS decreased from 30% to 9% but still favored the perimenstrual group. A shortcoming of both initial studies was the lack of reliability in determining the hormonal environment based on LMP versus the more accurate method of measuring via plasma hormone measurements. [76] However, studies utilizing plasma hormone measurements to determine menstrual cycle phase have been inconclusive, due at least in part to problems with classifying patients as being in the follicular versus luteal phase. In one study, up to 30% of the patients were unable to be classified in either group and were placed in a separate group altogether. [26] Given these difficulties, it may be more beneficial to administer progesterone in the perioperative setting rather than delaying surgery until certain plasma hormone thresholds have been achieved. [10]

HDE has also been used in the past as initial therapy for patients with advanced breast cancer but was replaced by hormone deprivation therapy due to equivalent survival and a better side effect

profile. Interestingly, some evidence has even pointed to the possible superiority of HDE as compared to tamoxifen for initial therapy rather than only in the long-term estrogen-deprived setting. An updated analysis of one study showed that 5-year OS was statistically significantly improved for women who were initially started on diethylstilbestrol (DES) versus tamoxifen (35% and 16%, respectively; p = 0.039). [77] Unfortunately regardless of initial therapy, patients with advanced breast cancer eventually develop resistance towards the end of endocrine sequencing therapy with hormone deprivation agents. In this setting, the results of the studies mentioned show that HDE may have potential utility as sequential salvage therapy in hormone therapy-resistant patients with advanced breast cancer. However further studies are needed and a study with alternating regimens of HDE and estrogen-deprivation therapy in patients is currently ongoing. [78] In addition to HDE, targeted therapy against mTOR and CDK 4/6 have also shown mortality benefit in those patients resistant to endocrine therapy and agents such as everolimus and palbociclib have recently received FDA approval for concurrent use with anti-hormonal agents in certain patient subgroups. Clinical trials for a wide-range of patient subgroups for both everolimus and palbociclib are in progress and phase 3 studies for other CDK4/6 inhibitors, such as ribociclib and abemaciclib, are ongoing as well.

Finally in patients with MBC, MDR1/Pgp inhibition has been one of the most researched and sought after therapeutic targets in breast cancer. Since its discovery, the Pgp efflux pump has been linked to MDR but reversal of this phenotype in breast cancer and other solid tumors has been mediocre. Although the advancement of newer agents has slowed considerably, some third generation inhibitors continue to be developed and have shown the potential to re-sensitize chemotherapy refractory tumors. Most studies to this point have only utilized MDR1 inhibiting agents in treatment refractory patients. As such, future studies should aim to select patient cohorts that may stand to benefit most from Pgp inhibition. Potentially including inhibitors as initial therapy may either delay or even prevent the manifestation of the MDR phenotype.

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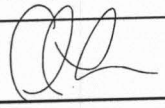
Author Signature:		Date:	3/1/17

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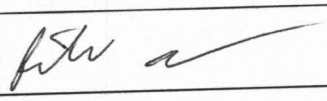
Advisor Signature:		Date:	3/1/17

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