Chemotherapy-Resistant Metastatic Breast Cancer

Carrie Marquette, MD
Lisle Nabell, MD

Address
2540 B 1824 6th Avenue South, Birmingham, AL 35294, USA
Email: Carrie.Marquette@ccc.uab.edu

© Springer Science+Business Media, LLC 2012

Keywords Combinatorial regimens · Microtubulins · Treatment of resistant breast cancer

Opinion statement
Remaining the most common cancer in women through the 21st century, breast cancer and the development of treatment strategies continue to highlight advances made in our understanding of the pathogenesis of cancer development and resistance to therapies. Despite significant progress in the treatment of breast cancer, resistance to chemotherapeutic agents remains a consistent obstacle in terms of treatment success. Anthracyclines, first used over 30 years ago, and the more recent addition of taxanes to the treatment armamentarium are integral components for both newly diagnosed and recurrent breast cancer. Unfortunately, along with other constituents of combination chemotherapy for metastatic breast cancer, these agents ultimately become ineffective in controlling disease. With the emergence of a resistant phenotype, tumors are deemed to be drug resistant - frequently multidrug resistant (MDR). A number of processes have been identified that can underlie clinical drug resistance; observations stemming largely from in vitro laboratory-based studies in human cancer cell lines. Recognized mechanisms of resistance include altered expression of the adenosine triphosphate-binding cassette (ABC) superfamily of transporters, alteration in DNA repair pathways, mutations in cellular targets, resistance to initiation of the apoptotic pathway and the development of constitutively activated signaling pathways. As our understanding of mechanisms of resistance expands, the ability to select specific drugs or drug combinations specific to the phenotype of the cancer will become more specific. Illustrative of these advancements are the reported benefits from the use of newer microtubule-targeting agents in triple negative breast cancer, such as eribulin and ixabepilone; drugs which may be less susceptible to common pathways of drug resistance. Likewise, the combination usage of agents which intersect in receptor crosstalk, such as between the estrogen receptor and the mammalian target of rapamycin (mTOR), have demonstrated synergy in antitumor effects. The recent report of exemestane used in combination with everolimus, have shown great promise in this regard. For patients with HER2 positive disease, a combination approach with trastuzumab and investigational agents such as pertuzumab appear to result in a more complete blockage of HER2 signaling, and improved progression free survival. Thus, as our understanding of the interconnectedness of signaling pathways in breast cancer improves, the ability to rationally design appropriate chemotherapy regimens and delay emerging resistance will improve.
Introduction

The treatment options for metastatic breast cancer have improved steadily as our knowledge of the interconnectedness of signaling pathways and understanding of biological behaviors has improved. However, for a patient with recurrent breast cancer, it remains inevitable that drug resistance will emerge. In addressing the dominant need to control the cancer and yet balance toxicity for the patient, the oncologist must employ strategies, which address the tumor phenotype and are tolerable. To date, despite intense effort, there are no agents clinically available that are capable of overcoming multidrug resistance. For patients with HER2 amplified recurrent disease, the judicious use of an aromatase inhibitor together with trastuzumab or lapatinib in ER (+) disease appears to confer a benefit in terms of progression-free survival. The studies of other agents, not yet approved for use, added to trastuzumab such as pertuzumab or T-DM1 appear to confer a benefit and offer some promise for the future. In the setting of ER (+) disease alone, the combination of an aromatase inhibitor together with the mTOR inhibitor everolimus has been an exciting result, which while it requires confirmation, suggests for the first time some benefit from inhibition of two pathways in this subset of breast cancer. Choosing agents for tumors that do not exhibit hormone positivity, or have lost initial expression is a more challenging problem. New generations of microtubule-targeting agents appear to have a clinical benefit possibly due to a decreased susceptibility to P-gp mediated drug efflux. Both ixabepilone and eribulin have demonstrated activity in resistant breast cancer, and offer a choice for patients with a good performance status who have progressed on an anthracycline and taxane (Table 1).

Mechanisms of resistance

Drug efflux mechanisms

The most well studied mechanism of cellular resistance is the ABC family of proteins, which include P-glycoprotein (P-gp), the multidrug resistance associated protein (MRP1), and breast cancer resistance protein (BCRP); these proteins function to translocate a variety of compounds across cell membranes using energy from adenosine triphosphate hydrolysis [1]. The energy released by hydrolysis results in a conformational change in the configuration of the transmembrane protein and subsequently a decrease in the intracellular retention of drugs [2]. Overexpression of the transport proteins, particularly P-gp, has been linked to clinical drug resistance and has sparked an intense search for inhibitors of the multidrug resistance (MDR) phenotype, given the wide array of drugs affected by this mechanism. One of the first-generation inhibitors recognized was

<table>
<thead>
<tr>
<th>Mechanisms of Drug Resistance</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug efflux mechanisms</td>
<td>ABC transporters: P-gp, MRP1, BCRP</td>
</tr>
<tr>
<td>Microtubule dysfunction</td>
<td>increased expression of β-III tubulin, failure to induce apoptotic signal</td>
</tr>
<tr>
<td>HER2</td>
<td>alternative signaling through EGFR members, activation of PI3K, loss of PTEN</td>
</tr>
<tr>
<td>PI3K pathway</td>
<td>activating mutations, amplification of Akt, loss of PTEN, Kras activation</td>
</tr>
<tr>
<td>DNA repair pathways</td>
<td>loss of heterozygosity, somatic mutations restoring BRCA1 function</td>
</tr>
<tr>
<td>Breast Cancer Stem Cells</td>
<td>MDR, anti-apoptotic molecules</td>
</tr>
<tr>
<td>Apoptotic Pathway</td>
<td>PTEN loss, Bcl-2 expression</td>
</tr>
</tbody>
</table>

P-gp, P-glycoprotein; MRP1, multidrug-resistant protein 1; BCRP, breast cancer resistant protein; EGFR, epidermal growth factor receptor; PI3K, phosphoinositol 3-kinase; PTEN, phosphatase and tensin homologue
Verapamil, a calcium channel blocker found to reverse MDR in vitro models. However, clinical trials of this agent were largely unsuccessful due in large part to significant clinical toxicity. Second-generation agents designed to inhibit MDR include valspodar a derivative of cyclosporine. Clinical trials of this agent however, resulted in significant serum concentrations of cytotoxic drugs with substantial toxicity [3]. Third-generation agents, which do not affect P450 isozymes nor pharmacokinetics of chemotherapeutic agents include LY335979 (Zosuquidar), XR9576 (Tariquidar) and GF120918 (Elacridar). Zosquidar, one of the most potent MDR modulators was recently tested in a clinical trial of older patients affected by acute leukemia but failed to show an improved outcome [4] over chemotherapy alone. Similar results were found in a phase II trial of docetaxel with or without zosquidar in women with metastatic breast cancer [5]. Evaluation of the MDR expression may serve in the future as an additional stratification for identifying high risk cancers, but is likely to be of limited value given the lack of effective inhibitors and the gradual up-regulation of these proteins with ongoing exposure to chemotherapy.

**Microtubule inhibition**

Resistance to microtubule inhibitors can occur from both the presence of the MDR phenotype or alteration in the microtubule molecular target. Composed of α and β tubulin heterodimers along with microtubule associated proteins, microtubules are critical components of the cytoskeleton and cell division. Disruption of microtubule processes through stabilization (taxane family) or destabilization (vinca family) is an important class effect of many agents, resulting in interruption of cellular processes and initiation of apoptosis; inhibition may occur through alteration of beta-tubulin isotypes, overexpression of β-III-tubulin (TUBB3), changes in microtubule-associated proteins and post-translational modifications of tubulin [6]. In particular, increased expression of β-III tubulin has been suggested to be a survival mechanism for tumors through up-regulation of the taxane binding site and diminished effective microtubule stabilization; correlative studies have found increased β-III tubulin expression in tumors exhibiting resistance to tubulin-binding agents [7]. Current clinical trials may help to clarify the prognostic and predictive value of β-III in choosing microtubulin-targeting agents as components of chemotherapy regimens.

Newer generations of microtubule-targeting agents exhibiting activity in taxane resistant cancers include the families of epothilones and halichondrins. Binding to a β-tubulin subunit in a similar fashion to the taxanes, epothilones exert an effect mimicking that of the taxane family with stabilization of microtubules and subsequent initiation of apoptosis. However, members of the epothilone family appear less susceptible to P-gp mediated drug efflux, mutations in tubulin, and mutations in β-III tubulin [8]. Ixabepilone, an epothilone originally isolated from the mycobacterium Sorangium cellulosum, is a member of this class of microtubule inhibitors and is now approved for use in advanced, refractory breast cancer based on objective response rates ranging from 10–50% [9, 10]. Major toxicities of this agent include neutropenia and neuropathy, limiting its application somewhat in taxane resistant patients.
However, demonstrated activity in heavily pretreated, resistant, triple negative cancers has suggested a role for ixabepilone in this particular subgroup [11].

Halichondrins, of which eribulin is a member, are derived from the Japanese sponge Halichondria okadai and are identified as natural products. They bind to β-tubulin at a site close to the vinca site, inhibiting polymerization and separating microtubules into nonfunctional aggregates [12, 13]. This unique mechanism of action appears to underlie the clinical activity seen with the use of this agent, separating it from the vinca, taxane, and epothilone families. Based on its activity in heavily pretreated patients, eribulin is now approved for refractory breast cancer as monotherapy [14].

Other novel taxanes under investigation, which exhibit unique mechanisms of action, include the semisynthetic taxane larotaxel (XRP 9881). While binding in a manner similar to other taxanes, larotaxel aggregates tubulin and importantly appears to have the ability to cross the blood–brain barrier in animal studies. Some authors have suggested that the minimal recognition of larotaxel by P-pg may account for its ability to penetrate the into the brain parenchyma, a feature that could be clinically very important [15]. Further trials are ongoing to explore the full potential of this agent.

Vinflunine is the newest member of the vinca alkaloid family. It differs from other members of this class however in possessing a lower binding affinity for microtubulins but conversely demonstrating antiangiogenic properties, which may impact the development of metastases [16]. In murine models, vinflunine exhibited activity against a broad range of transplanted tumors with activity superior to the well-established vinca alkaloid vinorelbine. Human trials have similarly suggested a high level of activity in bladder and breast cancer and the drug appear to have activity as a single agent in women with metastatic breast cancer which has progressed on anthracyclines and taxanes. Response rates have typically ranged from 12–30% with side effects that mirror those of other members of this family including neutropenia, fatigue and constipation [17, 18]. Combination studies are ongoing to test whether or not the novel mechanism of action will provide additional clinical benefit [19].

**BRCA1/2**

BRCA1/2 deleterious mutations were first identified as members of the breast cancer and ovarian cancer susceptibility genes through linkage analysis in high-risk families in 1994. Located on two different chromosomes, the genes appear to share a number of similarities in terms of maintaining chromosomal stability by repairing DNA strand breaks [20]. DNA repair is carried out through four main mechanisms: (1) base-excision repair, (2) nucleotide excision repair, (3) mismatch repair (MMR), and (4) recombinational repair using homologous (HR) and nonhomologous end joining. When single-strand DNA breaks occur, the complementary strand is used as a building scaffold for base-excision, nucleotide excision, and MMR. Poly-ADP-ribose polymerase-1 (PARP-1) is a critical component of the base excision repair pathway. Double-strand breaks, in contrast, are predominately repaired by HR. Germline tumors deficient in BRCA 1/2
may be initially more sensitive to chemotherapy, due to dysfunction of HR and an inability to accurately repair DNA double strand breaks. The concept of inhibiting BRCA1/2 associated tumors derives from the theory that tumors deficient in homologous recombination will rely on PARP1 mechanisms to repair DNA. Inhibition of PARP-1 converts single-stranded breaks to double strand breaks, and ultimately results in lethal chromosomal mutations and cell death [21]. Thus, BRCA1/2 tumors represent an ideal candidate for PARP inhibition. This idea has been extrapolated to triple negative phenotype tumors, given their shared molecular profile especially with BRCA1 cancers [22].

The first human study of mono-therapy with a PARP-1 inhibitor, olaparib, found response rates of up to 40% in advanced tumors, which were BRCA1/2 positive, with modest toxicity noted [23, 24]. Subsequent phase II trials have suggested much more limited activity and multiple trials are underway exploring PARP-1 inhibition in BRCA1/2 mutation carriers [25, 26]. Based on the molecular characterization of triple negative tumors as basal-like intrinsic subtype, an appearance shared by BRCA1 tumors, trials have been expanded to include this group, which constitutes a much larger slice of breast cancers. While initial results appeared quite positive, updated results of a chemotherapy backbone of carboplatin and gemcitabine in combination with the PARP-1 inhibitor iniparib, have suggested benefit limited to second or third line setting without a survival benefit [27]. Whether these results are a consequence of the PARP-1 inhibitor chosen for this trial, or represent the inclusion of triple negative tumors that may not be responsive to PARP-1 is not yet known.

Given some of the discrepancies in clinical trial results with grouping all triple negative tumors into one class for testing with PARP inhibition, there has been intense interest in delineating these tumors further at a molecular level to potentially identify responders. One such approach has been to identify a gene expression signature for tumors deficient in DNA repair in response to chemotherapy. Rodriguez et al. undertook this approach, developing a 69-gene signature indicative of defective DNA repair [28]. Testing their gene set in fixed tumors from treated patients they found a significant association with anthracycline response and taxane resistance [28]. If this approach proves successful, it may enrich our ability to select now, not at a phenotypic but a more precise genotypic level, those patients more likely to respond to PARP inhibition.

**HER2**

The HER family consists of human epidermal growth factor receptors including the epidermal growth factor receptor, EGFR (HER1), ErbB2 (HER2), ErbB3 (HER3), and ErbB4 (HER4), all of whom have identified ligands with the exception of HER2 [29]. Binding of the cognate ligand promotes the formation of homo-dimers or hetero-dimers and subsequent activation of the intracellular tyrosine kinase. These dimers can subsequently trigger a number of intracellular signaling pathways including PI3K/Akt/mTOR, Ras/Raf/Mek, and STATS, all of which play a role in critical tumor processes including proliferation, angiogenesis, and motility. HER2 can form dimmers with all of the class members but it is the HER2/HER3 hetero-dimer,
which is thought to be the most mitogenic and transforming [30, 31]. HER3 differs from other family members in that it lacks a tyrosine kinase receptor of its own but contains multiple binding sites for PI3K, thus serving as an effective scaffold to trigger the PI3K pathway. Over-expression of HER2 characterizes around 20% of all breast cancers; these cancers were more likely to be associated with a high risk of relapse until the advent of trastuzumab, a humanized monoclonal antibody directed at the extracellular portion of the HER2 protein [29]. Although the exact mechanism by which trastuzumab inhibits downstream signaling is not entirely clear, it is thought to inhibit hetero-dimerization and promote internalization and degradation of HER2. Suspected mechanisms of resistance to trastuzumab include heightened signaling through other epidermal growth factor receptor (EGFR) family members, alternative splicing of the extracellular domain, activation of the phosphoinositol 3-kinase (PI3K) pathway with subsequent constitutive activation, and loss of expression or function of the tumor suppressor gene phosphatase and tensin homologue (PTEN) which antagonizes the PI3K pathway [32].

Current efforts to overcome the development of resistance to trastuzumab include the use of humanized monoclonal antibodies to the extracellular domain such as pertuzumab, which targets a different epitope than trastuzumab. Binding to the junction component of the HER2 extracellular protein, pertuzumab blocks heter- and homo-dimerization and is thought to particularly block formation of the HER2/HER3 dimer with blockade of subsequent downstream signaling [33]. Results of early trials using pertuzumab as monotherapy or in combination with trastuzumab have had promising results [34, 35] and ongoing clinical trials of pertuzumab as in the NEOSPHERE and CLEOPATRA studies may further clarify the activity of this agent- particularly when given in combination- in overcoming intrinsic resistance.

Another agent which has shown promise in overcoming HER2 resistance, is the compound Trastuzumab-DM1 (T-DM1), a novel conjugate of the HER2 monoclonal antibody linked to the fungal toxin maytansine (DM1), an antimicrotubule agent, which inhibits assembly of cellular microtubules [29]. T-DM1 is unique among drug immunoconjugates in that it targets HER2. Thus in this system, trastuzumab functions to target HER2 expression in cells, delivering a potent cytotoxic agent, independent of downstream processes. Upon binding to the extracellular target, T-DM1 is internalized where it undergoes proteolytic degradation releasing maytansine intracellularly [36]. Results of early clinical trials suggest an improved response as compared to the co-administration of trastuzumab and docetaxel, with little evidence that free DM1 poses a problem [36]. Current clinical trials such as MARIANNE are investigating the addition of pertuzumab to T-DM1 (NCT01120184) thus seeking two slightly different targeting mechanisms to HER2 with the linkage of DM1 adding effective cytotoxicity.

Given the strong proliferative effect of the HER2/HER3 dimer, another potential therapeutic target in the HER family is HER3 and its physiologic ligand, heregulin (HRG). A number of anti-HER3 monoclonal antibodies are in development including MM-121, a fully humanized antibody that binds to HER3, preventing hetero-dimerization and subsequent phosphorylation [37]. As mentioned above, the dependence of HER2 activation on the PI3K pathway has focused attention on potential pathway inhibitors as a mechanism of overcoming trastuzumab resistance.
### Alternative Her2 targeted agents

Attacking HER2 though inhibition of tyrosine kinase activity, laptainib was the first small molecule inhibitor approved for use in HER2 amplified cancers. Lapatinib inhibits both HER2 and HER1, decreasing Insulin Growth Factor (IGF)-1 signaling [29]. Approved for use in conjunction with capecitabine after trastuzumab-based regimen failure, lapatinib offers the potential benefit of central nervous system penetration. In addition, lapatinib may specifically affect tumors co-expressing HER2 and the estrogen receptor (ER) limiting the cross-talk which occurs between the two signaling pathways [38]. Recent trials have demonstrated that the addition of lapatinib to letrozole nearly tripled progression-free survival rates in patients with breast cancer whose tumors co-expressed both hormone receptors and HER2 [39]. Current trials are underway seeking to define responses to lapatinib in combination, sequence, or direct comparison to trastuzumab.

### PI3K/Akt/mTOR pathway

The PI3K/Akt/mTOR pathway is emerging as one of the most critical pathways in cancer development and growth. Regulating multiple functions, including proliferation, cell growth, and survival, this pathway is pivotal in tumor formation and progression. Activation of the PI3K/Akt/mTOR pathway had been identified in several malignancies, including breast cancer, where increased expression levels have been associated with resistance to chemotherapeutic and hormonal agents and an increased likelihood of the development of metastatic disease [40]. Sitting at the interface of two signaling cascades, the mTOR molecule has engendered significant interest given the availability of inhibitors. In this pathway, mitogens binding to membrane receptor tyrosine kinases activate PI3K. Once activated, the kinase serves as a docking site for Akt, a serine/threonine kinase that is a central mediator in signal transduction of the PI3K pathway. Akt stimulates protein synthesis and growth by in turn activating the mammalian target of rapamycin (mTOR). A serine/threonine kinase also, mTOR is a critical regulator of protein translation and can induce a positive feedback loop further amplifying phosphorylation of Akt to enhance this activation [40]. Aberrant signaling has been observed at a number of junctures in this process including somatic mutations in the PI3K gene, amplification of Akt, and dysfunction of the tumor suppressor PTEN. Loss of PTEN occurs in approximately 40–50% of breast cancers and results in activation of mTOR [41, 42]. Like other aberrations in this pathway, loss of the negative regulation results in activation of the PI3K/Akt/mTOR pathway and is correlated with resistance to therapy and the presence of metastatic disease [41].

The most well developed inhibitors of this pathway are the mTOR inhibitors, including temsirolimus (Torisel), an agent approved for the treatment of recurrent renal cell cancer and everolimus (Certican/Afinitor) now approved for use of advanced refractory renal cell and progressive neuroendocrine tumors of pancreatic origin. Both agents have demonstrated anti-proliferation effects in breast cancer cell lines, which were ER positive, contained over-expressed HER2, or demonstrated loss of PTEN. Importantly, inhibitors of the PI3K pathway appear most effective when combined with inhibitors of receptor tyrosine kinase activity [43]. Building on these studies, clinical trials have been carried
out in comparable tumor groups. The first study of everolimus reported at the San Antonio Breast Cancer Symposium 2010, was a Phase I investigation demonstrating a 6-month improvement in response to everolimus combined with tamoxifen compared to tamoxifen alone (SABS 2010). Similar results were seen in a subsequent phase III trial, the BOLERO-2 trial (Breast Cancer Trials of Oral Everolimus-2), which compared everolimus with exemestane versus exemestane alone in women with advanced breast cancer refractory to either anastrozole or letrozole. In this trial enrolling 700 women, early results suggested an improvement in progression-free survival from 4.1 months to 10.6 months [44, 45]. Toxicities seen mirrored those of mTOR inhibitor pathways with stomatitis and anemia being the most prevalent side effects. Thus, these trials provide some proof in principal that duel pathway inhibition provides an optimal antitumor effect over a PI3K pathway inhibitor alone.

This approach of inhibition of downstream mTOR signaling has also been studied in HER2 positive breast cancers exhibiting resistance to trastuzumab. In a recent trial, women whose tumors had progressed on trastuzumab-based therapy were placed on daily everolimus with continued trastuzumab in an effort to overcome the resistant phenotype in a recently reported pooled analysis of two, phase I/II trials [42]. This study suggested some clinical benefit with partial responses or stable disease in up to 34% of patients. As might be expected, patients whose tumors expressed loss of PTEN had a decreased overall survival [42]. While these studies represent early forays into targeted pathway inhibition, they serve as a basis for development of mTOR specific kinase ATP-competitive inhibitors.

**Antiangiogenesis and VEGF inhibitors**

Defined as the growth of new blood vessels, angiogenesis has been viewed as a key regulator of tumor growth and invasion. Controlled by the vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) families of proteins and their receptors, these signaling pathways are key targets for breast cancer therapy. The ligand, VEGF, is elevated in metastatic breast cancer and has been associated with poor prognosis and decreased response to chemotherapy [46]. Anti-angiogenic targeting has been undertaken through neutralization of VEGF using the humanized monoclonal antibody, bevacizumab (Avastin), and through the use of small molecule tyrosine kinase inhibitors. Phase III trials have been conducted evaluating the benefit of adding bevacizumab to chemotherapy in the setting of metastatic breast cancer. A recently completed meta-analysis reviewed the available trials employing bevacizumab with chemotherapy and suggested that there was improvement in progression free survival and objective overall response but no significant change in overall survival. These reports along with the lack of survival benefit in E2100 have led to withdrawal of this agent by the FDA for metastatic breast cancer [46, 47]. However, the ability of agents such as bevacizumab to prevent metastasis is still under active investigation and ongoing clinical trials in this setting may shed more light on the optimal place to integrate anti-angiogenic therapy into the care of breast cancer patients [48]. Other avenues of investigation have sought to better define subgroups, which may benefit from VEGF inhibition. Based on preclinical studies
suggesting bevacizumab may reverse acquired hormone therapy resistance, clinical trials are ongoing in tumors with demonstrated acquired resistance and in the neoadjuvant setting [49, 50]. As suggested by discussions at the 2011 San Antonio Breast Symposium, bevacizumab in combination with other agents such as trastuzumab, may improve progression free survival for some women and confirmatory studies such as AVEREL are ongoing.

Attempts to utilize the multitargeted tyrosine kinase inhibitors in the inhibition of angiogenesis are also being investigated in refractory breast cancer. Blockage of the downstream signal cascade by agents such as sunitinib (Sutent) or sorafenib (Nexavar) have thus far failed to demonstrate substantive improvements in primary endpoints of clinical trials [51, 52]. These agents may not be successful in part due to angiogenic growth factor redundancy or rapid acquired resistance to VEGF-targeting drugs.

### Breast cancer stem cells

Breast cancer stem cells (CSC) are thought to be pivotal in tumors, making up a minority of the tumor but possessing the capability of limitless proliferation. Described first 2003 by Al-Hajj et al., breast CSCs have been postulated to be resistant to existing therapeutic agents and the drivers of distant metastatic disease [53]. Characterized by surface expression of CD44+ and CD24- these cells are recognized to have low rates of cell division and exhibit primary chemotherapy and radiation resistance [54]. Why this resistance exists is not clear though the cells appear to express anti-apoptotic proteins, MDR proteins, and possess efficient DNA repair mechanisms [55]. Targeting these cells has been an attractive concept in selectively reducing the driving force behind a tumor and eliminating stem cell renewal. Mechanistically however, accurate identification of stem cells and the ability to destabilize their sequestered dormant state remains elusive. Current approaches targeted towards affecting breast cancer stem cells include the addition of lapatinib to chemotherapy and inhibition of Akt downstream signaling in HER2 positive tumors to affect self-renewal pathways [56, 57].

### Apoptotic

The induction of programmed cell death or apoptosis is postulated to be one mechanism whereby an effect on stem cells might be exerted. Apoptosis is initiated through two main signaling pathways: the extrinsic system, triggered by binding of apoptotic membrane receptors, and the intrinsic system activated intracellularly [58]. Chemotherapy induced injury is generally viewed as initiating apoptosis through the intrinsic system by preventing cells from dividing and replicating; in contrast, proapoptotic receptor agonists initiate apoptosis through the extrinsic pathway. Currently there are two main forms of initiation of the extrinsic pathway: the naturally occurring apoptosis ligand 2/tumor necrosis factor-related apoptosis-inducing ligand (Apo2L/TRAIL) and monoclonal antibodies that bind to the Apo2/TRAIL death receptors (DR) 4 or 5 [59, 60]. Stimulation of the receptors results in activation of a caspase cascade, culminating in apoptotic cell death – a process that appears specific to tumor cells, sparing normal tissues.
Apo2L/TRAIL belongs to the superfamily of tumor necrosis factor (TNF) ligands at the surface of lymphocytes and natural-killer cells [59]. Thus far, there are five identified receptors including DR4, DR5, osteoprotegerin, and two decoy receptors [61]. The mechanism by which Apo2/TRAIL is selective for tumor cells is not entirely understood by may occur in part due to the presence of decoy receptors on normal cells that diminish binding of TRAIL to death receptors or sensitization of cancer cells when oncogenes such as MYC and RAS are present [6, 58]. The use of Apo2/TRAIL to initiate apoptosis has been demonstrated in a variety of in vitro and in vivo studies and appears specifically active in triple negative breast cell lines [62]. In addition, several studies have suggested that resistance to TRAIL-mediated apoptosis in breast cancer can be overcome with combinations of TRAIL with radiation, chemotherapy, and targeted agents [60, 63, 64]. Human trials are ongoing with recombinant Apo2L/TRAIL and monoclonal antibodies specific for DR activation that may offer a novel combination for basaloid, triple negative breast cancer [60, 63, 65].

### Heat shock protein inhibitors

Heat shock proteins (HSPs) are molecular chaperones in cells, serving to enhance the stability of a variety of proteins. Important in facilitating cell signaling and protein folding, these molecules are named for their increased synthesis in response to cellular stress. HSP90 is a stress protein upregulated in response to cellular stress; this molecule interacts with several client proteins, functioning in part by inhibiting stress-induced apoptosis. Interacting with a variety of molecules, including ER, PR, HER2, receptor kinases, and transcription factors, HSP90 appears to be a buffering agent, preventing potentially lethal events from culminating in cell death [66]. Utilizing HSP90 inhibitors has been an attractive approach, and several breast cancer specific trials are testing the activity of an HSP90 inhibitor, tanespimycin (17AAG) alone or in combination in refractory tumors. One study in HER2 amplified, trastuzumab refractory, patients reported meaningful responses and highlights the potential benefits of combining HSP inhibitors with other targeted therapies. [67].

### Unmet needs

While our understanding of mechanisms mediating drug resistance has improved, we have largely been unable to truly identify those patients whose tumors, on the basis of predictive testing, are vulnerable to specific interventions. Nevertheless, there are a number of potential strategies capitalizing on improved understanding of mechanisms of resistance, which offer modest improvements in the treatment of resistant breast cancer. Selecting intersecting pathways with combinatorial agents or regimens offers the hope of delaying the development of resistance in the treatment of breast cancer.

### Disclosure

No potential conflicts of interest relevant to this article were reported.
References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:
- Of importance
- Of major importance


Provides a succinct overview of approaches to a subgroup of breast cancer that epitomizes the improvements made in defining breast cancer at a molecular level.


One of the newest agents approved for use in the treatment of metastatic breast cancer, this class of drugs demonstrates activity in taxane resistant breast cancer.


Represents an early success story in the use of combining agents targeting intersecting pathways that are drivers for breast cancer proliferation.


