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# Signal Transduction Pathways in Breast Cancer – Drug Targets and Challenges

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## 1. Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in females worldwide [1]. Over 1.1 million cases of breast cancer are diagnosed across the world each year, compared with about 500,000 cases in 1975. This represents about 10% of all new cancer cases and 23% of all female cancers [2-3]. An annual prevalence of more than 4.4 million cases of breast cancer is expected worldwide by the year 2012 [4].

In the past years, our knowledge of the genetic changes that contribute to breast cancer development & progression has tremendously changed [5]. Our knowledge of alterations in the cancer cell have allowed us to identify the signaling pathways that when disrupted allow a cancer cell to escape from normal control mechanisms [6]. On the other hand, these aberrant processes have also provided many therapeutic points for intervention, which is the major topic of this chapter. Following the successful introduction of trastuzumab, the first human epidermal growth factor receptor (HER) targeted therapy to become widely used in breast cancer patients, other agents have been developed [7]. Other potential hallmarks of malignancy that represent a new opportunity for therapeutic targeting include abrogation of apoptosis, lack of senescence, angiogenesis, tumor invasion and metastasis [8]. Therefore, new compounds are being developed that may interfere with these hallmarks and that may prove to be effective in monotherapy or in combination with cytotoxic therapy or other targeted therapies. Novel agents are needed because many of the current therapies have limitations. These include drug resistance, lack of target receptor expression in tumors and relatively small improvements in survival [9-12].

Thus, this chapter provides an updated overview of the several signaling transduction pathways involved in development of breast cancer. In addition, it focuses on recent progress with the therapeutic strategies targeting these pathways contributing to their promising success in the clinical setting. Furthermore, molecular signals of its resistance phenotype and breast cancer stem cells & their therapeutic targeting are discussed briefly. Finally, the challenges facing the significant contribution of targeted therapeutics in breast cancer chemotherapy are also extensively discussed. Wherever possible, advances in drug analogs, accepted or controversial mechanisms described for their antitumor activity will be discussed within the framework of the current chapter.

**Section A** describes a reported panel of potential signal transduction pathways (STPs) that are altered in breast cancer and are undergoing research as targeted therapeutics. Some of these have yielded therapeutics that already had clinical success, while others are still in the experimental stages (**Fig. 1 & Tab.1**). Though many of the underlying mechanisms of the multi-drug resistance (MDR) phenotype are still not clearly identified, several potential molecular targets and pathways of activation have been suggested. The advances in this field provide an emerging picture of how MDR arises and how it could be therapeutically targeted. In **Section B**, we review the potential role of cancer stem cells molecular signaling in development of breast cancer & its resistance phenotype. It also discusses their putative modulation contributing to the success of targeted therapeutics in breast cancer chemotherapy.

| | STP | STI | Mechanism |
|---|---|---|---|
| 1 | **Receptor tyrosine Kinase:** **I- HER2 receptor** | -Trastuzumab<br><br><br><br>-Lapatinib, Erlotinib, Gefitinib and Neratinib | -Extracellular Targeted Therapeutic: Monoclonal Antibody inhibiting ligand binding to receptor<br>-Intracellular Targeted Therapeutic: HER Receptor tyrosine kinase catalytic inhibitor |
| | **Receptor tyrosine Kinase:** **II- Src receptor** | Dasatinib | Intracellular Targeted Therapeutic: Src Receptor tyrosine kinase catalytic inhibitor |
| 2 | **MAPK Signaling Pathway** | Tipifarnib | Farnesyl transferase inhibitor |
| 3 | **PI3K/Akt/mTOR pathway** | -Perifosine<br>-Temsirolimus and Everolimus<br>-Octreotide | -Inhibitor of Akt phosphorylation<br>-mTOR inhibitors<br><br>-Inhibitor of IGF-1R signaling |
| 4 | **HSP90 signaling pathway** | Tanespimycin and Alvespimycin | Potent inhibitiors of HSP90 function |
| 5 | **PARP signaling pathway** | Olapraib Veliparib | PARP inhibitors |
| 6 | **Apoptosis & Autophagy pathways** | | Inhibitors of the components of apoptotic & autophagic pathways |
| 7 | **Angiogenesis** | Bevacizumab | Extracellular Targeted Therapeutic: Monoclonal Antibody inhibiting ligand binding to receptor |
| 8 | **Multi-Target Therapeutics** | Sunitinib, Pazopanib, Sorafenib and Axitinib | Intracellular Targeted Therapeutic: Receptor tyrosine kinase catalytic inhibitors |
| 9 | **Estrogen signaling transduction** | -Tamoxifen<br>-Letrozole | -Anti-estrogen<br>-Aromatase inhibitor |
| 10 | **UPS signaling pathway** | Bortezomib | Proteasome inhibitor |

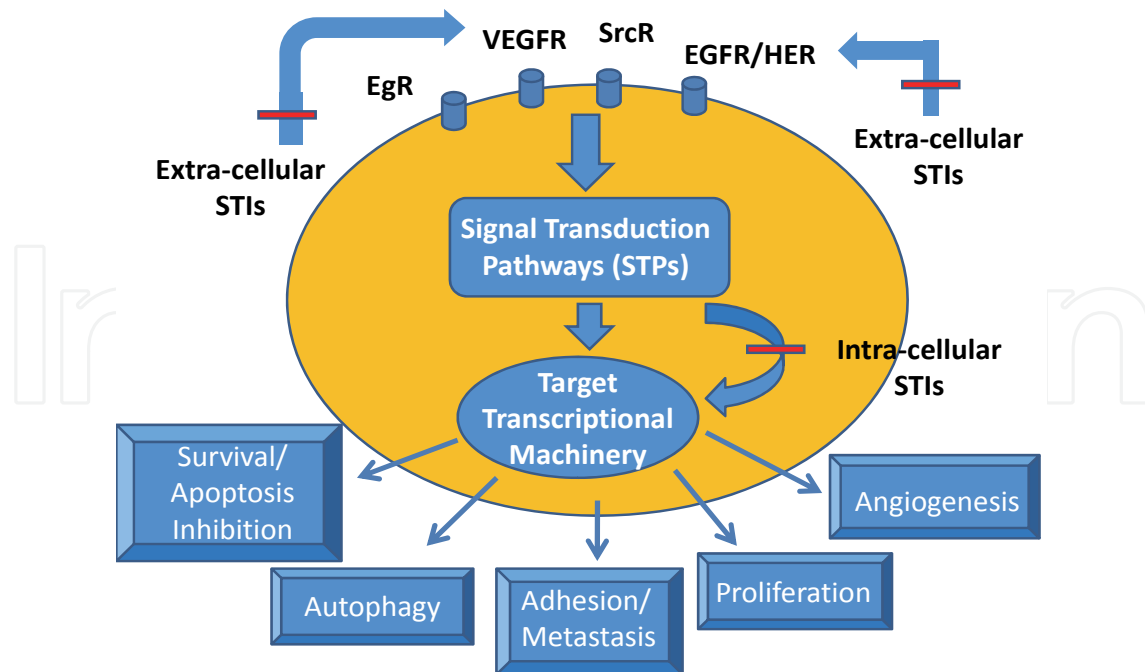Table 1. A list of the action site for signal transduction inhibitors (STIs) in breast cancer.

Fig. 1. Simplified schematic illustration of the multiple signal transduction pathways (STPs) in breast cancer.

## 2. Targeting signal transduction pathways in breast cancer

The mechanisms underlying the development of breast cancer are complex and vary among individual tumors [13]. Altered patterns of gene expression are associated with corresponding variations in growth rates, cellular composition and different prognoses [14]. Given the complex and varied factors that influence the development of breast cancer, and the use of increasingly sophisticated genetic analysis techniques, it is likely that more refined tumor subtypes and their associated prognoses will be identified[13, 15]. Advances in the understanding the etiology and biology of breast cancer have identified key targets among multiple signaling pathways involved in the development and survival of breast cancer cells. Thus, targeted therapies are among the most promising new agents for the treatment of breast cancer. Some of these reported breast cancer signaling transduction pathways (STPs) & their signal transduction inhibitors (STIs) are classified as follows:

### 2.1 STPs-1: Receptor Tyrosine Kinase (RTKs)
Altered patterns of gene expression can influence the activity of specific signaling pathways. For example, receptor tyrosine kinases (RTKs) are often aberrantly over-expressed or activated in human cancers. The epidermal growth factor receptor (EGFR) family is composed of cell surface tyrosine kinase receptors that are involved in the regulation of cellular proliferation and survival of epithelial cells. The EGFR (or also called HER) family includes four closely related receptors: EGFR/HER1/ErbB1, HER2 neu /ErbB2, HER3/ErbB3 and HER4/ErbB4 [16].

### 2.1.1 HER2 receptor
Members of the HER family are encoded by genes on different chromosomes and regulate normal breast growth and development. The HER family couples to multiple signaling

pathways that impact on all aspect of breast cancer biology. Through their interconnected cellular signaling network, the HER family regulates diverse biological processes, including cell proliferation, differentiation, and survival **[16-17].** Thus, they play a key role in the development and progression of breast cancer [16, 18-19].

Variations in the pathways associated with the HER family appear to be particularly important, not only in tumor development but also in treatment efficacy [20-21]. For example, in breast cancer, constitutive activation of the epidermal growth factor receptor (EGFR) and HER2 is found in approximately 16%–48% and 25%–30% of breast cancer tumors, respectively. Moreover, expression of HER family members in breast cancer tumors has a significant impact on tumor aggressiveness and patient survival. Importantly, their expression correlates with a more aggressive disease course, shorter survival time, and higher risk for resistance to endocrine therapies [10, 12, 22-27]. HER-3 expression, observed in approximately 18% of tumors, also correlates with shorter overall survival **[28].** Interestingly, expression of HER-4 (found in approximately 12% of tumors) has been associated with more favorable tumor characteristics and longer survival [27-28].

Each HER receptor has an extracellular domain (ECD) involved in ligand binding, a helical transmembrane segment, and an intracellular protein domain with tyrosine kinase activity. On ligand binding, the extracellular domains of the receptors undergo conformational changes, which allows them to form homodimers (consisting of two identical receptors) or heterodimers (consisting of two different receptors) of the HER family [16, 29]. Dimerization of HER receptors induces phosphorylation of their intracellular tyrosine kinase domains, which provide docking sites for adaptor proteins and signaling enzymes **[29].**These molecules act as a link between membrane receptor kinases and "downstream" intracellular protein kinases, which results in the activation of multiple signaling pathways, of which the MAPK and PI3K pathways are probably the best studied **[29].**

HER-2 is the preferred dimerization and signaling partner for all other members of the HER family, and it appears to function mainly as a co-receptor, increasing the affinity of ligand binding to dimerized receptor complexes [29-30]. With their multiple ligands, many dimerization combinations, and large number of downstream effectors, the HER family mediates an extensive range of signals, controlling a variety of cellular processes, including cellular proliferation, apoptosis, and angiogenesis [29, 31]. For example, HER2 receptor activation leads to the phosphorylation of the intracellular catalytic domains, and ultimately activation of signal transduction pathways that promote proliferation and survival, including the phosphatidylinositol 30-kinase (PI3K)/Akt/mTOR, the Erk1/2 mitogenactivated protein kinase (MAPK) and the Jak/Stat pathway **[32]**.

## 2.1.2 Src-family tyrosine kinases

The v-Src (Rous sarcoma virus) tyrosine kinase was the first oncogenic gene discovered **[33]**. The corresponding cellular gene, c-Src, is a non-receptor signaling kinase that functions as a hub of a vast array of signal transduction pathways that influence cellular proliferation, differentiation, motility and survival **[34]**. Several mechanisms lead to increased Src activity in tumors. Src is downstream in signaling from a number of growth factor receptors including PDGF receptor (PDGFR), epidermal growth factor receptor (EGFR) and insulin-like growth factor- 1 receptor (IGF-1R) **[35]**.

In many tumor types, over-expression of these receptors, their ligands or both is frequent **[36]**. For example, reported studies suggest an association between Src tyrosine kinase and the development, progression and metastasis of breast cancer [37-38]. In addition, it is

described that mice over-expressing the HER2 oncogene develop highly metastatic mammary tumors with elevated Src activity **[37].** Further supporting the role of Src in breast cancer, it has been demonstrated that Src activity is profoundly increased in human breast cancer tissues compared with benign breast tumors or adjacent normal breast tissues[38-39] and that elevated c-Src tyrosine kinase activity is correlated with early systemic relapse **[40]**. Taken together, these results strongly indicate that the Src may play an important role in the development and progression of breast cancer **[41]**.

## 2.2 STIs-1: Receptor Tyrosine Kinase Inhibitors (RTKIs)
## 2.2.1 HER2 receptor inhibitors

Numerous agents targeting individual members of the HER family have been developed for use in the treatment of breast cancer. Existing therapeutic approaches have largely focused on two classes of agents:

### 2.2.1.1 Extracellular targeted therapies

### Monoclonal antibodies

The first comprises monoclonal antibodies that bind to extracellular regions of HER to interfere with receptor function (e.g., trastuzumab, pertuzumab, and a number of pan-HER inhibitors). Trastuzumab binds to the juxtamembrane region of HER-2 with high specificity, but it is not currently known how it specifically interferes with HER-2 function **[42]**. Pertuzumab is the first in a class of HER-2 dimerization inhibitors. Binding to HER-2 inhibits its dimerization with other HER receptors and this is thought to result in slowed tumor growth [43].

Trastuzumab, an HER2 specific humanized monoclonal antibody was one of the first biologicals to be approved for metastatic breast cancer treatment [44]. In patients, HER2 is over-expressed and/or amplified in one-fourth of breast tumors and confers a more aggressive clinical course and a worse survival [25, 45]. The outcome of these highly aggressive tumors has markedly improved with the development of anti-HER2 therapies. Trastuzumab (Herceptin®) is a recombinant humanised monoclonal antibody that binds with high affinity to the extracellular juxtamembrane domain of HER2 and inhibits the proliferation of human tumor cells that over-express HER2 in vitro and in vivo [46-48].

Trastuzumab. Trastuzumab, administered as an i.v. infusion, is approved in the U.S. and Europe for the treatment of HER-2–over-expressing metastatic breast cancer (MBC) [7]. It is standard-of-care treatment for MBC patients with HER-2–over-expressing tumors, both as first-line treatment in combination with chemotherapy and as a single agent in women who have HER-2–over-expressing breast cancer that has progressed after chemotherapy for metastatic disease[44, 49]. Trastuzumab is approved for the adjuvant treatment of HER-2+, node negative (ER-/progesterone receptor [PgR-]) or node-positive breast cancer, either in combination with chemotherapy or as a single agent following multi-modality anthracycline-based treatment.

In patients with HER2 amplified tumors, trastuzumab has single agent activity and improves survival in the first-line setting when combined with chemotherapy in patients with advanced disease [44, 50]. Recently, a number of well powered clinical trials have demonstrated that administration of trastuzumab in the adjuvant setting, in combination and/or sequentially after chemotherapy, results in an improvement in disease-free survival as well as overall survival **[51-54].**

Trastuzumab efficacy in the metastatic setting has provided the rationale for several studies investigating the use of trastuzumab plus chemotherapy as adjuvant treatment for patients with earlystage HER-2+ breast cancer [52-53, 55-56]. Studies have evaluated a range of different trastuzumab-based combination regimens for the first-line treatment of MBC [50, 57]. Additionally, a number of trials are ongoing investigating trastuzumab in combination with hormonal therapy in MBC patients [58-59]. When administered as a single agent, trastuzumab has documented efficacy as a first-line therapy, with response rates typically in the range of 23%–33% [60-62]. Of those patients with MBC who do achieve an initial response, many experience disease progression within 12 months as a result of the high proportion of HER-2–over-expressing tumors that have intrinsic resistance to this agent **[63]**. Given the absence of known specific ligands for HER-2, thus there is no alternative approach to blocking this pathway except by trastuzumab. This has clinical implications as this may be related to the development of resistance to HER-2 blockade **[64]**.

On the other hand, changing the traditional treatment paradigm in patients progressing on trastuzumab and administering further trastuzumab-based therapy beyond disease progression may have clinical benefit [65-66].This "treatment beyond progression" approach is increasingly being studied in clinical trials by combining trastuzumab either with chemotherapy [67-68] or with another targeted agent, such as the RTKI lapatinib [69].

Trastuzumab-DM1 (T-DM1) is an anti–HER-2 antibody drug conjugate comprising trastuzumab linked to the maytansine derivative DM1. Combining these two agents facilitates anti–HER-2 activity as well as targeted intracellular delivery of a potent cytotoxic agent. Single-agent TDM1 as well tolerated, active and no dose-limiting cardiotoxicity was observed in a phase II study of 112 patients with pretreated MBC [7].

**Limitations of Trastuzumab therapy**

1.  Trastuzumab is an effective treatment for patients with HER-2+ disease, yet its use is limited to this group (approximately 25%) [25]. Thus, accurate patient selection for treatment is important, using an appropriate method, such as immunohistochemistry or fluorescence in situ hybridization, to detect HER-2 over-expression.

2.  Not all HER-2+ patients respond to treatment with trastuzumab, and the development of resistance is an issue. In the future, it may be possible to overcome resistance by combining trastuzumab with new therapies such as pertuzumab, by switching to an agent such as lapatinib that inhibits both HER-1 and HER-2 activity, or, if proven effective, the use of one of the pan-HER inhibitors currently in development.

3.  Trastuzumab is unable to penetrate the blood– brain barrier (Guy et al., 1994) and over-expression of HER-2 is known to be associated with a greater risk for central nervous system (CNS) metastases [70]. Patients with HER-2+ MBC treated with trastuzumab appear to be at greater risk for developing CNS metastases than those who do not receive trastuzumab therapy [71]. However, HER-2+ patients with CNS metastases who are treated with trastuzumab appear to have a longer overall survival duration than those who are HER-2- or those unselected for HER-2 status. This may reflect greater control of extra-cranial disease as a result of trastuzumab therapy [72].

4.  Treatment with trastuzumab is associated with a higher risk for cardiomyopathy (left ventricular dysfunction and congestive heart failure), particularly when used in combination with paclitaxel or anthracyclines [73]. However, these cardiotoxic effects appear to be reversible once trastuzumab treatment is discontinued or if they are managed with appropriate medical therapy [74-75]. The cellular mechanisms contributing to the cardiotoxicity observed with trastuzumab are still being explored. It

is known that HER-2 plays an important role in cardiomyocyte development and function, and trastuzumab- induced inhibition of HER-2 signaling in cardiomyocytes may be a central mechanism underlying the observed cardiomyopathy. However, the full explanation is likely to be more complex. Cardiotoxicity does not appear to be an issue with the RTKI lapatinib, which inhibits both HER-1 and HER- 2 [76]

5. Although cardiotoxicity is the primary safety concern with trastuzumab, potentially severe hypersensitivity reactions to infusion have also been reported [7].

Other example of novel anti-HER2 agents include antibodies that interfere with HER2 dimerisation. Pertuzumab is a recombinant, humanised MAb that targets an epitope within the HER2 dimerisation domain [77]. Once bound, pertuzumab inhibits ligand-activated HER dimerisation with HER2 and thereby inhibiting activation of intracellular signaling [78].

### 2.2.1.2 Intracellular targeted therapies

### Receptor tyrosine kinase inhibitors (RKIs)

The second class of HER-targeted agents comprises the small molecule receptor tyrosine kinase inhibitors (RTKIs) that inhibit enzyme function of HER family members intracellularly. Oral RTKIs include lapatinib, neratinib (both inhibit HER-1 and HER-2), erlotinib and gefitinib that target the intracellular domain of HER-1, and the irreversible pan-HER inhibitors PF-00299804 and canertinib, which inhibit the kinase signaling of multiple HER family members [79].

Another strategy to target HER2 is with low molecular weight tyrosine kinase inhibitors. Lapatinib (Tykerb®) is a dual EGFR and HER2 inhibitor that has been studied extensively in multiple clinical settings. Lapatinib is approved in the U.S. (March 2007) and European Union (EU) (June 2008) for use (oral administration) in combination with capecitabine for the treatment of patients with advanced breast cancer or MBC whose tumors over-express HER-2 and who have received prior therapy including an anthracycline, a taxane, and trastuzumab [7].

Lapatinib increases survival in patients with advanced HER2-over-expressing breast cancer when given in combination with the chemotherapeutic agent capecitabine compared to capecitabine alone in patients that had previously failed anthracyclines and taxanes [80]. The incidences of adverse events (including those leading to treatment discontinuation) and symptomatic cardiac events were similar in both treatment groups. The observation that fewer patients in the lapatinib group developed central nervous system (CNS) metastases together with lapatinib's low molecular weight and capacity to cross the blood-brain barrier has led to a clinical trial to study the role of lapatinib in the treatment of brain metastasis in patients with HER2-over-expressing breast cancer [25, 28]. Thus, contrary to trastuzumab, lapatinib has a putative activity against CNS metastases in patients with HER-2+ breast cancer [81]. These data suggest that, as a small molecule RTKI, it may be able to cross the blood– brain barrier to provide effective therapeutic concentrations in cerebrospinal fluid (unlike monoclonal antibodies such as trastuzumab).

Lapatinib appears to be associated with less cardiotoxicity than trastuzumab [82]. However, as lapatinib development is extended to include the treatment of patients with lower-risk primary breast cancer, it will be increasingly important to monitor cardiotoxic effects. The most common adverse effects associated with lapatinib treatment are gastrointestinal; lapatinib-related diarrhea generally occurs early in the course of treatment, is mild to moderate, and does not require treatment, although monitoring is important to identify patients who may need intervention [7].

Other examples of RTKIs include Erlotinib and Gefitinib. Recent clinical studies have not demonstrated any significant clinical benefit for erlotinib or gefitinib either as single agents or in combination with other agents in MBC [68, 83-85]. Given their lack of activity as monotherapy in MBC, studies continue to investigate the efficacy of erlotinib and gefitinib in combination with other targeted therapies, chemotherapy, or hormonal agents; however, tolerability issues may limit this approach.

Other anti-HER tyrosine kinase inhibitors in clinical development include HKI-272. Neratinib (HKI-272) is an orally administered, irreversible, pan-erbB kinase inhibitor [86] that covalently bind to the intracellular kinase domain. The observation that some patients with chronic myelogenous leukemia were developing resistance to the RTKI imatinib led to the development of neratinib. In preclinical models, neratinib has been shown to have promising antiproliferative activity in both HER-2–dependent cell lines and tumor xenografts.

Phase I/II data have confirmed that neratinib has antitumor activity in patients with HER-2+ MBC, either as a single agent in trastuzumab refractory patients or in combination with trastuzumab, and the safety profile of this agent has been manageable [87].

## 2.2.2 Src-family tyrosine kinases inhibitors

Currently, small-molecule Src inhibitors are in early phases of clinical development either as single agents, in combination with cytotoxic agents, biological therapies or in combination with hormonal treatment. Originally, dasatinib (BMS-354825) was selected as a synthetic small-molecule inhibitor of Src-family kinases; then, it was found to inhibit at least four other protein tyrosine kinases: bcr-abl, c-Kit, EphA2, PDGF-beta. Dasatinib is currently studied in clinical trials for the treatment of solid tumors, including breast cancer. Preclinical evidence suggests that dasatinib could be effective in breast cancer cell lines of basal-like subtype **[88]**. These findings provide scientific rationale for the clinical development of dasatinib in the treatment of patients with 'triple-negative' breast cancer, a tumor subtype that is categorised as being aggressive and lacking effective targeted treatments, such as endocrine therapies and anti- HER2 strategies. More recently, AZD-0530 a highly selective, dual-specific, orally available small-molecule inhibitor of Src kinase and Bcr-Abl has entered clinical trials **[89]**. In healthy volunteers, AZD-0530 has shown only mild adverse events.

### Targeting downstream effector molecules

Targeting HER receptors with extracellular monoclonal antibodies and intracellular RTKIs has shown promising clinical activity. There is, however, a need for better treatment of MBC patients because many of these current therapies are restricted to a subset of the MBC patient population. Targeting cellular signaling pathways, such as the MAPK and PI3K pathways, downstream of HER receptors may be an attractive avenue for novel treatments. Additionally, there is some evidence that targeting heat shock proteins (Hsps) and the apoptotic pathway may be viable options for future therapeutic strategies in MBC. Recent developments in this field are briefly discussed in the following sections.

## 2.3 STPs-2: Targeting downstream MAPK signaling pathway

The MAPK pathway, also termed the extracellular signal-regulated kinase (ERK) pathway, contains downstream effectorsof the HER family and other tyrosine kinases, and is a central

part of the signaling networks that control fundamental cellular processes, including cell proliferation, differentiation, and survival **[90]**.

### 2.4 STIs-2: Targeted therapies directed at the MAPK signaling pathway

The farnesyl transferase inhibitor tipifarnib (R115777) was evaluated in phase III trials for the treatment of breast cancer, although further development has now been terminated [91-93]. AZD6244 (ARRY-142886) is an inhibitor of the enzyme MEK, a component of the MAPK pathway. AZD6244 is currently in phase I clinical studies in several cancer types, including breast cancer.

### 2.5 STPs-3: Targeting downstream PI3K/Akt/mTOR pathway signaling pathway

The PI3K/Akt pathway plays a central role in diverse cellular functions including proliferation, growth, survival and metabolism. In addition to their physiological role, several isoforms of the PI3K family are implicated in tumor development, including cell proliferation, cell growth, cell motility, cell survival, and angiogenesis [29]. The relationship between dysregulated PI3K activity and the onset of cancer is well documented [94]. In particular, members of class 1A PI3Ks are often mutated in human cancer [95-100]. As a result of receptor tyrosine kinase RTK activation and phosphorylation, PI3K interacts with the intracellular domain of the receptors. Subsequent phosphorylation event by the mammalian target of rapamycin (mTOR)-complex is required for maximal Akt activity [101-102]. Akt is the central effector of the pathway. Akt reduces cell cycle inhibitors p27 and p21, and promotes cell cycle proteins c-Myc and cyclin D1, resulting in enhanced cellular proliferation. Its influence extends to a host of pro- and antiapoptotic proteins, such as the Bcl-2 family member Bad, limiting programmed cell death and boosting cellular survival. mTOR is a central regulator of cellular responses to multiple stimuli including amino acid availability and growth factor receptor signaling. In cells with sufficient nutrients, mTOR relays a signal to the translational machinery leading to an enhanced translation of mRNAs encoding proteins essential for cell growth and cell cycle progression [103-104].

There is growing evidence that uncontrolled activation of the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway, achieved via numerous genetic and epigenetic alterations, contributes to the development and progression of human cancers, including breast cancer [105]. In breast tumors, activating mutations in PIK3A, encoding the catalytic subunit of PI3K, or loss of PTEN, the negative regulator of PI3K activity, are very frequent and contribute to constitutive pathway activation and mTOR activity. Further, they may result in resistance to upstream anti-receptor agents. For example, trastuzumab depends on intact PTEN for its action in HER2-over-expressing breast cell lines, and PTEN loss predicts for trastuzumab resistance [106]**.** Therefore, this pathway is an attractive target for novel anticancer agents. Clinical trials are currently underway with mTOR, PI3K and Akt inhibitors.

### 2.6 STIs-3: Inhibitors of the PI3K/Akt/mTOR pathway

Therapies targeting the PI3K pathway include perifosine (KRX-0401), which inhibits Akt phosphorylation [107] and mTOR inhibitors are also further ahead in development within this class of agents. The rapamycin analogs that target mammalian target of rapamycin include temsirolimus (CCl-779) [108-109]. Rapamycin derivatives such as everolimus,

temsirolimus and deforolimus are potent inhibitors of mTOR and do not share the problems of poor solubility and chemical stability of rapamycin.

Recent data from two phase I trials suggest that everolimus can help overcome resistance to trastuzumab in women with HER-2+ MBC. Everolimus plus trastuzumab and weekly paclitaxel was shown to slow tumor growth in 77% of patients, and the combination of everolimus with trastuzumab and vinorelbine halted tumor growth in 62% of patients [110-111]. Although early indications suggest that targeting components of the PI3K pathway may have some activity in the treatment of MBC, additional data, including an understanding of combinations and patient selection, are required.

However, in unselected patients with breast cancer these agents have modest anti-tumor activity in the range of around 10% **[112]**. There is therefore a need to identify the subset of patients that may benefit from it, and PI3K/Akt/mTOR-dependency genetic signatures are being developed. In this direction, it has been recently observed that a majority of locally advanced and inflammatory breast cancers over-express the translation regulatory protein4E-BP1 and the initiation factor eIF4G, both of them are mTOR downstream targets. While additional studies are planned to further dissect this interaction, it does seem reasonable to explore the benefits of mTOR inhibitors in the treatment of locally advanced breast cancer [113].

Another potential explanation for the limited activity of mTOR inhibitors in breast cancer and other tumor types may be related to a 'collateral effect' of mTOR blockade. mTOR inhibition blocks the natural negative feed-back on IGF-1R signaling exerted on PI3K [114]. The result is an increase in PI3K and Akt activations which could potentially counteract the inhibition of mTOR. Thus, dual inhibition of both IGF-1 signaling, with either MAbs against the receptor or tyrosine kinase inhibitors, and mTOR results in superior anti-proliferative effect over each single strategy.

In the clinic there is indirect evidence that this approach may be fruitful as well. Octreotide has been shown to inhibit IGF-1R signaling. Although octreotide has limited activity in patients with refractory neuroendocrine tumors, it has been shown to that the combination of everolimus and octreotide has resulted in an impressive activity **[115]**.

## 2.7 STPs-4: HSP90 signaling pathway

Heat shock protein 90 (HSP90) is a molecular chaperone required for the stability and function of several conditionally activated and/or expressed signaling proteins ([116-117]. Many of these client proteins such as Akt, HER2, Bcr-Abl, c-Kit, EGFR and PDGFR-a are oncoproteins and important cell-signaling proteins [118-119]. As signal transducers and molecular switches, these client proteins are inherently unstable. HSP90 keeps unstable signaling proteins poised for activation until they are stabilised by conformational changes associated with the formation of signal transduction complexes. As such, it is a single molecular target that is a central integrator of multiple pathways important to cancer. Activation of HSP90-dependent client proteins proceeds through an ordered sequence of events linked to the ATPase activity of HSP90 and involves a variety of co-chaperone complexes **[120].**

HER2 is among the most sensitive client proteins of HSP90, demonstrating degradation within 2 h of HSP90 inhibition in cell culture experiments **[121]**. Geldanamycin analogues (17-allylamino- 17-demethoxygeldanamycin [17-AAG] and 17-dimethylaminoethylamino-

17-demethoxygeldanamycin [17-DMAG]) have demonstrated potent inhibition of HSP90 function in HER2-over-expressing cell lines, demonstrating significant anti-tumor activity in both cell culture and animal studies [121-122].

## 2.8 STIs-4: HSP90 inhibitors

(HSP90) is an exciting new therapeutic target, inhibition of which delivers a combinatorial attack on multiple oncogenic targets and pathways and on all of the hallmark traits of malignancy **[123]**. In the clinic, initial studies with the HSP90 inhibitor have demonstrated safety and anti-tumor activity and tolerability in combination with trastuzumab in patients with trastuzumab-refractory HER2- positive metastatic breast cancer patients **[120]**. It will be important to determine whether HSP90 inhibitors will have clinical activity as single agents in breast cancer patients.

## 2.9 STPs-5: PARP signaling pathway

Poly (ADP-ribose) polymerase 1 (PARP-1) is the initial and best characterised member of a family of enzymes largely associated with the maintenance of genomic stability. Activation of PARP-1 is part of the immediate cellular response to DNA strand breaks, converting them into an intracellular signal via poly (ADP-ribosylation) of nuclear proteins [124-125]. This results in a highly negatively charged target, which in turn leads to the unwinding and repair of the damaged DNA through the base excision repair pathway. In addition, PARP-1 is also known to bind dsDNA breaks (DSB) preventing accidental recombination of homologous DNA [126]. Upon binding DNA breaks, the catalytic activity of PARP-1 is stimulated >500- fold [127]. Enhanced PARP-1 expression or activity has been also observed in a number of different tumor cell lines and could provide a greater level of resistance to both endogenous genotoxic stress and to DNA damage-inducing therapeutic agents.

Studies of PARP expression in various tumor types identified that breast cancers with negative estrogen receptor, progesterone receptor and HER2 expression were much more likely to over-express PARP [128]. Additionally, it has been recently shown that BRCA1 and BRCA2 dysfunction sensitises cells to the inhibition of PARP enzymatic activity, resulting in chromosomal instability, cell cycle arrest and subsequent apoptosis. This seems to develop because the inhibition of PARP leads to the persistence of DNA lesions normally repaired by homologous recombination **[128]**.

## 2.10 STIs-5: PARP inhibitors

PARP inhibitors have been developed to investigate the role of PARP-1 in cell biology and to overcome DNA repair-mediated resistance of cancer cells to genotoxic agents [129]. These novel PARP inhibitors have been shown to enhance the anti-tumor activity of DNA-methylating agents, such as temozolomide, topoisomerase poisons and ionising radiation in preclinical studies [130], and to restore sensitivity of resistant tumors to methylating agents or topoisomerase I inhibitors, agents presently used for the treatment of breast cancer.

## 2.11 STPs-6: Apoptosis & autophagy signaling pathways

Apoptosis, the process of programmed cell death, is governed by complex, gene-directed pathways [131-133]. Dysregulation of apoptosis plays a key role in tumorigenesis and can allow tumor cells to become resistant to anticancer treatments [132-133]. Rationale for

targeting apoptosis in the treatment of breast cancer includes the over-expression of the Bcl-2 protein in 40%–80% of human breast tumors, which is associated with both resistance to chemotherapy **[134]** and a better prognosis after chemotherapy **[135].** Additionally, the association of Bcl-2 with ER and/or PgR, loss of expression of the gene for the pro-apoptotic protein Bax, and differential expression of tumor necrosis factor–related apoptosis-inducing ligand-receptor 2 have all been correlated with prognosis in breast cancer patients [134-137].

On the other hand, autophagy is an evolutionarily conserved lysosomal pathway for degrading cytoplasmic proteins, macromolecules, and organelles [138]. Many studies have described the role of autophagy as a protective mechanism for cell survival that is initiated in response to metabolic or therapeutic stress. Furthermore, autophagy may delay apoptotic cell death caused by DNA damaging agents and hormonal therapies [139-141].

### 2.12 STIs-6: Targeted therapies directed at the apoptotic & autophagic pathways

Anticancer agents targeting the components of apoptotic pathways are in the early stages of development, and no agent specifically targeting apoptosis has yet been approved for use in cancer treatment. A range of approaches is being tested, including antisense DNA oligonucleotides and antibody and small molecule inhibitors of the components of apoptotic pathways. Few clinical data are currently available in breast cancer; however, preclinical studies show that such agents do have anticancer activity, suggesting that this may be a promising approach, particularly when used in combination with chemotherapy [7].

On the other hand, recent studies have demonstrated that the inhibition of autophagy in cancer cells may be therapeutically beneficial in some circumstances, as it can sensitize cancer cells to different therapies, including DNA-damaging agents, antihormone therapies (e.g., tamoxifen), and radiation therapy. This supports the hypothesis that inhibiting autophagy can increase cell death when combined with anticancer agents, providing a therapeutic advantage against cancer proliferation & drug resistance [139-141].

### 2.13 STPs-7: Targeting the angiogenesis pathway

It is increasingly being accepted that tumor cell proliferation alone is insufficient to result in a substantial tumor mass. Angiogenesis is essential for tumors to develop into detectable localized masses, and for metastasis to occur [142-143]**.** The process of angiogenesis (the formation of new blood vessels from a pre-existing vascular bed) is complex and dynamic, and it is regulated by a range of pro- and anti-angiogenic molecules **[144].** The VEGF and platelet-derived growth factor (PDGF) families of proteins and their receptors (VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-$\alpha$, and PDGFR-$\beta$) appear central to the process **[144].** Activation of VEGFRs and PDGFRs initiates signaling that results in numerous cellular responses, including survival, mitogenesis, migration, proliferation, and differentiation [144-145]. Activation of the VEGF pathway also increases vascular permeability and the movement of endothelial progenitor cells from the bone marrow into the peripheral circulation [144]**.** Primary breast tumors express a variety of different angiogenic factors, with VEGF being the most abundant.

High VEGF expression appears to be correlated with poor clinical prognosis and response **[146]**. Levels of VEGF in breast cancer tumors are a prognostic factor for relapse-free and

overall survival in patients with both lymph node–negative and lymph node–positive disease [147-148], and they predict response to both tamoxifen and chemotherapy in advanced disease **[149]**. Similarly, a proportion of invasive breast cancers that over-express PDGFR-α have been associated with greater biological aggressiveness and a higher likelihood of lymph node metastasis [150].

Given their central roles in tumor angiogenesis and growth, the VEGF and PDGF signaling pathways are key targets for breast cancer therapy. However, with considerable redundancy in angiogenic signaling pathways, the inhibition of more than one receptor is likely necessary to block angiogenesis. It has been hypothesized that anti-VEGF agents may prevent the development of new tumor vasculature and induce normalization of existing, inefficient tumor vasculature (resulting from over-expression of VEGF) **[151]**. These agents, then, may allow better delivery of cytotoxic therapies to the tumor, suggesting a potential role for anti-VEGF therapy in conjunction with chemotherapy [151-152].

### 2.14 STIs-7: Targeted therapies directed at angiogenesis

Several therapies targeting angiogenesis are in development for breast cancer. These include monoclonal antibodies that act extracellularly by binding to receptors or their ligands, such as bevacizumab (Avastin®), and RTKIs that act intracellularly, such as sunitinib (Sutent®). Intracellular VEGF targeting therapeutics will be discussed later as they were proven to be targeting multiple sites.

**Monoclonal antibodies: Bevacizumab**

Bevacizumab is an anti-VEGF humanized monoclonal antibody administered as an i.v. infusion. It acts by binding to all VEGF isoforms, thus removing VEGF from the circulation and preventing activation of VEGFRs **[153]**. In 2008, the U.S. Food and Drug Administration (FDA) approved bevacizumab in combination with paclitaxel for the first-line treatment of locally recurrent breast cancer or MBC **[7]**.

Although bevacizumab has shown little activity as a single agent in MBC patients, combination therapy with chemotherapeutic agents has been associated with clinical activity in this patient population. Several phase III studies have investigated bevacizumab combined with chemotherapy [154-155]. These data suggest that VEGF inhibition combined with chemotherapy is a promising treatment strategy in this setting. Further studies are under way to explore the use of bevacizumab with different chemotherapeutic regimens, hormonal treatments, and other targeted therapies (including lapatinib and trastuzumab) in patients with MBC. Additionally, trials of bevacizumab are ongoing in the adjuvant and neoadjuvant settings, and preliminary reports suggest that this approach may be feasible.

Hypertension, bleeding, and thrombosis remain potential safety concerns with a number of anti-VEGF therapies, and this area requires further study. Future trials should focus on identifying those patients who will derive the most benefit from bevacizumab- based regimens and how best to combine bevacizumab with other cancer therapies (which therapies should be combined and whether sequential or concurrent administration is most effective). Overall, growing clinical experience with agents targeting angiogenic processes, such as bevacizumab, has provided proof of concept for the use of these treatments in MBC patients **[7]**.

## 2.15 STPs-8: Multi-target therapeutics

There appears to be extensive crosstalk between the pathways driving tumorigenic processes, and this provides a good rationale for inhibiting multiple pathways and processes with multi-targeted agents, either as single agents or in combination [7]. A number of single-agent RTKIs with multiple molecular targets have been developed as an alternative to combining multiple agents. These were developed based on previous studies showing that combining agents that target different pathways may have synergistic activity and delay or reverse resistance [156-158].

## 2.16 STIs-8: Multi-target inhibitors

A range of oral, antiangiogenic RTKIs with multiple targets is currently in development for MBC. These include sunitinib, pazopanib, sorafenib (Nexavar®) and axitinib.

**Sunitinib** selectively inhibits several receptor tyrosine kinases (VEGFR-1, VEGFR-2, VEGFR-3, PDGFR- α, PDGFR-β, Kit, FMS-like tyrosine kinase [FLT]-3, and colony-stimulating factor 1 receptor). It has both anti-angiogenic and anti-tumor activities. Sunitinib has been shown to have antitumor activity in breast cancer preclinical studies, both as a single agent and in combination with chemotherapy [159-163]**.**

Data from a phase II study of sunitinib monotherapy in patients with refractory MBC reported single-agent activity in heavily pretreated patients, previously exposed to an anthracycline and a taxane [164]. The toxicities were manageable [164]. Studies and case series evaluating sunitinib given in combination with taxane therapy for MBC have reported antitumor activity and a manageable and tolerable safety profile [165-166]. Phase III trials of sunitinib in combination with a variety of cytotoxic agents are under way in first- and second-line MBC therapy [7].

**Pazopanib** targets VEGFR, PDGFR, and Kit and is currently in development in a number of tumor types, including breast cancer. Although originally developed as a Raf inhibitor, sorafenib also inhibits the activity of VEGFR-2 and VEGFR-3, PDGFR-β, FLT-3, and Kit; thus, it may inhibit tumor growth both directly (through Raf and Kit) and indirectly, through inhibition of angiogenesis [167-168].

**Sorafenib** inhibited MAPK activity in breast cancer cell lines expressing mutations of K-Ras or B-Raf, and showed antitumor and antiangiogenic activity in a human breast cancer xenograft model [168]**.** Significant and sustained increases in blood pressure were reported in a study of sorafenib monotherapy in patients with metastatic solid tumors **[169]**. Current data suggest little activity for sorafenib as a single agent in MBC patients; ongoing studies are exploring combination treatment with paclitaxel and with anastrozole in MBC.

**Axitinib** inhibits all known VEGFRs, in addition to PDGFR-β and the stem cell factor receptor Kit, and is currently being investigated in a range of tumor types, including breast cancer. In preclinical studies, axitinib was shown to selectively block VEGF-stimulated receptor phosphorylation in vitro, resulting in the inhibition of endothelial cell proliferation and survival. In a human breast cancer xenograft model, it significantly inhibited tumor growth and disrupted tumor microvasculature as assessed by dynamic contrast-enhanced magnetic resonance imaging **[170]**.

To date, the mult-itargeted RTKIs discussed have not been validated in phase III trials in MBC patients, although there is preliminary evidence of clinical activity. Of the four agents

described above, three (pazopanib, sunitinib, and axitinib) appear to have the most clinical activity to date. Based on experience with other targeted agents in breast cancer, and with these RTKIs in other indications, combinations will hopefully show greater efficacy in the treatment of breast cancer [7].

## 2.17 STPs-9: Estrogen signaling transduction

Two of the genetic subtypes in breast cancer so far identified are those with gene expression characteristics typical of basal epithelial cells (which are predominantly estrogen receptor [ER-) and those with gene expression characteristics typical of luminal epithelial cells (which are predominantly ER+) [14, 171]. Typically, tumors of the ER-, basal subtype are associated with shorter relapse-free and overall survival times than those of the ER+, luminal subtype [171].

## 2.18 STIs-9: Estrogen signaling inhibitors

Endocrine therapy is widely used in the treatment of both early stage and recurrent/metastatic breast cancer. Tamoxifen and estrogen deprivation therapies such as aromatase inhibitors (AIs) and ovarian suppression have proved clinically effective and are generally well tolerated; they are the primary reason for the sustained improvement in survival for patients with early-stage [ER+] breast cancer [172]. Their long-term efficacy, however, is limited by relapse of disease and development of resistance following adjuvant endocrine therapy. This provides a strong rationale for using targeted agents against various growth factor and signaling pathways that are activated during endocrine resistance and for combining these targeted agents with ongoing endocrine therapy to either overcome endocrine resistance and enhance the efficacy of therapy for ER-positive breast cancer [173-174].

In this context, several selected targeted agents are being investigated in combination with endocrine therapy for patients with breast cancer in an attempt to overcome or prevent endocrine resistance. The role of EGFR/HER2 cross-talk with estrogen receptor (ER) signaling has been confirmed in preclinical studies [175] in which various inhibitors have yielded additive or synergistic effects when combined with endocrine agents. Recently, several results from clinical trials investigating this concept have been reported [59, 92].

## 2.19 STPs-10

Cancer progression is a multi-step process that enables tumor cells to invade through extracellular tissues and metastasize to distal organs **[8]**. Compelling recent evidence demonstrates that cooperation between signals from the extracellular matrix (ECM) and growth factors enhances malignant behaviors of aggressive cancer cells, such as proliferation, migration, survival, and invasion [176]. Examples of such cross talk signals include:

a.  **The STATs** are a family of transcription factors that relay the interactions of cytokines and growth factors with their receptors at the cell surface to mediate changes in gene expression. In normal situations STATs are transiently activated; however, in many tumors tyrosine-phosphorylated STATs, mainly STAT3 and STAT5, can be detected, suggesting constitutive pathway activation [6].

b.  **The NF-κB** pathway is triggered in response to microbial and viral infections and to pro-inflammatory cytokines. These agents activate the IκB kinase, which phosphoylates IκB allowing the liberated NF-κB family transcription factors to enter the nucleus. The pathway has an ever increasing interest for its role in human cancer [177]. The

association of NF-κB pathway activity with inflammation-associated tumor progression is well documented in mouse tumor models [178] and in human cancer cells [179].

c.  **The Notch network** in mammals consists of four Notch receptors and five ligands. Once activated, the receptor undergoes intramembrane proteolysis leading to release and nuclear translocation of the intracellular domain, which has transcriptional activity. The Notch-4 gene is frequently rearranged in mammary glands by MMTV proviral integration. This rearrangement leads to expression of the intracellular domain under MMTV promoter control [180] an early demonstration of Notch's ability to induce mammary cancer.

d.  **The UPS pathway**: The ubiquitin proteasome system (UPS) consists of several crucial enzymes contributing to most, if not all, cellular events. During the past decade, progress in endocrine therapy and the use of trastuzumab has significantly contributed to the decline in breast cancer mortality for hormone receptor-positive and HER2-positive cases, respectively. As a result of these advances, a breast cancer cluster with poor prognosis that is negative for the estrogen receptor (ER1), the progesterone receptor (PRGR) and HER2 (triple negative; basal like phenotype) has come to the forefront of medical therapeutic attention. DNA microarray analyses have revealed that this cluster is phenotypically most like the basal-like breast cancer that is caused by deficiencies in the **BRCA1 pathways**. BRCA1 acts as a hub protein that coordinates a diverse range of cellular pathways to maintain genomic stability [181]. Indeed, BRCA1 dysfunction in sporadic basal-like cancers has been reported [182]. Thus, investigating the BRCA1 pathway could be an important approach for the treatment of basal-like breast cancer.

### 2.20 STIs-10

To gain further improvements in breast cancer survival, new types of drugs might be required, and small molecules targeting the ubiquitin proteasome system have moved into the spotlight. The success of bortezomib in the treatment of multiple myeloma has sent encouraging signals that proteasome inhibitors could be used to treat other types of cancers [183]. In addition, ubiquitin enzymes involved in ER1, HER2 or BRCA1 pathways could be ideal targets for therapeutic intervention. The therapeutic effect of proteasome inhibitors on breast cancer remains to be determined but is greatly anticipated [184].

## 3. Targeting molecular signal in breast cancer stem cells responsible for development of multi-drug resistance (MDR)

Success in Breast cancer chemotherapy is challenged by the development of tumors having a multi-drug resistance (MDR) phenotype **[185]**. It is one of the major causes of failures to cancer chemotherapy. This phenotype is most prevalent in aggressive carcinomas as breast and ovarian carcinomas. By definition, MDR is a term used to describe the resistance developed by some tumors to protect themselves against a number of structurally and functionally unrelated chemotherapeutic agents. MDR is not only referred to the drug with which the patient has been treated but also to a wide range of other drugs used in cancer chemotherapy **[186]**.

Acquired drug resistance arises from exposure of tumor cells to chemotherapeutic agents. Random spontaneous mutations, acceleration of proliferation rate and alteration of cell

sensitivity to growth factors can occur in tumor cells under the influence of cytotoxic drugs. On the other hand, MDR does not only develop as the result of treatment of tumor cells by a drug. It may be intrinsic, i.e. connected with the type of cell differentiation or genetic profile of tumor cells **[187]**.

Studies of resistance of tumor cells to cytotoxic drugs are necessary for understanding the mechanisms for restoring back their sensitivity. Although, MDR is a multi-factorial problem i.e. multiple mechanisms were hypothesized to account for this phenomenon, some of them were frequently observed and their clinical significance was determined. These mechanisms are acting either alone or in concert with each other for the development of the MDR phenotype in breast cancer cells **[188]**. Over-expression of ABC transporters, detoxification enzymes (aldehyde dehydrogenase), low cell turn over rate and the ability to activate the DNA check point response are possibly all involved and previously described with other mechanisms **[189]**. Innovative therapies, based on a better understanding of cancer stem cells, should lead to enhanced and long-term cure rates in breast cancer.

Given that tumor resistance to chemotherapy is believed to account for the majority of treatment failure in breast cancer, research attention in the last two decades focused on developing agents to reverse MDR and enhance the response of tumors to chemotherapeutic agents. Although hundreds of compounds have been found in-vitro to be able to modulate the MDR phenotype, their clinical application was limited owing to their high toxicity in-vivo [190-191]. Accordingly, searching for compounds able to modulate the MDR phenotype and have low toxicity continues to be an important challenge for optimizing cancer chemotherapy. The development of several therapeutics targeting the MDR phenotype in breast cancer cells have been previously reviewed **[189]**.

Recently, the possible roles of cancer stem cells in carcinogenesis have become more obvious. Numerous investigations have recently provided evidence that the genetic alterations occurring in the multi-potent tissue-specific adult stem cells and/or their early progenies may lead to their malignant transformation into cancer progenitor cells also designated as cancer stem cells or cancer-initiating cells **[192]**. A small population of undifferentiated- or poorly differentiated cancer progenitor cells, which possesses the stem cell-like properties including their self-renewal ability and capacity to give rise to the bulk mass of further differentiated malignant cells, appears to represent the principal cancer cells that are responsible for tumor formation [193]. Accumulating genetic alterations in tumorigenic cancer progenitor cells occurring during cancer progression may also confer to them the invasive and resistant properties that are essential for their remission and migration to distant metastatic sites [194-195].

The role of cancer stem cells in breast cancer chemoresistance was recently reviewed [196]. One characteristic of cancer stem cells that differentiates them from other normal cells in the tumor is that they have high levels of ABC transporter proteins, in particular ABCG2 [197]. The ABC transporter molecules are responsible for protecting cells from drug damage via efflux pumping mechanisms. Thus, cancer stem cells, as a result of these biological properties, are rendered resistant to drug treatment, including chemotherapeutic drugs [198].

Since all the stem cell-like properties attributed to cancer progenitor cells may provide them with a higher resistance to current cancer therapies, thus they constitute a substantial obstacle to the successful treatment of cancer patients [196]. This finding underlines the critical importance of targeting the cancer progenitor cells and their early progenies as well as their local microenvironment in the earlier stages of cancer treatment to counteract the

rapid progression of certain cancer types and prevent the metastatic spread at distant sites. The simultaneous blockade of several oncogenic cascades activated in cancer progenitor cells during cancer progression may be essential for improving the current clinical treatments against high-risk, metastatic or relapsed breast cancers [189, 199].

Recent studies have revealed the possibility of using therapeutic agents targeting the EGFR, Wnt/β-catenin and/or Notch cascades to inhibit the ABC muli-drug efflux transporters and/or eliminate the cancer progenitor cells **[200]**. Moreover, one compound, salinomycin, reduces the proportion of cancer stem cells by >100-fold relative to paclitaxel, a commonly used breast cancer chemotherapeutic drug [201]. In addition, treatment of mice with salinomycin inhibits mammary tumor growth in vivo and induces increased epithelial differentiation of tumor cells **[199]**. What remains to be discovered is the extensive and in depth understanding of the molecular basis of cancer stem cell contribution to breast cancer development. Such extensive investigations enable the development of more effective and selective treatment strategies.

## 4. Concluding remarks and perspectives

Considerable progress has been made over the last several decades in understanding specific cellular, molecular and genetic mechanisms that contribute to cancer growth and progression **[202]**. In recent years, research efforts have focused on the signaling pathways involved in the growth and survival of breast cancer cells, leading to the development of a range of targeted agents with promising clinical activity. The encouraging success of trastuzumab, based on the identification of HER-2 as a molecular target, has provided the rationale for studying the array of targeted agents currently in clinical development for breast cancer **[7]**.

We have reviewed some of the most promising new targeted agents in breast cancer. This list, however, is far from complete. It should be pointed out that there are still many important additional classes of agents in clinical development in breast cancer. Examples include other tyrosine kinase inhibitors and also enhancers of apoptosis. We all hope that this list will keep developing. However, despite recent advances, there are still unanswered questions regarding the management of breast cancer with targeted agents.

With our knowledge of the molecular modifications in breast cancer and the increasing number of new targeted therapeutics, the current challenge is to select the best combinations of the so-called signal transduction inhibitors (STIs) for every patient [6]. The development of these targeted therapeutics will require a new set of challenging skills & investigations of unresolved issues **[120]**. These challenges are:

**First,** future studies are necessary to identify those patient subgroups likely to derive most benefit from a given therapy. Despite the temptation to use a targeted agent in all patients, identification of patient subgroups most likely to benefit must be a key goal and will be critical to the successful future use of these treatments **[7]**. These agents, unlike chemotherapy, will only work in the subset of tumors that show dependency on the target the therapy is being directed to.

If STI would have been developed in an unselected patient population, its anti-tumor activity would have been missed due to a dilutional effect brought in by the non-STPs-over-expressing population. This principle probably applies to the majority of classes of agents under study. The implication of this principle is that patient selection strategies will be of paramount importance in the development of these agents [120]. For example, accurate

patient selection based on HER-2 over-expression is essential for trastuzumab-based treatment and is likely to be important for other agents in this class. However, identifying suitable patients may prove more difficult for RTKIs, because receptor over-expression alone does not seem to predict response to treatment [16].

**Second,** future studies are necessary to determine the optimal combinations, doses, and schedules required to maximize clinical activity while minimizing toxicity. Also, in early clinical studies with these agents in addition to establishing their safety and optimal doses and schedules, it may prove to be instrumental to also check for the presence of the target in the studied tumors and to seek for indications of target engagement with the study agent using careful analysis of [120]**2007**). The need to determine target engagement is currently being taken into account with the majority of clinical trials with novel agents that are moved into the clinic.

**Third,** Predictive markers are factors that are associated with upfront response or resistance to a particular therapy. Predictive markers are important in oncology as tumors of the same tissue of origin vary widely in their response to most available systemic therapies. Currently recommended oncological predictive markers include both estrogen and progesterone receptors for identifying patients with breast cancers likely to benefit from hormone therapy, HER-2 for the identification of breast cancer patients likely to benefit from trastuzumab [203]. Thus, the drive to identify new biomarkers of target engagement and sensitivity with these novel agents is also promoting the search for new clinical study designs in a minimally pre-treated population **[120]**.

**Fourth,** future studies will need to address how best to incorporate these agents into existing treatment regimens and to determine when and in which combinations targeted therapy should be administered **[7]**. Some of these agents will have limited activity by themselves and yet have the capacity to markedly enhance the anti-tumor activity of conventional agents like chemotherapy or even other biological agents. For example, anti angiogenesis MAb bevacizumab that has no activity as a single agent and yet is clinically active when combined with chemotherapy. Furthermore, HER-targeted agents may need to be used in combination with chemotherapy to provide clinically relevant activity.

**Fifth,** the therapy end-points with these agents also need to be revisited. Some of these agents are not expected to result in tumor shrinkage (or response), and therefore we cannot propose a unified definition of clinical benefit as it has been done with chemotherapy in the past. Furthermore, genetic assay techniques used to provide information on clinical outcomes, including the risk for tumor recurrence and individual benefit from a particular chemotherapy also needs further critical development **[204]**.

**Sixth,** Targeting HER-2 is associated with cardiac toxicity, which is an especially important consideration in the adjuvant setting and when combining anti–HER-2 agents with cardiotoxic chemotherapeutic drugs. Targeting HER-1 in combination with HER-2, as with the RTKIs lapatinib and neratinib, appears to reduce the risk for cardiotoxicity, although the exact mechanisms underlying this observation remain unclear **[7]**.

**Finally,** with the increase in the number of available lines of therapy in breast cancer, there is a danger that novel agents will be tested in a heavily pre-treated patient population. While there is little debate if patients with advanced disease receiving multiple lines of therapy may be appropriate study participants in early clinical studies. There is also a growing concern that patients with advanced disease may not be the ideal population to detect the anti-tumor activity of novel agents since their tumors may have become highly resistant to any type of therapy **[120]**.

In summary, the contribution of STIs in cancer chemotherapy continues to be encouraging, and clinical trials are currently addressing whether the promising preclinical activities of STIs will translate into benefits for patients. Hopefully, the previously mentioned suggestions and mechanisms will ultimately be put into practice. Moreover, cellular responses to cancer chemotherapeutic agents are complex and several mechanisms are commonly associated with the resistance of laboratory human breast cancer cell lines. Clinical testing of breast cancer STIs like other modern day targeted therapeutics must be rationally developed with a firm basis in the lessons learned in the laboratory and with proper selection of patient populations in which the predictive power and the potential for benefit is greatest.

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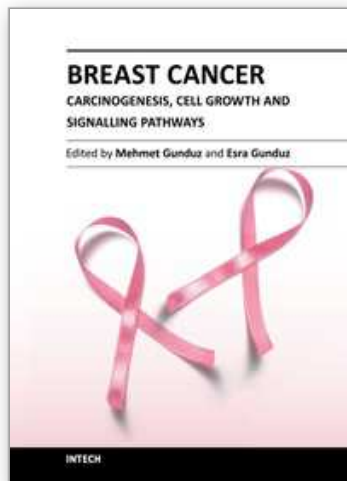
**Breast Cancer - Carcinogenesis, Cell Growth and Signalling Pathways**

Edited by Prof. Mehmet Gunduz

Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In this book, we discussed various aspects of breast cancer carcinogenesis from clinics to its hormone-based as well as genetic-based etiologies for this deadly cancer. We hope that this book will contribute to the development of novel diagnostic as well as therapeutic approaches.

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