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Review article

Novel Targets and Breast Cancer Treatment

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Abstract

Breast cancer is considered as second most common cancer in women, after skin cancer. One out of every 8 woman in the United States is predisposed to develop breast cancer. It is usually caused by genetic mutations, using certain medication and few even hereditary disorders. Surgery, radiation therapy and chemotherapy are the traditional and mainstay of treatment of breast cancer. However, novel therapies such as targeted therapy have been subject to serious consideration. Targeted therapy mainly focuses on the molecular pathways responsible in process of carcinogenesis, tumor promotion and metastasis. In this paper, we discuss the causes, epidemiology, and potential targeted therapies techniques to treat breast cancer.

Keywords: Breast Cancer; Immunotherapy; Estrogen; Monoclonal Antibodies; T-Cell Therapy; Vaccines; Checkpoint Inhibitors

Introduction/Epidemiology

Breast cancer is one of the leading causes of death worldwide. National Cancer Institute estimated that there would be 232,670 new cases of breast cancer in females and 2,360 new cases in males in 2014. It is also estimated that there would be 40,000 cases of deaths in females and 430 cases of deaths in males in the United States in the same year [1]. As per the statistical analysis, in the United States of America (USA), breast cancer is the second most common leading cause of death in women and around 1 in every 8 women will develop invasive breast cancer in their lifetime [2]. The annual age-standardized incidence rates in various countries are as follows: North America, 90; Central America, 42; Western Europe, 78; Northern Europe, 73; Southern Europe, 56; South and Eastern Europe, 49; East Asia, 18; North Africa and Western Asia, 28; South-East Asia, 26; South Central Asia, 22; Oceania, 74; and sub-Saharan Africa, 22 per 100,000 females. The USA has higher incidences as compared to rest of the world [3].

Generally, breast cancer initiates in the lining of the ducts or lobules that supply them with milk. A breast cancer that initiates in the ducts is called a ductal carcinoma. If it initiates in the lobules, it is called as lobular carcinoma [1]. Race, ethnicity, breast feeding, hormone therapy, oral contraceptives, age at menarche, parity, first live birth, menopausal status, alcohol, diet, anthropometric factors, physical activity, environmental exposures, occupational exposures and mammographic breast density are the various risk factors of breast cancer [4].

White women are more prone to breast cancer than African-American women. But African-American women are more likely to develop more advanced-stage breast cancer, be diagnosed at a young age and are more likely to die from this cancer. However, in women under 45 years of age, breast cancer is more common in African-American women. Asian, Hispanic, and Native-American women have a lower risk of developing and dying from breast cancer. Breastfeeding for a year or more slightly reduces a woman's overall risk of breast cancer.

Results from observational studies indicate that hormone replacement therapy after menopause increases the risk of breast cancer. Use of a regimen that includes both progesterone and estrogen has been linked with a higher risk of breast cancer than the use of estrogen alone. Women

who do not currently use hormone therapy may also undergo screening mammography less frequently as it plays a role in declining the incidence of breast cancer. Various studies have found that women using oral contraceptives (birth control pills) have a slightly greater risk of breast cancer than women who have never used them. Once women stop taking these pills, the risk might reduce over time. Women who stopped using oral contraceptives for more than 10 years do not appear to have any increased breast cancer risk.

The use of alcohol is clearly linked to an increased risk of developing breast cancer. The increase in risk is directly proportional to the amount of alcohol intake. High-fat diets can lead to being overweight or obese, which is a breast cancer risk factor. A diet high in fat has also been shown to influence the risk of developing several other types of cancer. Also, intake of certain types of fat is clearly related to heart disease risk. Women who have had more menstrual cycles because of early menarche (menstruating before 12) and/or went through menopause late (after age 55), have a slightly higher risk of developing breast cancer. The increase in risk may be due to a long exposure to estrogen and progesterone.

Pathophysiology and molecular basis

Well-defined molecular subtype's of breast cancer are luminal (luminal A and luminal B) or hormone-sensitive, HER2-positive, molecular apocrine subtype and triple negative tumors [5]. The luminal molecular subtype includes estrogen (ER) and progesterone (PR) receptor [6]. The identification of Luminal B subtype is done by the co-expression of HER2, in addition to ER and PR, HER2-negative luminal-A subtype, or by higher proliferative activity [6,7]. There is a lack of expression of ER and PR in HER2 positive breast cancer, but it is defined by the overexpression of HER2 protein by immunohistochemistry and/or HER2/neu gene amplification [6]. Breast cancer negative for ER, PR and HER2 protein expression is called triple negative. It partially overlaps with basal-like subtype showing expression of basal cytokeratins that is normally present in the basal cell of mammary ducts.

New molecular subtypes are recently noted in breast cancer. The claudin-low subtype includes triple negative breast cancers, which lacks cytokeratin 5/6 and epidermal growth factor receptor in contrast to basal triple negative subtype [6,8]. The molecular apocrine breast cancers are characterized by ER negativity and androgen receptor positivity in addition to apocrine morphology with the presence of intracellular vacuoles [9].

Both HER2-positive and triple negative breast cancers have a higher tendency to develop metastases in visceral locations or in the central nervous system. The molecular type also act as an indication for treatment: luminal type can be targeted by hormone therapy, HER2-positive tumor – by anti-HER2 agents, and triple negative – by chemotherapy. Triple-negative breast cancer cells also are dependent on poly ADP Ribose polymerase (PARP) to repair single strand breaks in DNA. Additionally, PARP inhibition also serves as an effective treatment modality [5].

A. Tyrosine kinase inhibitors:

a. FDA approved Tyrosine Kinase Inhibitors:

1. Lapatinib[10]:
Lapatinib is indicated in combination with capecitabine for the treatment of advanced and metastatic breast cancer patients whose tumors overexpressed HER2 and patients who have received prior therapy with trastuzumab, an anthracycline and a taxane. It is also indicated in combination with letrozole for the treatment of postmenopausal women with hormone receptor positive, metastatic, breast cancer that overexpresses the HER2 receptor for whom hormonal therapy is also indicated.

The peak plasma concentration (Cmax) of lapatinib occurs around 4 hours after the administration. Steady state is received in 6-7 days. The area under the curve (AUC) is 2 fold higher with separated daily dose as compared to the single dose administration. When it is taken with a high or low fat meal, the AUC improves 3 to 4 fold, respectively. Lapatinib is highly bound to albumin and alpha-1 glycoprotein more than 99%.

The most common adverse effects during the treatment of breast cancer with Lapatinib were diarrhea, hand, foot and mouth disease, nausea, rash, vomiting, fatigue or weakness, loss of appetite, indigestion, unusual hair loss or thinning, epistaxis, headache, dry skin, itching, nail disorders, such as nail bed changes, nail pain, infection and swelling of the cuticles. Lapatinib has been reported to decrease LVEF. The dose of Lapatinib should be reduced during the pre-existing hepatic impairment. Lapatinib should be discontinued in patients who are suffering from interstitial lung disease or pneumonitis. Lapatinib can cause fetal harm. It should be avoided in pregnant women. Hepatotoxicity may occur after some days of treatment. If there is any change in the liver function, then the treatment with Lapatinib should be stopped.

2. Palbociclib [11]: It is a cyclin-dependent kinase (CDK) inhibitor with potential antineoplastic activity. Palbociclib selectively inhibits cyclin-dependent kinase 4 (CDK4) and 6 (CDK6), thereby inhibiting retinoblastoma (Rb) protein phosphorylation early in the G1 phase leading to cell cycle arrest. This suppresses DNA replication and decreases tumor cell proliferation. CDK4 and 6 are serine/threonine kinases that are upregulated in many tumor cell types and play a key role in the regulation of cell cycle progression.

Palbociclib is indicated in combination with letrozole for the treatment of postmenopausal women with estrogen receptor (ER) positive, human epidermal growth factor receptor 2 (HER2)-negative, advanced breast cancer as initial endocrine-based therapy for their metastatic disease. The volume of distribution of palbociclib was 2583 L and it was 85% bound to human plasma protein. It is metabolized through CYP3A and SULT2A1 and its half-life was 29 hours.

The most common adverse effects are neutropenia, leukopenia, anemia, peripheral neuropathy, nausea, diarrhea, vomiting, fatigue, asthenia and alopecia. Decreased neutrophil counts have been observed in clinical trials with palbociclib. Pulmonary embolism has been reported at a higher rate in patients treated with palbociclib plus letrozole (5%). Treatment should be discontinued. Palbociclib can cause fetal harm. It should not be administered in pregnant women.

b. Kinase inhibitors under trial: Many of the kinase inhibitors are under clinical trials phase I-III as listed in table 1.

1.Neratinib: This is a 6,7-disubstituted-4-anilinoquinoline-3-carbonitrile irreversible inhibitor of the HER-2 receptor tyrosine kinase with potential antineoplastic activity. Neratinib binds to the HER-2 receptor irreversibly, thereby reducing autophosphorylation in cells by targeting a cysteine residue in the ATP-binding pocket of the receptor. Treatment of cells with this agent results in inhibition of downstream signal transduction events and cell cycle regulatory pathways; arrest at the G1-S (Gap 1/DNA synthesis) phase transition of the cell division cycle; and ultimately decreased cellular proliferation. Neratinib also inhibits the epidermal growth factor receptor (EGFR) kinase and the proliferation of EGFR-dependent cells.

2. LEE011: It is a cyclin-dependent kinase (CDK) inhibitor targeting cyclin D1/CDK4 and cyclin D3/CDK6 cell cycle pathway, with potential antineoplastic activity. CDK4/6 inhibitor LEE011 specifically inhibits CDK4 and 6, thereby inhibiting retinoblastoma (Rb) protein phosphorylation. Inhibition of Rb phosphorylation prevents CDK-mediated, G1-S phase transition, thereby arresting the cell cycle in the G1 phase, suppressing DNA synthesis and inhibiting cancer cell growth. Overexpression of CDK4/6, as seen in certain types of cancer, causes cell cycle deregulation.

3. PLX3397: This is a small-molecule receptor tyrosine kinase (RTK) that has potential antineoplastic activity and inhibits KIT, CSF1R and FLT3. Multitargeted tyrosine kinase inhibitor, PLX3397, binds to and inhibits phosphorylation of stem cell factor receptor (KIT), colony-stimulating factor-1 receptor (CSF1R) and FMS-like tyrosine kinase 3 (FLT3), which may result in the inhibition of tumor cell proliferation and down-modulation of macrophages, osteoclasts and mast cells involved in the osteolytic metastatic disease. FLT3, CSF1R and FLT3 are overexpressed or mutated in many cancer cell types and play major roles in tumor cell proliferation and metastasis.

4. KX2-391: This is a small molecule, Src kinase inhibitor with potential antineoplastic activity. Unlike other Src kinase inhibitors, which bind to the ATP-binding site, Src kinase inhibitor KX2-391 specifically binds to the peptide substrate binding site of Srckinase; inhibition of kinase activity may result in the inhibition of primary tumor growth and the suppression of metastasis. Src tyrosine kinases are upregulated in many tumor cells and play important roles in tumor cell proliferation and metastasis.

5. LY2780301: It is an inhibitor of the serine/threonine protein kinase, Akt (protein kinase B), and has potential antineoplastic activity. Akt inhibitor LY2780301 binds to and inhibits the activity of Akt, which may result in inhibition of the PI3K/Akt signaling pathway. This will lead to inhibition of cell proliferation and the induction of apoptosis in tumor cells. Activation of the PI3K/Akt signaling pathway is frequently associated with tumorigenesis. Dysregulated PI3K/Akt signaling may contribute to tumor resistance to a variety of antineoplastic agents.

6. Ruxolitinib: This is a Janus-associated kinase (JAK) inhibitor with potential antineoplastic and immunomodulating activities. Ruxolitinib specifically binds to and inhibits protein tyrosine kinases JAK 1 and 2, which may lead to a reduction in inflammation and an inhibition of cellular proliferation. The JAK-STAT (signal transducer and activator of transcription) pathway plays a key role in the signaling of many cytokines and growth factors and is involved in cellular proliferation, growth, hematopoiesis, and the immune response. JAK kinases may be upregulated in inflammatory diseases, myeloproliferative disorders, and various malignancies.

7. Refametinib: This is a selective MEK inhibitor with potential antineoplastic activity. Refametinib specifically inhibits mitogen-activated protein kinase, kinase 1 (MAP2K1 or MAPK/ERK kinase 1), resulting in inhibition of growth factor-mediated cell signaling and tumor cell proliferation. MEK, a dual specificity threonine/tyrosine kinase, is a key component of the RAS/RAF/MEK/ERK signaling pathway that regulates cell growth. Constitutive activation of this pathway has been implicated in many cancers.

8. CLR457: This is a pan inhibitor of phosphatidylinositol-3-kinase (PI3K), with potential antineoplastic activity. Upon oral administration, pan-PI3K inhibitor CLR457 inhibits all of the PI3K kinase isoforms, which may result in apoptosis and growth inhibition in tumor cells overexpressing PI3K. Activation of the PI3K pathway promotes cell growth, survival, and resistance to both chemotherapy and radiotherapy.

9. Cabozantinib: This is a small molecule receptor tyrosine kinase (RTK) inhibitor with potential antineoplastic activi-

ty. Cabozantinib strongly binds to and inhibits several RTKs, which are often overexpressed in a variety of cancer cell types, including hepatocyte growth factor receptor (MET), RET (rearranged during transfection), vascular endothelial growth factor receptor types 1 (VEGFR-1), 2 (VEGFR-2), and 3 (VEGFR-3), mast/stem cell growth factor (KIT), FMS-like tyrosine kinase 3 (FLT-3), TIE-2 (TEK tyrosine kinase, endothelial), tropomyosin-related kinase B (TRKB) and AXL. This may result in an inhibition of both tumor growth and angiogenesis, and eventually leads to tumor regression.

10. Dovitinib: It is a benzimidazole-quinolinone compound with potential antineoplastic activity. Dovitinib strongly binds to fibroblast growth factor receptor 3 (FGFR3) and inhibits its phosphorylation, which may result in the inhibition of tumor cell proliferation and the induction of tumor cell death. In addition, this agent may inhibit other members of the RTK superfamily, including the vascular endothelial growth factor receptor, fibroblast growth factor receptor 1, platelet-derived growth factor receptor type 3, FMS-like tyrosine kinase 3, stem cell factor receptor (c-KIT), and colony-stimulating factor receptor 1, this may result in an additional reduction in cellular proliferation and angiogenesis and the induction of tumor cell apoptosis. The activation of FGFR3 is associated with cell proliferation and survival in certain cancer cell types.

11. Gefitinib: This is an anilinoquinazoline with antineoplastic activity. Gefitinib inhibits the catalytic activity of numerous tyrosine kinases including the epidermal growth factor receptor (EGFR), which may result in inhibition of tyrosine kinase-dependent tumor growth. Specifically, this agent competes with the binding of ATP to the tyrosine kinase domain of EGFR, thereby inhibiting receptor autophosphorylation and resulting in inhibition of signal transduction. Gefitinib may also induce cell cycle arrest and inhibit angiogenesis.

12. MK2206: It is an allosteric inhibitor of the serine/threonine protein kinase, Akt (protein kinase B), that also has potential antineoplastic activity. Akt inhibitor MK2206 binds to and inhibits the activity of Akt in a non-ATP competitive manner, which may result in the inhibition of the PI3K/Akt signaling pathway and tumor cell proliferation and the induction of tumor cell apoptosis. Activation of the PI3K/Akt signaling pathway is frequently associated with tumorigenesis and dysregulated PI3K/Akt signaling may contribute to tumor resistance to a variety of antineoplastic agents.

13. Trametinib: It is an inhibitor of mitogen-activated protein kinase kinase (MEK MAPK/ERK kinase) with potential antineoplastic activity. Trametinib specifically binds to and inhibits MEK 1 and 2, resulting in an inhibition of growth factor-mediated cell signaling and cellular proliferation in various cancers. MEK 1 and 2, dual specificity threonine/tyrosine kinases often upregulated in various cancer cell types, play a key role in the activation of the RAS/RAF/MEK/ERK signaling pathway that regulates cell growth.

14. Crizotinib: This is an aminopyridine-based inhibitor of the receptor tyrosine kinase anaplastic lymphoma kinase (ALK) and the c-Met/hepatocyte growth factor receptor (HGFR). It also has antineoplastic activity. Crizotinib, in an ATP-competitive manner, binds to and inhibits ALK kinase and ALK fusion proteins. In addition, crizotinib inhibits c-Met kinase, and disrupts the c-Met signaling pathway. Altogether, this agent inhibits tumor cell growth. ALK belongs to the insulin receptor superfamily and plays an important role in nervous system development. ALK dysregulation and gene rearrangements are associated with a series of tumors.

15. Dacomitinib: A highly selective, second-generation small-molecule inhibitor of the pan-epidermal growth factor receptor (EGFR) family of tyrosine kinases (ErbB family) with potential antineoplastic activity. Dacomitinib specifically and irreversibly binds to and inhibits human EGFR subtypes, resulting in inhibition of proliferation and induction of apoptosis in EGFR-expressing tumor cells. EGFRs play major roles in tumor cell proliferation and tumor vascularization, and are often overexpressed or mutated in various tumor cell types.

16. Lucitanib: This is a novel dual inhibitor targeting human vascular endothelial growth factor receptors (VEGFRs) and fibroblast growth factor receptors (FGFRs) with antiangiogenic activity. Lucitanib inhibits VEGFR-1, -2, -3 and FGFR-1, -2 kinases in the nM range, which may result in the inhibition of tumor angiogenesis and tumor cell proliferation and the induction of tumor cell death. Both VEGFRs and FGFRs belong to the family of receptor tyrosine kinases that may be upregulated in various tumor cell types.

17. LY2606368: This is an inhibitor of checkpoint kinase 1 (chk1) with potential antineoplastic activity. Upon administration, LY2606368 selectively binds to chk1, thereby preventing activity of chk1 and abrogating the repair of damaged DNA. This may lead to an accumulation of damaged DNA and may promote genomic instability and apoptosis. LY2606368 may potentiate the cytotoxicity of DNA-damaging agents and reverse tumor cell resistance to chemotherapeutic agents. Chk1, a serine/threonine kinase, mediates cell cycle checkpoint control and is essential for DNA repair and plays a key role in resistance to chemotherapeutic agents.

18. Cobimetinib: This is a small-molecule inhibitor of mitogen-activated, protein kinase, kinase 1 (MAP2K1 or MEK1), with potential antineoplastic activity. Cobimetinib specifically binds to and inhibits the catalytic activity of MEK1, resulting in inhibition of extracellular signal-related kinase 2 (ERK2) phosphorylation and activation and decreased tumor cell proliferation. Preclinical studies have demonstrated that this agent is effective in inhibiting the growth of tumor cells bearing a B-RAF mutation, which has been found to be associated with

many tumor types. A threonine-tyrosine kinase and a key component of the RAS/RAF/MEK/ERK signaling pathway that is frequently activated in human tumors, MEK1 is required for the transmission of growth-promoting signals from numerous receptor tyrosine kinases.

19. Icotinib: This is a quinazoline-based inhibitor of epidermal growth factor receptor (EGFR) with potential antineoplastic activity. Icotinib selectively inhibits the wild-type and several mutated forms of EGFR tyrosine kinase. This may lead to an inhibition of EGFR-mediated signal transduction and may inhibit cancer cell proliferation. EGFR, a receptor tyrosine kinase, is upregulated in a variety of cancer cell types.

20. Pazopanib: This is a small molecule inhibitor of multiple protein tyrosine kinases with potential antineoplastic activity. Pazopanib selectively inhibits vascular endothelial growth factor receptors (VEGFR)-1, -2 and -3, c-kit and platelet derived growth factor receptor (PDGF-R), which may result in inhibition of angiogenesis in tumors in which these receptors are upregulated.

21. Selatinib: It is an analog of the quinazolinelapatinib and dual inhibitor of epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (ErbB-2 or HER-2), with potential antineoplastic activity. Upon administration, selatinib reversibly blocks phosphorylation of both EGFR and ErbB2, thereby suppressing tumor growth in EGFR/ErbB-2-overexpressing tumor cells. The tyrosine kinases EGFR and ErbB2 have been implicated in the growth of various tumor types.

22. Indoximod: It is a methylated tryptophan that acts as an immunosuppressant. It acts as indoleamine 2,3-dioxygenase (IDO) inhibitor. This leads to the tryptophan degradation. Reduced amounts of tryptophan are responsible for immunosuppression.

23.Uparlisib: This is an inhibitor of the pan-class I phosphatidylinositol 3-kinase (PI3K) family of lipid kinases with potential antineoplastic activity. Buparlisib specifically inhibits class I PIK3 in the PI3K/AKT kinase (or protein kinase B) signaling pathway in an ATP-competitive manner, thereby inhibiting the production of the secondary messenger phosphatidylinositol-3,4,5-trisphosphate and activation of the PI3K signaling pathway. This may result in inhibition of tumor cell growth and survival in susceptible tumor cell populations. Activation of the PI3K signaling pathway is frequently associated with tumorigenesis. Dysregulated PI3K signaling may contribute to tumor resistance to a variety of antineoplastic agents.

24. Abemaciclib: It is a cyclin-dependent kinase (CDK) inhibitor that targets the CDK4 (cyclin D1) and CDK6 (cyclin D3) cell cycle pathway, with potential antineoplastic activity. Abemaciclib specifically inhibits CDK4 and 6, thereby inhibiting retinoblastoma (Rb) protein phosphorylation in early G1 phase of the cell cycle. Inhibition of Rb phosphorylation prevents CDK-mediated G1-S phase transition, thereby arresting the cell cycle in the G1 phase, suppressing DNA synthesis and inhibiting cancer cell growth. Overexpression of the serine/threonine kinases CDK4/6, as seen in certain types of cancer, causes cell cycle deregulation.

25. BBI608: This is a cancer cell stemness inhibitor with potential antineoplasticactivity. Even though the exact target has yet to be fully elucidated, BBI608 appears to target and inhibit multiple pathways involved in cancer cell stemness. This may ultimately inhibit cancer stemness cell (CSC) growth as well as heterogeneous cancer cell growth. CSCs, self-replicating cells that is able to differentiate into heterogeneous cancer cells, appear to be responsible for the malignant growth, recurrence and resistance to conventional chemotherapies.

26. BKM120: It is an inhibitor of the pan-class I phosphatidylinositol 3-kinase (PI3K) family of lipid kinases with potential antineoplastic activity. Buparlisib specifically inhibits class I PIK3 in the PI3K/AKT kinase (or protein kinase B) signaling pathway in an ATP-competitive manner, thereby inhibiting the production of the secondary messenger phosphatidylinositol-3,4,5-trisphosphate and activation of the PI3K signaling pathway. This may result in inhibition of tumor cell growth and survival in susceptible tumor cell populations. Activation of the PI3K signaling pathway is frequently associated with tumorigenesis. Dysregulated PI3K signaling may contribute to tumor resistance to a variety of antineoplastic agents.

F. mTORinhibitors:

1. Everolimus [37]: This is a derivative of the natural macrocyclic lactone sirolimus with immunosuppressant and anti-angiogenic properties. In cells, everolimus binds to the immunophilin FK Binding Protein-12 (FKBP-12) to generate an immunosuppressive complex that binds to and inhibits the activation of the mammalian target of rapamycin (mTOR), a key regulatory kinase. Inhibition of mTOR activation results in the inhibition of T lymphocyte activation and proliferation associated with antigen and cytokine (IL-2, IL-4, and IL-15) stimulation and the inhibition of antibody production.

Everolimus is a kinase inhibitor that has gained FDA approval for the treatment of postmenopausal women with advanced hormone receptor-positive, HER2-negative breast cancer (advanced HR+ BC) in combination with exemestane after failure of treatment with letrozole or anastrozole. It is 74% bound to human plasma protein and it is metabolized through CYP3A4. Its half-life is 30 hours.

The most common adverse effects are stomatitis, infections, rash, fatigue, diarrhea, edema, abdominal pain, nausea, fever, asthenia, cough, headache and decreased appetite. Mouth

Drugs	Clinical trial identifier number	Phase	Study design	Target
Neratinib	NCT01808573	Phase III	Randomized, Open Label, Safety/Efficacy Study	EGFR
Buparlisib	NCT01633060	Phase II	Randomized, Double blind, Efficacy Study	PIK3
Abemaciclib	NCT02107703	Phase II	Randomized, Double blind, Efficacy Study	CDK 4/6
BBI608	NCT01325441	Phase I/II	Non-Randomized, Open Label, Safety/Efficacy Study	CSC
Indoximod	NCT01792050	Phase II	Randomized, Double blind, Efficacy Study	IDO1
LEE011	NCT01958021	Phase II	Randomized, Double Blind, Efficacy Study	CDK4 and 6
PLX3397	NCT01596751	Phase I/II	Open Label, Safety/Efficacy Study	CSF1R, KIT, FMS
KX2-391	NCT01764087	Phase I/II	Open Label, Safety/Efficacy Study	Kinase activity
LY2780301	NCT01980277	Phase I/II	Open Label, Safety/Efficacy Study	Akt
Ruxolitinib	NCT02041429	Phase I/II	Open Label, Safety/Efficacy Study	JAK 1 and 2
Refametinib	NCT02168777	Phase I/II	Non-Randomized, Open Label, Safety/Efficacy Study	MAP2K1
CLR457	NCT02189174	Phase I/II	Non-Randomized, Open Label, Safety/Efficacy Study	PI3K
Cabozantinib	NCT01441947	Phase II	Open Label, Efficacy Study	MET, KIT, VEGFR-2/3
Dovitinib	NCT01528345	Phase II	Randomized, Double Blind, Efficacy Study	c-KIT, FGFR3,
Gefitinib	NCT01732276	Phase II	Open Label, Safety/Efficacy Study	EGFR
MK2206	NCT01776008	Phase II	Open Label, Efficacy Study	PI3K/Akt signaling pathway
Trametinib	NCT01964924	Phase II	Open Label, Efficacy Study	MEK 1 and 2
Crizotinib	NCT02034981	Phase II	Open Label, Efficacy Study	ALK kinase
Dacomitinib	NCT02047747	Phase II	Open Label, Safety/Efficacy Study	EGFR
Lucitanib	NCT02202746	Phase II	Randomized,Open Label, Safety/Efficacy Study	VEGFR-1, -2, -3 and FGFR-1, -2
LY2606368	NCT02203513	Phase II	Open Label, Efficacy Study	DNA
Cobimetinib	NCT02322814	Phase II	Randomized, Double Blind, Safety/Efficacy Study	MEK1
Icotinib	NCT02362230	Phase II	Open Label, Safety/Efficacy Study	EGFR
Pazopanib	NCT01548144	Phase I	Non-Randomized, Open Label, Safety/Efficacy Study	VEGFR)-1, -2,-3, c-kit, PDGF-R
Selatinib	NCT01931943	Phase I	Open Label	EGFR/ErbB-2

Table 1. Non-FDA approved kinase inhibitor [12-36].

ulcers, stomatitis, and oral mucositis have occurred in patients who were treated with everolimus at an incidence ranging from 44-86% across the clinical trial experience. Several cases of renal failure (including acute renal failure), some with a fatal outcome, have been observed in patients treated with everolimus. Treatment should be discontinued in case of any increase in serum creatinine level. Exposure to everolimus is increased in patients with hepatic impairment. Everolimus should be used at a reduced dose if the desired benefit outweighs the risk.

There are few mTOR inhibitors are under clinical trials in phase I-III as in Table 2 below:

1. Temsirolimus: This is an ester analog of rapamycin. Temsirolimus binds to and inhibits the mammalian target of rapamycin (mTOR), resulting in decreased expression of mRNAs necessary for cell cycle progression and arresting cells in the G1 phase of the cell cycle. mTOR is a serine/threonine kinase which plays a role in the PI3K/AKT pathway that is upregulated in some tumors.

2. MLN0128: This is an inhibitor of raptor-mTOR (TOR complex 1 or TORC1) and rictor-mTOR (TOR complex 2 or TORC2) with potential antineoplastic activity. TORC1/2 inhibitor MLN0128 binds to and inhibits both TORC1 and TORC2 complexes of mTOR, which may result in tumor cell apoptosis and a decrease in tumor cell proliferation. TORC1 and 2 are upregulated in some tumors and play an important role in the PI3K/Akt/mTOR signaling pathway, which is frequently dysregulated in human cancers.

3. AZD2014: This is an inhibitor of the mammalian target of rapamycin (mTOR) with potential antineoplastic activity. The mTOR kinase inhibitor, AZD2014, inhibits the activity of mTOR, which may result in the induction of tumor cell apoptosis and a decrease in tumor cell proliferation. mTOR, a serine/threonine kinase that is upregulated in a variety of tumors, plays an important role downstream in the PI3K/Akt/mTOR signaling pathway.

Drugs	Clinical trial identifier number	Phase	Study design	Target
Temsirolimus	NCT01111825	Phase I/II	Non-Randomized, Open Label, Safety/Efficacy Study	Mtor
MLN0128	NCT02049957	Phase I/II	Non-Randomized, Open Label, Safety/Efficacy Study	mTOR
AZD2014	NCT02216786	Phase I/II	Randomized, Open Label, Efficacy Study	mTOR

Table 2. mTOR inhibitors in phase I-III [38- 40].

H. Apart form the mentioned classifications of targeted therapies under clinical development for the treatment of breast cancer, there are other molecular targets that has been recognized and investigated as a potential therapeutic approach in breast cancer in early preclinical and clinical studies. Table 3 summarizes the available molecules and the clinical tirlas on these specific therapeutic approaches.

1. AZD4547: This is an inhibitor of the fibroblast growth factor receptor (FGFR) with potential antineoplastic activity. FGFR inhibitor AZD4547 binds to and inhibits FGFR, which may result in the inhibition of FGFR-related signal transduction pathways, and, inhibits tumor cell proliferation and tumor cell death. FGFR, up-regulated in many tumor cell types, is a receptor tyrosine kinase essential for tumor cellular proliferation, differentiation and survival.

2. SNX-5422: It is a synthetic prodrug targeting the human heat-shock protein 90 (Hsp90) with potential antineoplastic activity. Although the mechanism of action remains to be fully elucidated, Hsp90 inhibitor, SNX-5422, is rapidly converted to SNX-2112, which accumulates more readily in tumors relative to normal tissues. SNX-2112 inhibits Hsp90, which may result in the proteasomal degradation of oncogenic client proteins, including HER2/ERBB2, and the inhibition of tumor cell proliferation. Hsp90 is a molecular chaperone that plays a key role in the conformational maturation of oncogenic signaling proteins, such as HER2/ERBB2, AKT, RAF1, BCR-ABL, and mutated p53, as well as many other molecules that are important in cell cycle regulation or immune responses.

3. GRN1005: This is a peptide-drug conjugate containing the taxane paclitaxel covalently linked to the proprietary 19 amino acid peptide, angiopep-2, in a 3:1 ratio, with potential antineoplastic activity. Upon administration, LRP-1-targeted peptide-drug conjugate GRN1005, via angiopep-2 moiety, binds to LRP-1 (low density lipoprotein receptor-related protein 1), which is highly expressed in blood brain barrier (BBB) and glioma cells. This binding allows the transcytosis of the agent across the BBB and the delivery of the cytotoxic agent paclitaxel. Compared to paclitaxel alone, GRN1005 is able to increase the concentration of paclitaxel in the brain and is also able to specifically deliver paclitaxel to LRP-1-overexpressing tumor cells, both in the brain and in the periphery.

4. Erismodegib: This is a small-molecule smoothened (Smo) antagonist with potential antineoplastic activity. Erismodegib selectively binds to the Hedgehog (Hh)-ligand cell surface receptor, Smo, which may result in the suppression of the Hh signaling pathway and the inhibition of tumor cells in which this pathway is abnormally activated. The Hh signaling pathway plays an important role in cellular growth, differentiation and repair. Inappropriate activation of Hh pathway signaling and uncontrolled cellular proliferation, as is observed in a variety

of cancers, may be associated with mutations in the Hh-ligand cell surface receptor, Smo.

5. IGF-methotrexate conjugate: This is a conjugate containing the antimetabolite and antifolate agent methotrexate conjugated to insulin-like growth factor (IGF), with potential antineoplastic activity. After intravenous administration, the IGF moiety of the IGF-methotrexate conjugate binds to and is internalized by IGF receptors (IGFR) on the surface of tumor cells. Following cell entry, the methotrexate then binds to and inhibits the enzyme, dihydrofolatereductase, which catalyzes the conversion of dihydrofolate to tetrahydrofolate. This results in both the inhibition of DNA and RNA synthesis and the induction of death in rapidly dividing cells. Binding to IGFR can localize the cytotoxic effect of methotrexate in tumor cells. This may increase its efficacy while decreasing its toxicity to normal, healthy cells. IGFR is overexpressed in many types of cancer cells and has been implicated in metastasis and resistance to apoptosis.

Drugs	Clinical trial identifier number	Phase	Study design	Target
AZD4547	NCT01791985	Phase I/II	Randomized, Open Label,	FGFR
SNX-5422	NCT01848756	Phase I/II	Open Label, Safety/Efficacy Study	Hsp90
GRN1005	NCT01480583	Phase II	Non-Randomized, Open Label, Efficacy Study	Cancer cells
Erismodegib	NCT01757327	Phase II	Randomized, Double blind, Efficacy Study	Cancer cells
IGF-methotrexate conjugate	NCT02045368	Phase I	Open Label, Safety Study	Dihydrofolatereductase

Table 3. Other targeted agents in phase I-III [41- 45].

Conclusion

Targeted therapy has proven to be effective in the treatment of breast cancer, but there are some kinase inhibitors targeting EGFR, PI3K, Hoedghog as well as cycline dependent kinases that are under clinical trials for the FDA approval in immunotherapy. Our success in treating breast cancer is increasing and advancing with the knowledge of molecular pathways, involve in carcinogenesis as well as tumor promotion and metastasis. The recent activities have increased our understanding of the tumor microenvironment, various molecular targeted modalities or combination therapy (like chemotherapy with immunotherapy). Additionally, the effects of such modalities in combination with immunotherapy in cancer patients are still in the exploratory phase. The complete perspective of immunotherapy treatment has not been realized and utilized. Proper preclinical and clinical designs are the important pillars in understanding the future of immunotherapy in treating breast cancer patients.

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