

Breast Cancer Progression and Current Therapeutic Approach

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Abstract

Breast cancer is the most common malignancy and a leading cause of deaths of women around the globe. Tumor originated from the mammary gland are the most recurrently diagnosed malignancies worldwide and register around 14% of all deaths, making breast cancer the most fatal neoplasm in women. Breast cancer can start as a local disease however it can metastasize to distant part of body particularly to lymph node, bone and spine which makes it deadliest. Breast cancer can be categorized into a number of types on the basis of size, possession of specific marker, grades of metastasis etc. Genetic predisposition marks an important place in list of risk factors associated with breast cancer but the sporadic forms are remarkable. Breast cancer is curable if diagnosed at very early stage although there are several therapies like chemotherapy, hormonal therapy, radiation therapy, and targeted therapy etc. which improve expectancy and quality of life in later stages also.

Keywords: Breast cancer, Molecular marker, Therapy, Risk factor

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Introduction

Breast Cancer is the most frequent cancer among the women now a day. It is most reported cancer globally. By far Breast cancer is the most spotted cancer among women. Globally, more than 20% enhancement in breast cancer since 2008 with a newly diagnosed cases of 1.7 million in 2012; and there are 6.3 million women alive with breast cancer with 14% increase in mortality rate in preceding 5 years [1]. In Asia, mostly breast cancer occurs in women in their forties [2,3]. In Africa it is also usually around 48 years in which two third cases are premenopausal whereas in Europe majority cases happens in postmenopausal stage [4].

Normal human women breast consists of milk producing lobules, tiny ducts that carry milk from lobule to the nipple and stroma which contains fatty tissues and connective tissues surrounding ducts, lobules, blood vessel and lymphatic vessels [5]. Breast cancer is an assemblage of very diverse group of diseases [6]. The Basis of classification of breast cancer was started with histological features, then based on estrogen receptor(ER) expression and later according to presence of Human Epithelial Growth Factor receptor (HER2) [7]. Molecular sub typing of breast cancer carries important prognostic and predictive values related to HER2, ER and progesterone receptor (PR) thus help in initial process of

breast cancer diagnosis [6]. Mostly all breast cancer in human is mainly carcinomas (epithelial cells cancer) or specifically adenocarcinomas (carcinoma that starts in glandular tissues). Breast cancer in specifically begin in lobules (lobular cancer) or ducts (ductal cancer). Keeping in mind the metastatic capability, breast cancer can either be *in situ*/non invasive or invasive. *in situ* breast cancer can have different origin and grouped accordingly as Ductal carcinoma *in situ* (DCIS) or Lobular carcinoma *in situ* (LCIS). DCIs are the most common type of *in situ* carcinoma while LCIS can be termed as pre cancer. There is some other *in situ* type of breast cancer having characteristics of both ductal and lobular carcinomas and have an unknown origin [5]. Implying the same criteria as noninvasive, invasive breast cancer can either be invasive ductal carcinoma (IDC) or invasive lobular carcinoma (ILC). Using molecular sub typing, IDCs are either ER positive or ER negative. ER positive IDCs have variation in expression of ER, PR and other receptors and are further identified as Luminal A and Luminal B. Luminal A having high expression of ER and PR related gene, GATA binding protein 3, low expression of proliferation related gene, lack expression of Her-2. Luminal B having decreased expression of ER, over expression of Her-2 and higher expression of proliferation associated genes. The ER negative one is either Basal subtype or ERBB2 type. Basal Subtype having

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high grade tumor displaying necrosis, prominent lymphocytic infiltration and pushing border, lack of expression of ER, PR and Her-2. ERBB2 having high grade tumor, excess expression of Her 2, GRB 7, lack expression of ER and PR [8]. Other uncommon varieties of breast cancer embrace inflammatory breast cancer, triple negative breast cancer etc. Inflammatory breast cancer usually has no single lump or tumor, high chance of spreading and might not show up in mammography. Triple negative breast cancer are invasive ductal carcinoma whose cells lack ER and PR and do not have an excess of the Her 2 protein on their surface, spreads more quickly than any other types of cancer and nonresponsive to cancer therapies except chemotherapy. Other rare types of breast cancers are Paget disease of nipple, phyllodes tumor, angiosarcoma etc. Certain benign breast lumps include non-proliferative lesions (fibrosis and/or simple cyst, mild hyperplasia, adenosis), proliferative lesion without atypia, and proliferative lesion with atypia [5].

Breast Cancer Progression

The conventional tumor initiation hypothesis demonstrates the origin of a single genetically transformed cells via various transforming events like TP53 mutation or epigenetic changes which further gets clonally expanded along with accumulation of additional genetic changes [9].

There are two models are available to explain the origin of breast cancer cells, the first one is sporadic clonal evolution model and the second one is cancer stem cell (cSC) model. The first model proposes that mutation can occur randomly in any breast cancer epithelial that over time acquires many genetical and epigenetical changes for cancer progression. The second model found only stem and progenitor cells eligible for initiation and progression of breast cancer [8].

Yet another model, Carcinogenesis model suggests that all the genetic changes whether it is germline or somatic those are differentially presents in different types of breast cancer make cells susceptible to epigenetic changes that can be an initial cause of breast cancer progenitor cell development. An epigenetic trigger is required for transformation of predisposed breast cancer cell to a breast cancer progenitor cell. Not only the initiation, during the metastasis the differential activation and inactivation of various genes under controlled by epigenetically can further leads to a rapid growth and further spread of cancer [10].

It has been postulated that for the development of ductal carcinoma, tumor starts with flat epithelial atypia (FEA), advances into atypical ductal hyperplasia (ADH) and finally to the ductal carcinoma *in situ* (DCIS). Thereafter may develop into lethal and highest stage of breast cancer i.e. invasive ductal carcinoma (IDC) [8,11]. The second model proposes epithelial ducal hyperplasia (UDH) as an intermediate Stage between FEA and DCIS. But recent studies deny the second model. Model for lobular carcinoma identifies atypical lobular hyperplasia (ALH) as a precursor of lobular carcinoma *in situ* [8].

Based on the theories of cancer origin, invasive cancer may origin from primary tumor before it get metastasize, alternatively,

metastatic cancer my origin from dispersed tumor cells, independent of primary tumor [12]. Metastasis is a complex and multistage process that include detachment of cells from their origin, invading through basement membrane, blood vessel or lymphatic intravasation, transit through the vasculature and ability to establish new secondary tumor at organs such as bone, lung, liver or brain. Cancer cells show dramatic phenotypic changes during invasive metastatic cascade referred as EMT (epithelial to mesenchymal transition) [13]. EMT is an essential process during morphogenesis at embryonic developmental stage and also in wound healing [14] but the improper activation of EMT can be a cause of tumor progression and also for drug resistance in some cases [13]. Epithelial to mesenchymal cell switching is a multifaceted phenomenon that includes several complex modifications like loss of cell adhesion, rearrangement of cytoskeleton architecture, phenotypic change from typical cuboidal to elongated spindle shape, adoption of migratory and invasive phenotype, loss of epithelial cell adhesion molecule E-cadherin and gain of mesenchymal markers such as vimentin, N-cadherin and fibronectin. Several growth factors secreted by stromal cells promote the EMT process. These factors include TGF β , hepatocyte growth factor, platelet-derived growth factor, fibroblast growth factor, and Wnt and Notch ligands [13]. The progression of localized DCIS to invasive and ultimately metastatic breast cancer is a vital landmark for the clinical management and outcome of the disease. One of the fundamental programmes for this progression is angiogenesis [9]. Angiogenesis is massively observe during embryonic development but is not restricted to embryonic condition; it is also observe in adults under specific physiological task. In case of tumor, the foremost cause is switching from avascular to vascular state due to hypoxia and malnutrition [15]. Vascular Endothelial Growth Factor is the most important factor for tumor angiogenesis activated during hypoxia [9].

Breast cancer progression is the phenomena not only associated with the cancer cells. Several recent and promising *in vivo* and *in vitro* studies indicate it a signaling interplay between malignant and surrounded non epithelial cells in tumor microenvironment [8]. Fall of pH in due to induction of hypoxia related gene leads to activation of adaptive immune response. The adaptive immune system of our body recruits some immune cells like regulatory T-cells (Tregs), myeloid-derived suppressor cells (MDSCs). This cell along with their derivatives suppresses our immune system and also shows anti-autoimmunity response under the influence of tumor-secreted cytokines, enzymes, and antigens [16].

Genetics of breast cancer

Family history or hereditary back background increases the risk of breast cancer. Having one first degree relative (mother, sister or daughter) with breast cancer approximately doubles a women risk. Having two first degrees relative increases her risk about 3 fold. The exact risk is not known, but women with a family history of breast cancer in a father or brother also have increased risk of breast cancer. Women with one breast cancer have risk of developing in the other breast cancer [5]. Loss of heterozygosity-based genomic studies of premalignant stages of breast cancer progression show major genetical changes that trigger the origin

and progression of breast cancer [17]. The underlying genetic causes in primary breast cancers are chromosomal anomaly includes either the whole arm gain (in 1q and 8q) or increase in copy number of some regions (in 17q and 20q) [18]. The examples are known breast cancer oncogenes on chromosomes 8q (MYC) and 17q (HER2/neu [ERBB2]). Different type of oncogenes and tumor suppressor gene related to breast cancer are tabulated (Table 1). In DCIS, chromosomal gain is not alone responsible, chromosomal loss is also happens. Chromosomal gains are mainly observed in 1q, 8q, and 17q, whereas losses include most commonly 8p, 11q, 13q, 14q, and 16q [19]. In invasive breast cancer (IDC), a gain includes 1q, 6p, 8q, 11q, 16p, 17q, and 20q frequently. Chromosomal losses have been identified in 1p, 8p, 11q, 16q, 18q, and 22 [20]. Copy number changes frequently associated with BRCA1 predisposed cases are gain of 3q, 7p, 8q, 10p, 12p, 16p, 17q and loss of 2q, 3p, 4p, 4q, 5q, 12q, 16p and 18q while those associated BRCA2 are gain of 8q, 17q, 22q and loss of 8p, 6q, 11q and 13q [21]. According to the recent studies the predisposition factors are grouped according to their association with occurrence of disease. For example BRCA1 and BRCA2 (In the normal cells these genes prevent the cells from growing abnormally) are high penetrance genes according to genome wide linkage analysis and positional cloning [22]. Along with BRCA1 and BRCA2, TP53, CDH1, STK11 are some rare yet highly penetrable genes that causes nearly 25% hereditary breast cancer cases [21,23,24]. Mutation in BRCA1 increases the risk by 80% and mutation in BRCA2 increases the risk by 45%. Breast cancer related to this mutation occurs more often in younger women [5]. Mutations in these two genes are mainly associated with cancer at an early stage [25]. Three well described mutation associated with BRCA1/2 viz 187 delAG and 5385 in cG (with BRCA1) and 6174 del T (with BRCA 2) [26]. Other genes associated with breast cancer are CHK2, ATM, BRIP, RAD50, NBS1, CDH1, STK11/LH31, BRIP 1 and PALB responsible for rare mutation and possess intermediate risk of possession. Association study further identified 8 other gene with low penetrance [21,22].

The progression of breast cancer to advanced stages accounts for loss of genes related to cell adhesion, cell division, DNA damage response etc. Loss of FAT1, a surface membrane protein for cell adhesion, promotes the progression of DCIS [27]. Other genes which are mutated in DCIS are mainly TP53, PIK3CA and AKT. All PIK3CA genes where present in high number during DCIS to IDC progression. The mutated version of 3 genes i.e. PIK3CA, AKT1 and PTEN is mainly responsible for progression of DCIS to IDC. The mutation of PTEN is associated BRCA1 mutation [28] and also responsible for early age breast cancer [19]. The BRCA1 repair the mutated PTEN gene. Unrepaired PTEN gene causes uncontrolled cell division, inhibition of apoptosis, metastasis, angiogenesis etc [28-31].

Causes of breast cancer

The hereditary breast cancer covers nearly for 5 to 10 % of total cases of the disease [5,21,23] but genetic predisposing is not the only risk factors [22]. The epidemiological study always indicates the increased level of estrogen as an important risk factor of breast cancer. Females have more estrogen and progesterone which can promote breast cancer cell growth than males [5]. Hormone level in women fluctuates drastically and the magnitude of risk increases with it. Early menarche increases the risk of breast cancer. Nulliparous women have increased risk than parous one. Postmenopausal obesity also increases the risk of breast cancer [32]. First pregnancy after 30 years increases the risk while breast feeding for long time decreases the risk [5]. Chances of breast cancer increases with increase in age, consumption of alcohol and by consumption of oral contraceptive pill [32]. Women with dense breast have higher risk of breast cancer than women with less dense breast tissue [5]. But these are not the only cause, Worldwide studies has recognized many factor associated with breast cancer, including stress and other psychosocial factors, grievance to the breast, chemicals, food additives, viral or bacterial infection [33,34].

Table 1 Genetics of breast cancer.

Oncogene [29]			
Name	location	Type of mutation	Function
Her2	17q	Increase in copy number	Transmembrane tyrosin kinase growth factor receptor.
c-myc	8q	Whole arm gain	Transcriptional regulator involve in cell proliferation differentiation and apoptosis
Tumor suppressor genes [30]			
TP53 (p53)	17p13	Intragenic mutation, deletion	DNA repair, cell cycle, apoptosis and angiogenesis regulator.
Rb1	13q14	Intragenic deletion, mutation,	Cell cycle inhibitor
PTEN	10q23	Intragenic deletion, mutation,	Dual-specific phosphatase
BRCA1	17q21	Intragenic deletion, mutation,	DNA repair
BRCA2	13q12	Intragenic deletion, mutation,	DNA repair
ATM	11q22	Intragenic deletion, mutation,	DNA repair

Stages and grades of breast cancer

Staging of breast cancer depends upon several aspects. The most common system describe by The American Joint Committee on Cancer (AJCC) to describe the stages of breast cancer is TNM staging system which classifies cancers based on their T, N and M stages where: T describes the tumor’s size and spread to skin while increasing number (0-4) behind it indicates the increasing tumor size and extent of spread to skin. N indicates the involvement of lymph nodes and the followed by numbers (0-3) indicates affected lymph nodes. M indicates the metastatic state of the cancer followed by a 0 describes no metastasis and M followed by one indicates that the tumor is metastatic. **Table 2** shows further staging of breast cancer and **Table 3** shows histological grades of breast cancer.

Diagnosis of Breast cancer

Diagnosis of breast cancer starts with physical examination. Nipple discharge, formation of any masses or lump should be included under this [35]. Diagnostic imaging and image-guided needle biopsy are the central mean for diagnosis and screening of breast cancer. Newly diagnosed breast cancers are either imaged with mammography or ultrasonography or both. Women having palpable mass in their breast or having a previous history of breast cancer undergoes diagnostic mammograms. The breast imaging reporting and database system (BI-RADS) is the consistent technique for coverage of mammographic findings [35]. Though mammography is the basic imaging study for the breast cancer diagnosis, other examinations procedures are also there for better detection and understanding about staging, surgical planning and evaluation of treatment response. MRI

(Magnetic resonance imaging) is a integral part of breast cancer diagnosis for some patients. MRI is applicable to those cases where mammographical assessment is limited like like if patient have and saline implants and silicone injections or if we have to measure the extent of disease at the initiation time or the patients having inconclusive mammography or ultrasonography or the patients having high risk of breast cancer. MRI can also be used to determine the effect if neoadjuvant therapy to track the residual disease [34].

Molecular diagnosis and prognosis

Identification of different molecular markers is very much essential for the mode of different treatment and recurrence chance. Estimation of the expression of Estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2(HER2) are now in routine practice for the diagnosis and management of breast cancer. Recently several molecular markers or panel of molecular markers have been developed for the prognosis of breast cancer (**Table 4**). In conjugation with traditionnal clinico-pathological tests, these markers are very useful for the determination of risk of recurrence and treatment [35].

Breast cancer therapy

Multiple variables including genetic predisposition, disease burden, tumour markers, receptor status and patients preference are integral to decision making to each individual patient [36]. The Trial Assigning Individualized Options for Treatment (TAILORx) is a method for probing molecular markers often linked with risk of recurrence in early stage breast can be used as the most suitable and efficient treatment or not [37].

Table 2 TNM staging system [5].

Stages of Cancer	Characteristics	Description
Stage 0	Tis, N0, M0	Tx: Not assessable primary tumor T0: primary tumor in evident;
Stage I: IA IB	T1, N0, M0 T0 or T1, N1mi, M0	Tis: Carcinoma of in situ (DCIS, LCIS, or Paget disease of nipple without associated tumor mass); T1 : diagonally tumor size is less than 2 cm or less ; T2: crosswise tumor size is more than 2 cm but less than 5 cm;
Stage II IIA IIB	T0 or T1, N1 (but not N1mi), M0 or T2, N0, M0 T2, N1, M0 OR T3, N0, M0	T3: crosswise tumor is greater than 5 cm; T4: involvement of chest wall or skin to any size tumor,
Stage III IIIA IIIB IIIC	Either(T0 to T2, N2, M0) or (T3, N1 or N2, M0) T4, N0 to N2, M0 any T, N3, M0	NX: in assessable nearby lymph nodes (for example, if they were removed previously); N0 [include N0 (i+) and N0 (mol+)]: spreading of cancer has not occur to nearby lymph nodes; N1:cancer has extended to 1 to 3 axillary lymph node or internal mammary lymph node shows few cancerous cells during sentinel lymph node biopsy; N2: extension of cancer to 4 to 9 lymph nodes under the arm or enlarged internal mammary lymph node due to cancer ; N3: spreading of cancer to 10 or more axillary lymph node with at least one area spread over greater than 2 mm;N3b: at least one axillary nymph node is infected with enlarged internal lymph node and at least one area has spreading of more than 2 mm space or spreading of cancer to 4 or more axillary lymph node, internal mammary lymph node shows few cancer cells on sentinel lymph node biopsy and at least one area of cancer has a spreading of greater than 2 mm ;N3c: extension of cancer to the lymph nodes situated above the clavicle and having at least one area spread over greater than 2,
Stage IV	any T, any N, M1	MX: in assessable distance spread; M0: No distant spread is found on x-rays (or other imaging procedures) or by physical exam; M1: Cancer has spread to distant organs. (The most common sites are bone, lung, brain, and liver.)

Table 3 Histological Grades of breast cancer [33].

Grade	Characteristics
Grade 1	Well differentiated(high homology to the normal breast terminal duct lobular unit, tubule formation, a mild degree of nuclear pleomorphism, and low mitotic count)
Grade 2	Moderately differentiated
Grade 3	Poorly differentiated (with a marked degree of cellular pleomorphism and frequent mitoses and no tubule formation)

Treatment Options Available for Diagnosed Breast Cancer Patients are Various Type that Include Following

1. Surgery
2. Radiation Therapy
3. Chemotherapy
4. Hormone Therapy
5. Targeted Therapy

These therapies can either used as local therapy (therapy intended to treat a tumor at the site without affecting the rest of the body) like surgery and radiotherapy or systemic therapy (drugs which can be given by mouth or directly into the bloodstream to reach cancer cells anywhere in the body) like chemotherapy, hormone therapy and targeted therapy [5].

Early detection and removal of breast cancer is sometimes is not the solution because of resurgence of cancer. To cure or minimize this adjuvant therapy has been invented [6]. Adjuvant therapy is given to the patient after primary therapy (therapy use to lessen or eradicate the cancer like surgery) to increase the chance of survival. Patients with high risk of cancer reoccurrence are generally given the adjuvant therapy but the patients have no detectable cancer after surgery is also often given adjuvant therapy for precaution. Adjuvant therapy includes chemotherapy, hormone therapy, targeted therapy, radiation therapy or a combination of therapies. Adjuvant therapy can be given orally (usually hormone therapy) or directly into blood vessels (usually chemotherapy). Adjuvant therapy is given in cycles with a treatment phase followed by recovery phase and its duration is decided by consulting doctor [5]. The chief adjuvant therapies are cytotoxic chemotherapy and endocrine therapy. Anthracyclin and taxane are the main drugs used during cytotoxic chemotherapy. Endocrine therapy related agents can be categorized into two groups: selective estrogen receptor modulators (SERMs) and aromatase inhibitor (AIs). SERMs compete with to bind estrogen receptor with estrogen which in turn interfere DNA synthesis by recruiting co-repressor, and inhibit G_0 to G_1 cell cycle progression. AIs inhibit formation of estrogen by inhibiting an enzyme named aromatase which converts circulating testosterone to estradiol (E2) and androstenedione to estrogen by arometisation. It only works when primary source of estrogen is not present. Exemestane, anastrozole and letrozol are are three main drugs of this category [6].

Some patients have given chemotherapy or hormone therapy before surgery. This is called neoadjuvant therapy [5]. Women

having stage IIA or IIIB tumor and not willing for mastectomy should consider for neoadjuvant therapy [6]. According to National Cancer Institute a woman may take delivery of neoadjuvant chemotherapy for breast cancer to minimize a tumor that is untreatable in its contemporary state for surgical removal. A woman may receive neoadjuvant therapy before mastectomy to contract the tumor sufficient to allow breast-conserving operation. Neoadjuvant therapy also reduces the need of adjuvant therapy after primary treatment as it reduces the chance of cancer reoccurrence [14]. Neoadjuvant therapy provides additional prospect of translational study along with important prognostic biomarkers [38].

Surgery

Surgery has been the focal modality for the breast cancer for centuries [37]. Surgery not only includes breast but also the lymph nodes which are associated with metastasis. For breast it is mainly of two type- Breast conserving surgery (lumpectomy) or mastectomy [5]. Standard care for breast conserving surgery has become lumpectomy with radiation [39]. Mastectomy is of many types like simple, skin sparing, radial and modified radial. For lymph node it is of two types – axillary lymph node dissection and sentinel lymph node dissection [5]. The identification and biopsy of sentinel lymph node has become a standard method of treatment of stage I and stage II in last decade [40].

Radiation Therapy:

Radiation therapy is treatment of with high energy rays or particle which destroys the cancer cells. It is mainly given after breast conserving surgery for lowering the local recurrence or may be given after mastectomy in patients either with a cancer larger than 5 cm, or when cancer is found in the lymph nodes or cancer has metastasize to other part of body like bone, brain etc [5]. Radiation may cover whole breast (whole breast irradiation, WBI) or part of it (accelerated partial breast irradiation, ABPI) [41] and can deliver externally (external beam radiation) or internally (brachytherapy) which is either interstitial or intracavitary [5]. WBI along with lumpectomy usually combined with lumpectomy to extend life span [42] and also reduces the chance of recurrence by 60-70% in comparison to lumpectomy alone [43]. In comparison to WBI, ABPI use low, uniform and accelerated wave and reduces the related side effects [43]. External beam radiation begins 3 to 6 weeks following surgery if systematic chemotherapy is given and for treating breast and lymph node, four to six MV photon energy is most commonly selected [44]. Interstitial brachytherapy has been applied as a boost therapy following whole breast external beam therapy [45].

Chemotherapy

Chemotherapy can be given as a part of adjuvant or neoadjuvant therapy and its duration is often 3 to 6 months depending upon the drug used or may be longer in case of advance cancer and based on its side effects [5].

Preclinical and early clinical research suggests that specific classes of chemotherapy may be more effective in mutation carriers. PARP inhibitors represent a novel therapeutic strategy that exploits the weakness of BRCA1/2-associated malignancies [46].

Table 4 Different panel of molecular markers used for the predication of the recurrence of breast cancer [35].

Name of the test /Panel [Company]	Description	Sample source
Genomic Grade Index (GGI) [Qiagen Marseille, Marseille, France]	97 gene-based assay using DNA micro array.	Fresh frozen material is used to perform the analysis.
Oncotype Dx Recurrence Score [Genomic Health, USA]	21 gene-based expression profile score using qRT-PCR (16 cancer genes, 5 housekeeping genes).	formalin-fixed paraffin-embedded block for RNA extraction
Breast Cancer Index (BCI) [bioTheranostics, USA]	Combination of two biomarkers HOXB13/IL17BR (H/I) and molecular grade index (MGI). Multi-gene assay using qRT-PCR.	formalin-fixed paraffin-embedded block for RNA extraction
EndoPredict [Sividon, Germany]	12 gene-based expression profile score using qRT-PCR (8 cancer genes, 4 housekeeping genes).	formalin-fixed paraffin-embedded block used to extract RNA to perform analysis.
MammaPrint [Agendia, Irvine, CA, USA]	70 gene-based expression profile using DNA microarray.	Fresh frozen material is used to perform analysis.

Chemotherapy used can be group on several bases like chemical structure, working mechanism, correlation with other drugs etc. If the working mechanism of drugs is known, they can be used in combination with other drugs for better treatment. If working mechanism is considered, chemotherapy can include:

Alkylating agents directly damages the genetic material of each cell by work by working on each phase of cell cycle [5] like Cyclophosphamide (alkylates the N⁷ position of guanine to create enol tautomer which pairs with thymine that induces apoptosis) [47].

Antimetabolites works primarily during S phase of cell cycle when genetic material is duplicating [5]. Examples are Methotrexate and 5-flourouracil (inhibit production of thymidin 5' monophosphate by blocking N⁵, N¹⁰-methylenetetrahydrofolate synthesis and hence no production of thymidine triphosphate that ultimately leads to any DNA synthesis and cell death [47].

Anti-tumor antibiotics are not like general antibiotics. They work via DNA [5]. Anthracyclins (mainly causes DNA damage creates DNA cleavage mediated by topoisomerasell isoform. This anomaly leads to cell death) [45] like doxorubicin, epirubicin represents this group [5].

Mitotic inhibitors mainly interfere during M phase of cell cycle but have capability of interfering other phases also [5]. Taxanes (binds to β tubulin of tubulin heterodimer hence disrupts spindle formation as well as cell division) like paclitaxel, docetaxel represents this group [47].

Hormone therapy

Most of the hormone therapy includes drugs that either lower the estrogen level or prevent estrogen acting on breast cancer cells. The anti-estrogen therapies are selective estrogen receptor modulators (SERMs) such as tamoxifen, raloxifene and toremifene, which block activity of ER; selective estrogen receptor down regulators (SERDs) such as fulvestrant, which induce destabilization and degradation of ER; and aromatase inhibitors (AIs), including steroidal/irreversible (anastrozole and letrozole) and nonsteroidal/reversible (exemestane) inhibitors, which decrease estrogen production in peripheral tissues and within the tumors through inhibition of the enzyme aromatase

[48]. There are a number of drugs that helps hormone therapy to work improved manner like Palbociclib (blocks CDK4 and 6 in hormone receptor positive cells) and everolimus (blocks mTOR) [5]. Aromatase inhibitor binds to enzyme aromatase(enzyme responsible for estrogen biosynthesis) [47]. It is drug of choice for postmenopausal women. In case of premenopausal women it activates the hypothalamic-pituitary-adrenal axis results in increase in ovarian androgen production and work against the intended outcome of drug [39].

Targeted therapy

Current treatment is moving towards highly specific, nontoxic and targeted therapies that can be personalized to an individual patient's tumor. Taken as example, trastuzumab, lepatinib, T-DM1, pertuzumab, MM-111 are drugs mainly used in tumors expressing high level of HER2 while that only shows HER2 expression were challenged with tamoxifen, Als, fulveatrant, cabozantinib etc. PARP inhibitors are forBRCA1 and BRCA2 mutant tumor. Anti-PI3K, anti- AKT and m-TOR targeting therapies are used in PI3K mutant as well as in HER2 over-expressing and HER2 positive tumor [49,50].

Immunotherapy

Relationship between of immune system and clinical response to some standard systemic breast cancer therapies has been demonstrated in past. For example greater disease free survival is seen among the individuals having high level of intraepithelial CD3⁺ T lymphocytes when treated with adjuvant anthracyclin based therapy while there is no such relationship between intratumoral CD3⁺ T lymphocytes and cyclophosphamide, methotrexate and 5-flourouracil [51]. So, treatment of breast cancer used to rely on immunogenic nature of the disease [52]. The first step toward the development of breast cancer immunotherapy was development of vaccines against tumor associated antigens [51]. The key tumor associated antigens that draw attention are human epithelial growth factor receptor 2 (HER2), carbohydrate antigens, telomerase reverse transcriptase (hTERT), and mucin-1 (MUC-1) [51]. Monoclonal antibodies like Trastuzumab (a humanized mouse monoclonal immunoglobulin G₁ that binds to HER2 and prevents further signal transduction that promotes cell cycle progression and inhibit apoptosis) also holds its own existence [42].

Antigen-specific vaccination with peptides or protein subunits is not the only option. Several other distinct platforms like vaccine platforms derived from cell extracts or whole tumor cells, Cell-based vaccines [52], DNA based vaccine; dendritic cells based vaccines are also admired [53]. In clinical trials the most studied HER2-derived peptide is E75 (HER2 amino acids [aa] 369–377) derived from the extracellular domain of HER2 and characterized by HLA-A2 restriction. The other one is AE37, an HER2 (aa 776–779)-derived peptide modified with a “li-Key” motif in order to elicit a combined CD4+ T- and CD8+ T-cell response. Another HER2-derived peptide tested in clinical trials is GP2, derived from the transmembrane domain of HER2 (aa 654–662). This is a nine-aa, HLA-A2-restricted peptide, with a lower affinity to HLA-A2 [46]. MUC-1 is a membrane glycoprotein tumor antigen comprised of a protein that is aberrantly glycosylated by transformed cells from secretory tissues, including the breast epithelium. MUC-1 epitope is given in conjugate form with keyhole limpet hemocyanin [52]. hTERT is a complex of hTERT and a RNA template which is with broad expression in cancer cells while little or no expression in somatic cells. Vaccination shows specific CD8+ T-cell response in more than 50% of the patients) [53]. Combination of Immunotherapeutic approaches along with other novel strategies like chemotherapy, radiotherapy, vaccination, immunomodulating agents enhances the treatment [52].

TLR Ligand Clinical Trials

Numerous TLR agonists have been demonstrated to produce anticancer effects [54]. Like TLR 7 agonist, imiquimod, shows a regression in cancer in neu transgenic mouse as well in a model of human HER-2/neu(+) breast cancer [55]. TLR2

agonist polysaccharide krestin (PSK) when administrated orally significantly inhibit breast cancer growth in neu transgenic mice [56]. TLRs also contribute to tumor-cell resistance to apoptosis and increased invasiveness [57]. Effect of TLR agonists are well studied in Human breast cancer cell line MDA-MB-231 as it expresses all the TLRs (TLR1-TLR10) like Knockdown of TLR4 MDA-MB-231 cells show huge reduction in breast cancer cell viability and inhibition of IL-6 and IL-8 cytokines, compared with vector control [58] While activated form of TLR9 promotes cell invasion by promoting MMP13 but not MMP in same cell line [59,60].

Above mentioned therapies are beneficial but also contains shortcoming and side effects. **Table 5** shows side effects and criteria of different therapeutic approaches mentioned above.

Conclusion and Future Prospects

Even though Breast cancer is the root of numerous deaths in every part of the world however that does not be a sign of losing hope and it will not win the race from human kind in future. Different therapeutic trial’s success denies overpower of breast cancer and bring new anticipation in human kind to win the race with breast cancer. Recent application of antibody blocking programmed death 1 pathway (PD-1) and its ligand programmed death ligand 1(PD-L1) have shown revolutionizing result in case of metastatic melanoma. In recent time combinational therapies with PD-1 overpower the effect of single agents’ activity [61].

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Table 5 Side effects of different therapeutic approaches [5,49,60].

Therapy	Criteria	Side effects
Chemotherapy	Adjuvant chemotherapy- for patients having high or moderate risk of recurrence. Preoperative chemotherapy is a favored choice for advanced or inflammatory breast cancer.	Risk of infection, anemia, bruising and bleeding, hair loss and thinning, nausea and vomiting, fatigue, cracked skin and nail, cognitive impairment, menopausal defects.
Radiation therapy	Local breast irradiation after healing of surgery wound, radiation should start with 12 week of surgery. In case of breast conserving surgery, radiotherapy boost is recommended for patients aged 50 or under at diagnosis In high grade intensive cancer, age of limit >50.	Skin reaction, swelling (oedema) of the breast, pain in breast area, tiredness and fatigue, lymphoedema, weakening of bones in the treated area, damage to the nerve in arm.
Hormone therapy	Premenopausal women with hormone receptor positive breast cancer should be treated with tamoxifen. For premenopausal women, duration of tamoxifane in hormone positive breast is at least 5 years. But there is evidence to support up to 10 years of use.	Menopausal defects, joint pain and stiffness, nausea
Targeted therapy	Tumor expressing high HER2-, Trastuzumab: lepatinib, T-DM1, pertuzumab, MM-111. For BRCA1 and BRCA2 mutant tumor: PARP inhibitors	Trastuzumab: flu like symptoms, nausea, diarrhea Pertuzumab: diarrhea, skin rash, sore mouth, a drop in number of white and red blood cells, alopecia, fatigue.

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