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Breast Cancer- It's All in the DNA

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1. Introduction

Breast cancer is the leading cause of cancer death in women, the second most common cancer worldwide, and the fifth most common cause of cancer-related deaths [1-3]. Not only are the incidence rates of breast cancer increasing, partly due to improved screening and detection techniques, but also the global burden of breast cancer exceeds all other cancers. So it is imperative to improve the quality of life of these patients.

Our knowledge of the process of tumorigenesis has increased significantly over the last decade thanks to continued funding from federal and private organizations, improved technologies enabling affordable sequencing of the entire genome, analysis of large data sets as well as gene expression profiles of human tumor samples, and improved animal models that attempt to resemble tumor formation in humans. The predisposing risk factors, precancerous lesions, and disease progression vary significantly across the tissues of origin. However, common themes have been described that drive a normal cell to undergo transformation and generate a tumor. We plan to lay the groundwork for our discussion utilizing the widely recognized models of colorectal cancer by Bert Vogelstein, the two hit hypothesis by Alfred Knudson, and the common characteristics of cancer cells described by Doug Hanahan and Robert Weinberg.

Furthermore, in this chapter we aim to discuss the early events that cause a normal breast epithelial cell to initiate the process of tumor formation and delineate them from later stage insults to the cell that cause it to progress to advanced metastatic disease. We particularly plan to focus on the role of oxidative stress and one major environmental agent i.e. ionizing radiation inducing DNA damage and chromosomal instability. At the same time we will discuss the cell cycle changes that ensue and the implications of loss of a tumor suppressor gene. Concurrently, there are morphological changes that can be witnessed in experiments performed with cancer cells in vitro which we will tie in with the underlying molecular mechanisms. We will trace

the damaged cell along its course to metastasis by focusing on the molecular mechanisms that cause loss of cell-cell adhesion, loss of cellular polarity, ability to migrate through the stroma and gain access to the vascular or lymphatic system, resistance to anoikis and ability to seed a tumor in a new environment. A myriad of hypotheses exists in literature that attempts to explain the process of cancer formation and progression.

Next, we will classify breast tumors as malignant or non-malignant while describing the subtypes of each in a concise manner. Since the therapeutic options available in the clinic are targeted to particular genetic subtypes such as BRCA1 positive, estrogen receptor (ER) positive or triple negative (Her2-/-, ER -/-, PR -/-) etc., we will also discuss these molecular signatures. The clinical diagnosis criteria and imaging modalities will be mentioned concisely. A limited number of clinical trials that have a promising premise behind the study and considered to be ground breaking will be described.

Therapeutic options for breast cancer have expanded in the past 10 years to improve the survival outcomes for the disease. Existing FDA approved pharmacologic agents, small molecule inhibitors in clinical trials and drugs shown to have efficacy in preclinical studies will be methodically described in the final section. In the process, we hope to summarize where we are now with respect to this potent disease that affects millions.

2. How does cancer arise?

As a cell achieves a neoplastic phenotype, its genetic sequence is usually vastly altered and multiple genes are mutated, amplified, or lost. Several models have been proposed regarding what leads to tumorigenesis. One of the models proposed by Dr. Bert Vogelstein proposes the loss of function of tumor suppressors [4-7]. According to his model, loss of function of tumor suppressors such as p53 leads to genomic instability which eventually leads to tumorigenesis via alterations in metabolism, loss of sensitivity to apoptotic signals, and increased invasiveness [8, 9]. Loss of function of the tumor suppressor, p53, is associated with the development of most, if not all, tumor types [10-12]. An inactivating mutation in a tumor suppressor not only leads to hyper-proliferation of epithelial cells, it may also inactivate DNA repair genes. Mutations in proto-oncogene can either create an oncogene or lead to a cascade of inactivation of several more tumor suppressor genes before resulting in cancer. Figure 1 shows this model for colon carcinogenesis.

An alternate theory that accounts for both hereditary and non-hereditary cancer is the two-hit theory of cancer causation proposed by Dr. Alfred Knudson [13, 14]. Normal cells have two undamaged chromosomes, one inherited from each parent. People with a hereditary susceptibility to cancer inherit a damaged gene on one of the chromosomes at conception which is their 'first hit' or mutation. Others receive the 'first hit' in their lifetime. Damage to the same gene on the second chromosome in their lifetime may lead to cancer. An overview of this model is given in Figure 2 and is seen in cancer such as retinoblastoma.

Weinberg and Hanahan have proposed the hallmarks of cancer which helps explain oncogenesis. These are biological capabilities acquired during the complex multistep development of
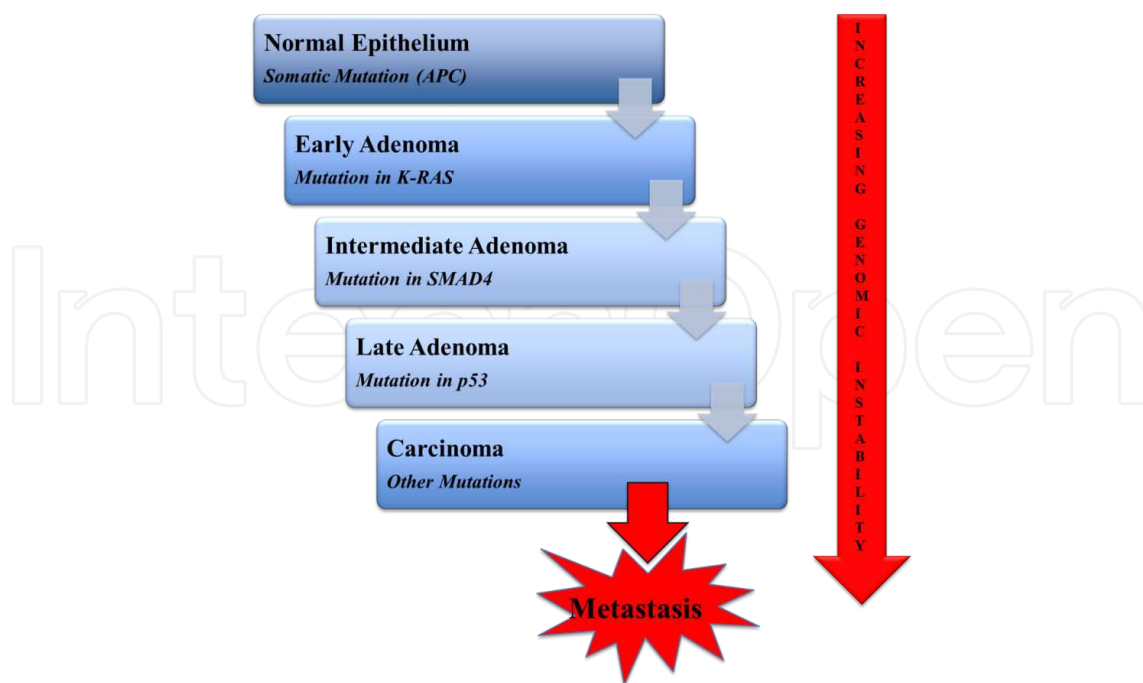
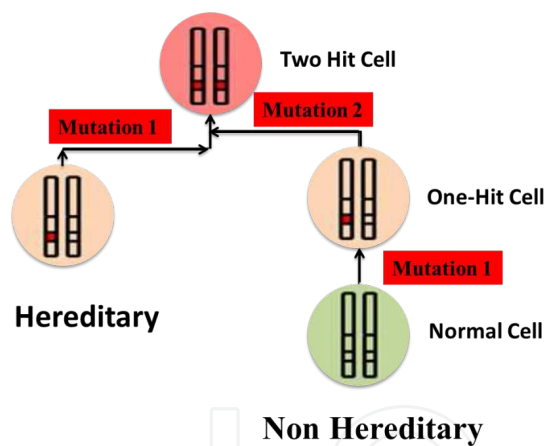**Figure 1.** The cascade of events that lead to oncogenesis.

Figure 2. The two-hit model of carcinogenesis.

cancer. Figure 3 summarizes the 8 hallmarks of cancer. They include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming of energy metabolism, and evading immune destruction [15]. All these hallmarks lead to genomic instability and persistent inflammation, possibly fueling further genetic diversity, as well as propagation, acquisition and fostering of multiple hallmark functions.

A possible contributing factor that hasn't gained much attention is the role of fragile sites. Common fragile sites (CFSs) are regions of the genome with a predisposition to DNA double-strand breaks in response to intrinsic (oncogenic) or extrinsic replication stress. CFS breakage
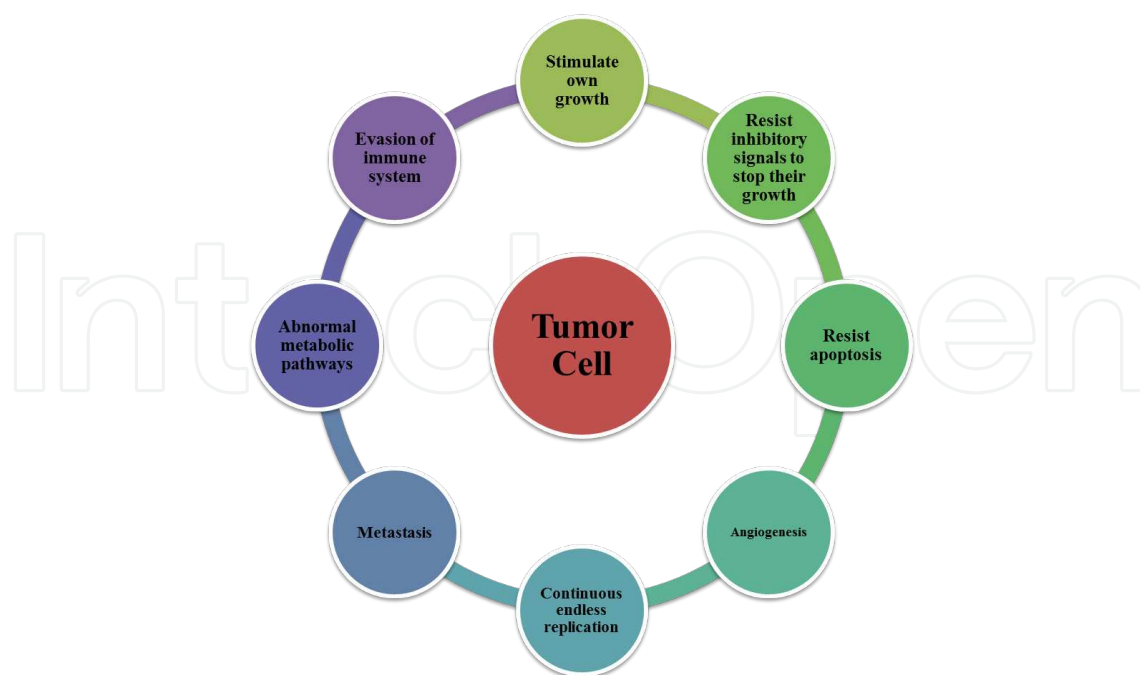
Figure 3. The 8 possible hallmarks of cancer.

is a common feature in carcinogenesis from its earliest stages and through its evolutions. In a recent article the association of several fragile sites stability with key DNA damage response (DDR) and DNA repair proteins like breast cancer type 1 susceptibility protein (BRCA1), Ataxia telangiectasia and Rad3 related (ATR), and Ataxia telangiectasia mutated (ATM) opens another possibility for the induction and/or acceleration of instability in breast tissue [16]. For example *FRA3B*, one of the most frequently expressed fragile sites in the human genome, is located within the tumor suppressor gene *FHIT* region. Deletions within *FHIT* have been associated with various human cancers including breast [17].

3. Events that cause a normal breast epithelial cell to start the process of tumor formation and eventually progress to advanced metastatic disease

A proto-oncogene is a normal gene that can convert to an oncogene due to mutations (generally dominant mutations) or increased expression [18-20]. Proto-oncogenes function in promoting cell division and inhibiting cell differentiation. Oncogenes, however, promote all the markers of a cancer cell such as increased cell division and replication stress, decreased cell differentiation, and inhibition of cell death (usually apoptosis). A proto-oncogene can convert into an oncogene due to various reasons including chromosomal translocation (such as BCR-ABL that is seen in leukemia), gene amplification, point mutations, deletions, alterations in promoter region leading to increased transcription, and insertions that lead to a hyperactive gene product. Human epidermal growth factor receptor 2 (HER2) is a proto-oncogene that is amplified in about 30% of breast cancer [18]. This is discussed in detail in a subsequent section.

To balance the effect of oncogenes, tumor suppressors are present as well to regulate cell growth and cell death but mutations in them can lead to tumor formation. The guardian of the genome, p53, is the most commonly mutated tumor suppressor gene in human cancer [21, 22]. It is involved in multiple pathways including maintenance of genomic stability by causing cell cycle arrest as the cell attempts to repair the damaged DNA, apoptosis, tumor progression, and metastasis [23]. Not surprisingly, a lot of breast cancers harbor mutations in this transcription factor as well. Since p53 has been linked to how BRCA1 dictates DNA repair and cell death, it may have a role in tumor response to treatment as well [24].

Checkpoints are present throughout the cell cycle that halt further progression of DNA replication and cell division, either permanently (senescence) or transiently, when damaged DNA is detected. This activates specific DNA repair pathways (discussed below). ATM and ATR are key proteins in the DNA damage response pathway. ATM is recruited to and activated by DNA double strand breaks while ATR is recruited to and activated by replication protein A-coated double stranded DNA. Two of the best studied ATM/ATR targets are the protein kinases checkpoint kinase 1 (CHK1) and checkpoint kinase 2 (CHK2). Together with ATM and ATR, these proteins reduce cyclin dependent kinase (CDK) activity which slows down or arrests cell-cycle progression at the G1–S, intra-S and G2–M cell cycle checkpoints allowing more time for DNA repair before progression of replication or mitosis. Moreover, ATM/ATR can promote DNA repair by a variety of methods including induction of DNA repair proteins transcriptionally or post-transcriptionally, by recruiting repair factors to the damage-site, and by activating DNA-repair proteins by modulating their post-transcriptional modifications such as phosphorylation, acetylation, ubiquitylation or SUMOylation.

Continuous DNA damage checkpoint activation may lead to selective suppression of the DNA-damage response-induced antitumor barriers. This may be due to inactivating mutations. This process promotes genomic instability and tumor progression [25-28]. Prolonged overexpression of licensing factors such as hCdt1 and hCdc6 prevent cell death and lead to a more aggressive phenotype. Overexpression of the replication licensing factor Cdc6 led to phenotypic changes with mesenchymal features and loss of E-cadherin. Analysis in various types of human cancer revealed a strong correlation between increased Cdc6 expression and reduced E-cadherin levels [29]. Cells possessing re-replicated DNA above a critical threshold are typically neutralized by cell death mechanisms but cells with re-replicated elements below a critical threshold are prone to recombination processes leading to genomic instability. As a result these cells are much more resistant to therapy [30].

DNA can be damaged spontaneously during replication stress and cell division as well as due to exogenous/environmental agents. This leads to thousands of DNA lesions/cell per day. In some cases of high oxidative or environmental stresses, repair resistant complex DNA damage can be induced as analytically discussed in a recent review by Kryston et al. 2011 [31]. As little as one unrepaired DNA double strand break can be lethal to the cell. Thus, the DDR and DNA repair pathways are in place to maintain the genomic integrity. This response pathway detects the DNA damage, signals their presence to recruit repair factors and halt cell cycle progression, and promote DNA repair. DNA lesions can block genomic replication and transcription and lead to mutations. Most of the time, cells undergo death in the form of apoptosis or necrosis

when there is unrepaired DNA. Cells defective in DNA repair are hypersensitive towards DNA damaging agents. For example, breast cancer cells with defective BRCA proteins are sensitive to poly ADP ribose polymerase (PARP) inhibitors. This is an active area of research with promising results thus far. This is discussed further in a later section. DNA repair pathways include base excision repair (BER), nucleotide excision repair (NER), double strand break repair via homologous recombination (HR) or non-homologous end joining (NHEJ), and mismatch repair (MMR) [32-34]. Frequently, multiple proteins are involved in the repair of the damaged DNA. The repair pathways are briefly described below.

In MMR-mediated repair, nuclease, polymerase and ligase enzymes fix a single-strand cut that is induced upon detection of mismatches and insertion/deletion loops. DNA glycosylase detects a damaged base in BER-mediated repair. This is subsequently removed before nuclease, polymerase and ligase proteins complete the repair. NER-mediated repair recognizes helix-distorting base lesions. The damage is excised as a 22–30-base oligonucleotide, producing single-stranded DNA that is a substrate for DNA polymerases and associated factors. The process ends with ligation. There are 2 major DNA double strand break repair pathways. NHEJ is predominantly used in the repair of radiation induced DNA damage. It is highly efficient but error-prone. The Ku proteins recognize and bind to the damaged site and activate the protein kinase DNA-PKcs, leading to recruitment and activation of end-processing enzymes, polymerases and DNA ligase IV. In contrast, HR uses sister-chromatid sequences as the template to mediate faithful repair. It is used in repair of replicative stress-induced lesions, stalled replication forks, and inter-strand DNA crosslinks. HR starts with single strand DNA generation, which is promoted by various proteins including the MRE11–RAD50–NBS1 (MRN) complex. In events catalyzed by RAD51 and the breast-cancer susceptibility proteins BRCA1 and BRCA2, the single strand DNA then invades the undamaged template and, following the actions of proteins mentioned above such as polymerases, nucleases, helicases, etc., the DNA is repaired.

One of the most famous mutations in cancer is the BRCA family of genes which are critical for HR-mediated repair of DNA double strand breaks [35, 36]. Mutations in the BRCA genes lead to an increased risk for breast cancer as part of the hereditary breast-ovarian cancer syndrome. Women with mutated BRCA1 or BRCA2 gene have up to a 60% risk of developing breast cancer [37, 38]. Hypermethylation of the BRCA1 promoter may be an inactivating mechanism for BRCA1 expression [39, 40]. Many of the mutations in BRCA1 or BRCA2 that predispose to breast cancer cause premature termination of the amino acid coding sequences, resulting in a truncated, dysfunctional protein.

Mutations in ATM, a critical DNA repair protein, lead to Ataxia Telangiectasia (AT). As mentioned above, ATM is a serine/threonine protein kinase that is recruited and activated by DNA double strand breaks and phosphorylates proteins that initiate activation of the DNA damage checkpoint, leading to cell cycle arrest, DNA repair or apoptosis. Several of these targets, including p53, CHK2 and H2AX are tumor suppressors which explains why AT sufferers are predisposed to breast cancer and are hypersensitive to radiation [41, 42]. Another example is the Werner syndrome which is marked by mutations in Werner syndrome ATP-dependent helicase (WRN) and Rad51 genes leading to deficiency in HR- and NHEJ mediated

DNA double strand break repair which, as expected, leads to increased incidence of breast cancer.

Breast cancer often metastasizes to bones, lungs, liver and brain [43-47]. The metastatic cascade is a series of biological steps that tumor cells must complete to exit the primary tumor and develop a new tumor at a distant site. One of the most critical steps involves invasion of the basement membrane and surrounding tissue and enter the bloodstream or lymphatic system. Cells that survive, eventually move into the tissue and establish a new colony that may form a tumor down the line. The host defense system is able to fend off millions of cancer cells that enter the blood stream but a few may escape nonetheless. Invasion involves the loss of cell-cell adhesion which may be mediated by matrix metalloproteinases and urokinases which break down integrins which attach tumor cells to their microenvironment and plasminogen respectively [48-54]. Cadherins are an intricate part of cell-cell adhesion and so downregulation of e-cadherin and upregulation of n-cadherin, involved in epithelial and mesenchymal phenotypes respectively, can promote metastasis [55-60].

Circulating tumor cells (CTCs) which like breast cancer is a heterogeneous population on cells, have a crucial role in the metastatic cascade, tumor dissemination and progression. Epithelial-to-mesenchymal transition (EMT) has an important role in the generation of CTCs and the acquisition of resistance to therapy [61-63]. Fibroblasts and myofibroblasts represent the majority of stromal cells within breast cancer. These cells promote the growth of cells by creating the perfect environment for cell survival and proliferation including enhanced angiogenesis. Tumor cells can express chemokine receptors that not only help direct migrating tumor cells to specific sites, they also determine if the cells will thrive and colonize at those sites. The bloodstream is highly unfavorable to tumor cells owing not only to the presence of immune cells, but also physical forces and anoikis, which combats metastasis. Interestingly, binding of tumor cells to coagulation factors, including tissue factor, fibrinogen, fibrin and thrombin, creates an embolus and facilitates arrest in capillary beds followed by the establishment of metastasis [64].

EMT is an important process in metastasis. Here, epithelial cells lose cell-to-cell contacts and cell polarity, downregulate epithelial-associated genes, upregulate mesenchymal-genes, and undergo major changes in their cytoskeleton. This confers greater motility and invasiveness. Expression of stem-cell markers and acquisition of stem-cell characteristics are important processes in this pathway as well. Once the tumor cells seed at the secondary site, they undergo redifferentiation to an epithelial phenotype [65]. One of the factors involved in EMT is epithelial derived growth factor (EGFR) which induces tissue factor which in turn promotes tumor seeding via the process described above. The transcription factor Twist-related protein 1 (TWIST1), the receptor ligand tumor derived growth factor β (TGFβ), Hypoxia-inducible factor 1 (HIF1), HER2, and Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/ Protein kinase B (AKT) signaling pathways have also been implicated in metastases. In preclinical models, expression of TWIST reduces metastasis and number of CTCs. CTCs often express NOTCH1 which confers self-renewal abilities. Some cells also express Aldehyde dehydrogenase 1 (ALDH1), another gene associated with stem cell like properties. Interleukin 6 (IL6) and Interleukin 8 (IL8) attract CTCs while Matrix metalloproteinase-1 (MMP1)–collagenase 1 and

the actin cytoskeleton component fascin 1 help CTCs infiltrate into tumors. Overexpression of the chemokine receptor C-X-C chemokine receptor type 1 (CXCR1) in CTCs is associated with decreased metastases and may be a therapeutic target.

4. Risk factors for breast cancer

Risk factors for malignant breast tumors include increased estrogen exposure which can be due to a number of reasons. For example, a woman can be exposed to increased estrogen due to increased total number of menstrual cycles, older age at 1st live birth, and obesity (increased estrogen exposure as adipose tissue converts androstenedione to estrone). BRCA1 and BRCA2 gene mutations also increase the risk of breast cancer and much research has been done in this avenue. Interestingly, increased incidence of triple negative breast cancer is seen in the African American population. Breast cancer risk is also increased with increased alcohol intake. Research suggests alcohol stimulates tumor growth by fuelling the production of growth factors that promote angiogenesis and by suppressing the immune system [66].

5. Classification of breast tumors as malignant or non-malignant

The breast is an organized organ and diseases may arise at any of its structural subunits. The stroma provides a supporting environment and this is where fibroadenoma and phyllodes tumor can arise. The smallest subunit is the lobule where we can see lobular carcinoma. Lobules give rise to terminal ducts where we can see tubular carcinoma. Next are major ducts where fibrocystic changes, DCIS, and invasive ductal carcinoma are often seen. These join to form the lactiferous sinus where intraductal papilloma may arise. Finally, Paget disease can be seen at the nipple. Figure 4 summarizes the different breast pathologies.
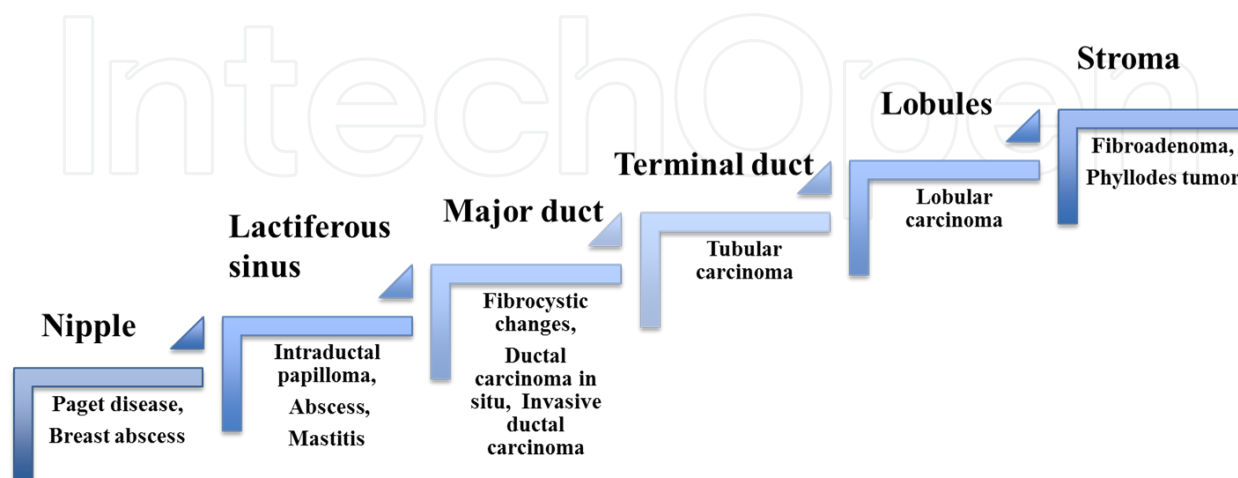

Figure 4. Pathologies that can affect the different breast tissues.

Not all breast tumors are malignant. Fibroadenoma are small, mobile, firm mass with sharp edges. They are most common in those <35 years old and increase in size and tenderness in response to estrogen as is seen in pregnancy and prior to menstruation. As mentioned, it does not lead to breast cancer. Similarly, intraductal papillomas are small benign tumors that grow in lactiferous ducts, typically beneath the areola. They can cause serous (faintly yellow and thin) or bloody nipple discharge. Of note, they do increase the risk for carcinoma be approximately 2-fold [67]. Phyllodes tumor are large bulky mass of connective tissue and cysts with leaf-like projections. They are most common in the 6th decade of life and similar to intraductal papilloma, can become malignant.

Malignant breast tumors are more common in postmenopausal women. They usually arise from terminal duct lobular unit. Overexpression of different proteins such as HER2 and EGFR are often seen. As discussed in a later section, receptor status can affect the therapy and prognosis. Since approximately 70% of the breast is drained by the axillary lymph node, involvement of this node indicating metastasis is the single most important prognostic factor. Since there is more tissue in the upper outer quadrant of the breast, tumors often arise here.

Malignant breast tumors can be subdivided into noninvasive and invasive tumors. Noninvasive tumors include ductal carcinoma in situ (DCIS), Paget disease, and comedocarcinoma. Comedocarcinoma is a subtype of DCIS where ductal caseous necrosis is seen. DCIS fills the ductal lumen and arises from ductal atypia. They are often seen as microcalcification on mammography due to necrosis. Paget disease results from underlying DCIS and results in eczematous patches on the nipple. Invasive breast tumors include invasive ductal and lobular cancer. A firm, fibrous mass with sharp margins and small, glandular, duct-like cells are seen in invasive ductal tumors. They are the worst and most invasive of the tumors as well as the most common, comprising of over 70% of all breast cancer. Invasive lobular cancer often presents bilaterally with multiple lesions in the same location. Pathologically, they present as an orderly row of cells. Fleshy, cellular lymphocytic infiltrate is seen with medullary breast carcinoma and it has a good prognosis. Finally, inflammatory breast tumor presents with dermal lymphatic invasion and has approximately 50% survival at 5 years. Due to blockage of the lymphatic drainage, Peau d'orange is often seen with this condition.

The classification is important because treatment varies based on the type of cancer. When a tumor is diagnosed as benign, it is often left alone. With malignant tumors, biopsy is performed to determine the severity and aggressiveness of the tumor.

5.1. Subtypes of breast cancer

Molecular subtypes of breast cancer may be useful in planning treatment and developing new therapies and so a lot of research is being conducted in this field. Figure 5 depicts some of the more common subtypes. Most studies divide breast cancer into six major molecular subtypes:

i.   Luminal A

ii.   Luminal B

iii. Triple negative/basal-like

iv.   HER2 positive

v.   Claudin low
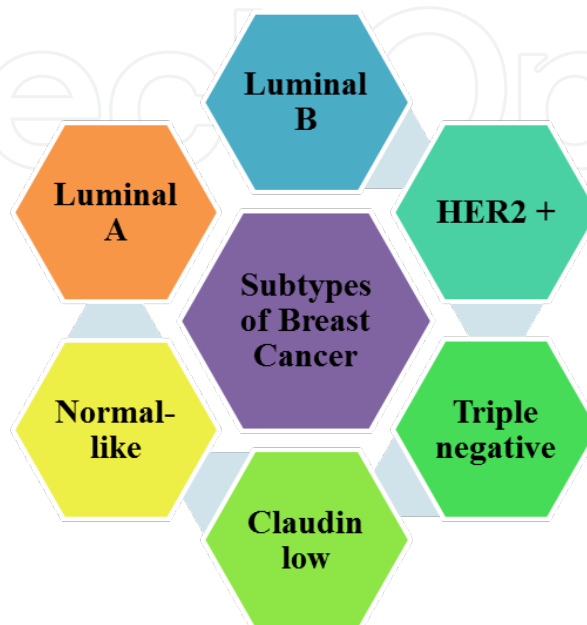
vi.   Normal-like

Figure 5. Subtypes of breast cancer.

Some of the less common subtypes include apocrine molecular type. Molecular apocrine breast cancers are aggressive estrogen receptor negative tumors overexpressing either HER2 or gross cystic disease fluid protein-15 (GCDFP15) [68]. Breast cancers that do not fall into any of these subtypes are often listed as unclassified.

i.   Luminal A

Most breast cancers are luminal tumors. Luminal tumor cells look the most like the cells of breast cancers that start in the inner (luminal) cells lining the mammary ducts. Luminal A tumors tend to be ER+ and/or PR+, HER2-, and tumor grade 1 or 2. Less than 15% of luminal A tumors have p53 mutations. Hence, luminal A tumors tend to have the best prognosis, with fairly high survival rates and fairly low recurrence rates. Since luminal A tumors tend to be ER+, treatment often includes hormonal therapy which is discussed in a subsequent section.

ii.   Luminal B

As mentioned above, luminal tumors have cells that look like those of breast cancers that start in the inner (luminal) cells lining the mammary ducts. Luminal B tumors tend to be ER+ and/or PR+. Since they have highly mitotically active cells, they are positive for Ki67. They are often HER2+ as well. Interestingly, women with luminal B tumors are often diagnosed at a younger age than those with luminal A tumors and have a poorer prognosis due to poorer tumor grade,

larger tumor size and lymph node involvement. About 30% of the tumors also have mutations in p53.

iii.   Triple negative/basal-like

Triple negative breast cancers are: ER-, PR-, and HER2-; hence the name triple negative. There are several subsets of triple negative breast cancer. One subset is referred to as basal-like because the tumors have cells with features similar to those of the outer (basal) cells surrounding the mammary ducts. Most basal-like tumors have mutations in p53. About 15 to 20% of breast cancers are triple negative or basal-like. These tumors tend to occur more often in younger and African American women. Of note, most BRCA1 breast cancers are both triple negative and basal-like. Triple negative/basal-like tumors are often aggressive and have a poorer prognosis. These tumors are usually treated with some combination of surgery, radiation therapy and chemotherapy.

iv.   HER2 type

The molecular subtype HER2 type is not the same as HER2+ and is not used to guide treatment. Although most HER2 type tumors are HER2+ (and named for this reason), about 30 percent are HER2-. HER2 type tumors tend to be ER-, PR-, with lymph node involvement and poor tumor grade. About 10% to 15% of breast cancers fall under this category and about 75% of HER2 type tumors contain p53 mutations. HER2 type tumors have a fairly poor prognosis and are prone to early and frequent recurrence and metastases. Women with HER2 type tumors appear to be diagnosed at a younger age than those with luminal A and luminal B tumors. HER2/neu-positive tumors can be treated with the drug trastuzumab (Herceptin) and this is discussed in further detail in a subsequent section.

v.   Claudin-low

Claudin low is often triple-negative, but distinct in that there is low expression of cell-cell junction proteins including E-cadherin and frequently there is infiltration of lymphocytes. It is also enriched in mesenchymal and stem cell features [69].

vi.   Normal-like

About 6 to 10% of all breast cancers are classified as normal-like. These tumors are usually small and tend to have a good prognosis.

6. Clinical diagnosis criteria and imaging modalities for breast cancer

Breast cancer is divided into different stages. Table 1 summarizes these stages.

The extent of cancer can be used to stratify patients. Patients with clinical stage I, IIA, or a subset of stage IIB disease (T2N1 where T= tumor, N= node) are classified as having early-stage breast cancer. Patients with a T3 tumor without nodal involvement or stage IIIA to IIIC disease are classified as having locally advanced breast cancer. Stage IV is when there are distant metastases present and is seen in about 5% of newly diagnosed patients.

Stage	Description
0	Restricted to membrane of the milk duct (DCIS, LCIS)
1	<2cm tumor restricted to the breast
2	2-5 cm tumor +/- metastasis to draining lymph node
3	Metastasis to the lymph nodes +/- superficial skin and surrounding muscles
4	Metastasis to other parts of the body

Table 1. Stages of breast cancer

i.   Early-stage breast cancer

The surgical approach to the primary tumor depends on the size of the tumor, whether or not multifocal disease is present, and the size of the breast. Options include breast-conserving therapy or mastectomy and both have similar outcomes.

The risk for metastatic disease in the regional nodes is related to tumor size, histologic grade, and the presence of lymphatic invasion within the primary tumor. As mentioned above, the axillary nodes drain most of the breast tissue. Tumor characteristics are used to select adjuvant treatment for patients with breast cancer. Patients with hormone receptor-positive breast cancer should receive adjuvant endocrine therapy. For patients with triple-negative breast cancer, treatment option includes adjuvant chemotherapy if the tumor size is >0.5 cm. Patients with HER2-positive breast cancer >1 cm in size typically receive a combination of chemotherapy plus HER2-directed therapy. Following chemotherapy, patients with ER-positive disease generally receive adjuvant endocrine therapy.

ii.   Locally advanced breast cancer

Most patients with locally advanced, inoperable breast cancer should receive neoadjuvant systemic therapy rather than proceeding with primary surgery in an attempt to shrink the tumor. Typically, these patients are usually not candidates for breast conservation. Neoadjuvant treatment improves the rate of breast conservation without compromising survival outcomes and so most patients get chemotherapy in the neoadjuvant setting rather than endocrine therapy. Due to its greater toxicity to cancer cells, chemotherapy is associated with higher response rates in a faster time frame. As mentioned earlier, HER2-directed agent (ie, trastuzumab) should be added to the chemotherapy regimen for tumors that are HER2-positive. Following surgery, all patients who undergo breast-conserving surgery generally undergo adjuvant radiation therapy (RT) to maximize locoregional control. Some patients treated by a mastectomy should receive postmastectomy RT in order to kill any cancer cells that may have escaped during the procedure.

Patients with hormone receptor-positive breast cancer should receive adjuvant endocrine therapy. The selection of endocrine therapy is made according to menopausal status. In patients with ER-positive breast cancer, in whom surgery is not an option or life expectancy is limited, primary hormonal treatment with either tamoxifen or an aromatase inhibitor without surgery is generally used.
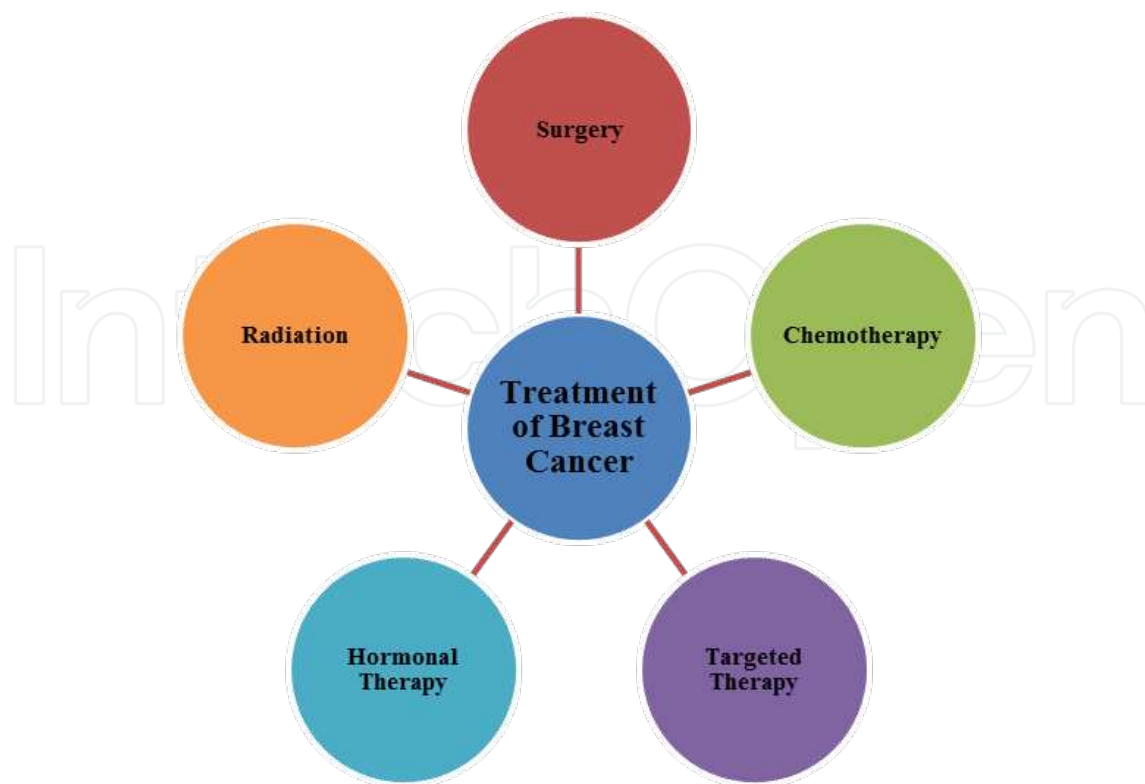
Figure 6. Different treatments available for breast cancer.

7. Therapeutic options for breast cancer

The heterogeneity of breast cancers makes it a challenge to diagnose and treat this solid tumor.

The main types of treatment for breast cancer are:

i.   Surgery

ii.   Radiation therapy

iii.   Chemotherapy

iv.   Hormone therapy

v.   Targeted therapy

Treatments can be classified into broad groups (Figure 6), based on how they work and when they are used.

a.   Local and systemic therapy

As the name implies, local therapy is intended to treat a tumor at the site without affecting the rest of the body. Examples include surgery and radiation therapy. Systemic therapy refers to drugs which can be given by mouth or directly into the bloodstream to reach cancer cells anywhere in the body. Chemotherapy, hormone therapy, and targeted therapy are systemic therapies that are widely used.

b.   Adjuvant and neoadjuvant therapy

Since even in the early stages of breast cancer, cancer cells may break away from the primary breast tumor and begin to spread, adjuvant therapy is often given to patients with no detectable cancer after surgery. A small number of cells can't be 'felt' on a physical exam or seen on X-rays or other imaging tests, and they cause no symptoms until they reach a certain number but, menacingly, they can go on to become new tumors in nearby tissues, other organs, and bones. Hence, adjuvant therapy is a mainstay following surgery. Both systemic therapy like chemotherapy, hormone therapy, and targeted therapy, and radiation can be used as adjuvant therapy.

In neoadjuvant therapy, patients are treated with chemotherapy or hormonal therapy prior to surgery. The goal of this treatment is to shrink the tumor in the hope it will allow a less extensive operation to be done. This also lowers the chance of the cancer coming back later.

i.   Surgery

For both DCIS and early-stage invasive breast cancer, doctors generally recommend surgery to remove the tumor. To make sure that the entire tumor is removed, the surgeon will also remove a small area of normal tissue around the tumor until a negative margin is achieved. A lumpectomy is the removal of the tumor and a small cancer-free margin while a mastectomy is the removal of the entire breast. It is important to lower the risk of recurrence and to get rid of any remaining cancer cells that can lead to both local and distant recurrence of cancer. Adjuvant therapies include radiation therapy, chemotherapy, targeted therapy, and/or hormonal therapy which are described below. Surgical treatment for breast cancer involves removal of the lymph nodes and can also include resection of the surrounding axillary nodes.

ii.   Radiation therapy

This involves killing the cancer cells by inducing clustered DNA damage using ionizing radiation. By overwhelming the cell with DNA damage, the cell undergoes apoptosis. As little as one DNA double strand break can be lethal to the cell. By giving multiple doses of radiation broken up into fractions, the hope is to prolong survival. Some of the side effects include dermatologic issues, fibrosis, nausea etc. due to the radiation. Although most side effects usually go away after radiation therapy has been concluded, some long-term side effects may occur months or even years after treatment ends. These late effects which usually associate with persistent inflammation and oxidative stress may include develop-ing a secondary primary cancer. However, we must mention that the risk of developing a second cancer because of radiation therapy is relatively low, and this risk is generally outweighed by the benefit of treating the primary, existing cancer and offering survival to the patient.

iii.   Chemotherapy

This involves using drugs and small molecules to selectively kill the cancer cells. Examples include: carboplatin, cisplatin, cyclophosphamide, docetaxel, doxorubicin, fluorouracil (5-FU), gemcitabine, methotrexate, paclitaxel, etc. A patient may receive one drug at a time or

combinations of different drugs at the same time. Research has shown that combinations of certain drugs are sometimes more effective than single drugs for adjuvant treatment and so combinations are often used. Carboplatin and cisplatin are alkylating agents and belong to the group of platinum-based antineoplastic agents. They interact with DNA to interfere with DNA repair. These drugs cross-link with the DNA strands, mostly to guanine groups. This causes intra- and inter-strand DNA cross-links, resulting in inhibition of DNA, RNA and protein synthesis. Antimetabolites, such as methotrexate, are more active against S-phase cells where they block DNA synthesis whereas vinca alkaloids are more active in the M-phase where they inhibit spindle formation and alignment of chromosomes. Antimetabolites are compounds that bear a structural similarity to naturally occurring substances such as vitamins, nucleosides or amino acids. They compete with the natural substrate for the active site on an essential enzyme or receptor. Methotrexate competitively inhibits dihydrofolate reductase, which is responsible for the formation of tetrahydrofolate from dihydrofolate. This plays an important role in the synthesis of, among others, purines and methionine. Anthracyclines such as doxorubicin intercalate with DNA and affect the topoisomerase II enzyme. This DNA gyrase splits the DNA helix and reconnects it to overcome the torsional forces that would interfere with replication. The anthracyclines stabilize the DNA topoisomerase II complex and thus prevent reconnection of the strands. Paclitaxel promotes assembly of microtubules and inhibits their disassembly which interferes with cell division.

One of the more recent treatment options for breast are PARP inhibitors which showed initial promise in patients with tumors that have BRCA1 or BRCA2 mutations and therefore deficient double strand break repair. PARP inhibitors achieve an enhanced or synthetic lethality for tumor cells by blocking DNA repair pathways. PARP, which has multiple family members, detects single strand DNA breaks and participates in BER. It forms poly (ADP-ribose) polymers on itself and a number of substrates which can alter a number of pathways including DNA repair. Inhibition of PARP leads to persistent single strand break which converts to a double strand break as the cell attempts to replicate the DNA. Normal cells have an intact HR-mediated repair pathway and so are able to repair the DNA double strand break. However, in the absence of intact HR-mediated repair pathway which can happen with loss of or mutation in BRCA proteins, the cell is unable to repair the double strand break. As a result, typically, the cell undergoes apoptosis. A phase II study of the PARP inhibitor olaparib in patients with advanced breast cancer with BRCA1 or BRCA2 mutations has shown promising results with a response rate of 11/27, a progression-free survival of 5.7 months, and a median objective response duration of 144 days [70]. Phase III trials are currently in progress to evaluate olaparib in breast cancer [71]. TNBC also demonstrates BRCAness and so PARP inhibitors may be useful in this setting as well. Data from clinical trials have not been conclusive in this regard thus far.

Phosphatase and tensin homolog (PTEN) regulates RAD51 mediated DNA repair to maintain genomic stability. PTEN mutations, which occur in 30–50% of breast cancers, cause genomic instability similar to that seen in BRCA-deficient cells and so may be targets of PARP inhibitors as well [72].

iv.   Hormonal therapy

Hormonal therapy is widely used in breast cancer treatment. These are used in the setting of ER+ and PR+ tumors. Since these tumors use hormones to fuel their growth, blocking the hormones can help prevent or at least slow down the growth of the tumor.

Selective estrogen receptor modulators (SERMs) are a class of compounds that act on the estrogen receptor. Tamoxifen blocks estrogen from binding to breast cancer cells. It is effective for not only lowering the risk of recurrence in the breast that had cancer, it also reduces the risk of developing cancer in the other breast, and the risk of distant recurrence. It is also approved to reduce the risk of breast cancer in women at high risk for developing breast cancer and for lowering the risk of a local recurrence for women with DCIS who have had a lumpectomy. Tamoxifen is also an effective treatment for metastatic hormone receptor-positive breast cancer. However, chronic Tamoxifen use has been linked with some toxicity and adverse effects like persistent oxidative stress and others as reviewed in [73].

Aromatase inhibitors (AIs) decrease the amount of estrogen made by tissues other than the ovaries in postmenopausal women by blocking the aromatase enzyme, which converts androgens into estrogen. These drugs include anastrozole and exemestane. Similar to Tamoxifen, AIs are also an effective treatment for metastatic hormone receptor positive breast cancer.

Fulvestrant, a SERM, is an additional hormonal therapy approved for patients with metastatic breast cancer. Fulvestrant is an estrogen-receptor targeting therapy that is used for the treatment of advanced-stage breast cancer in postmenopausal women with endocrine-sensitive cancer [74-77].

v.   Targeted therapy

Targeted therapy is a treatment that targets specific genes or proteins. One of the advantages of this is that it limits damage to healthy cells. Trastuzumab, a monoclonal antibody, is approved for both the treatment of advanced breast cancer and as an adjuvant therapy for early-stage HER2+ breast cancer. Trastuzumab does have cardio toxic effects. Pertuzumab is a monoclonal antibody marketed by Genentech for the treatment of HER2+ breast cancer, in combination with trastuzumab and docetaxel. It inhibits the dimerization of HER2 with other HER receptors, which reduces tumor growth. Lapatinib, a dual tyrosine kinase inhibitor which interrupts the HER2/neu and epidermal growth factor receptor (EGFR) pathways, is commonly used for women with HER2-positive metastatic breast cancer when trastuzumab and pertuzumab in combination with docetaxel are no longer effective at controlling the cancer's growth. Lapatinib decreases tumor-causing breast cancer stem cells and inhibits receptor signal processes by binding to the ATP-binding pocket of the EGFR/HER2 protein kinase domain, preventing auto-phosphorylation and subsequent activation of the signal mechanism.

Table 2 lists some of the current trials evaluating different therapies for breast cancer.

ClinicalTrials.gov Identifier	Description
NCT00065325	Compare the efficacy of Faslodex (fulvestrant) to Aromasin (exemestane) in hormone receptor positive postmenopausal women with advanced breast cancer.
NCT00103181	Compare whole breast radiation therapy to partial breast radiation therapy in treating women who have undergone surgery for ductal carcinoma in situ or stage I or stage II breast cancer.
NCT00176488	Evaluate epirubicin (an anthracycline) together with vinorelbine (an anti-mitotic drug) in treating patients with stage II, stage III, or stage IV breast cancer.
NCT00281697	Evaluate the efficacy and safety of bevacizumab when combined with standard chemotherapy compared with chemotherapy alone in subjects with previously treated metastatic breast cancer.
NCT00372710	Evaluate the safety and efficacy of zoledronic acid (a bisphosphonate) when added to standard therapies in breast cancer patients with metastatic bone lesions.
NCT00399529	Examine combination therapy with Trastuzumab, Cyclophosphamide, and an allogeneic Granulocyte-macrophage colony-stimulating factor (GM-CSF)-secreting whole cell breast cancer vaccine in patients with stage IV HER2/neu-overexpressing breast cancer.
NCT00454532	Assess the toxicity, maximum tolerated dose, safety and preliminary efficacy of BZL101, an aqueous extract from herba Scutellaria Barbata D. Don of the Lamiaceae family, for the treatment of advanced metastatic breast cancer.
NCT00466102	Determine whether RAD001 can inhibit growth of tumor cells and/or stop the formation and activity of bone degrading osteoclasts.
NCT00494234	To see if the drug KU 0059436 (olaparib) is effective and well tolerated in treating patients with measurable BRCA1- or BRCA2-positive advanced breast cancer and for whom no curative therapeutic option exists.
NCT00503841	How well does erlotinib work in treating women undergoing surgery for stage I, stage II, or stage III breast cancer?
NCT00629616	Efficacy of Anastrozole with fulvestrant in treating postmenopausal women with stage II or stage III breast cancer that can be removed by surgery.
NCT00817362	Efficacy and safety of IPI-504 (heat shock protein 90 inhibitor) with Trastuzumab in pretreated, locally advanced or metastatic HER2+ breast cancer
NCT00817531	Efficacy of Dasatinib in locally advanced triple negative breast cancer patients
NCT01031446	Evaluate cisplatin and paclitaxel together with everolimus and to see how well it works in treating patients with metastatic breast cancer
NCT01132664	Assess the safety and efficacy of BKM120 (PI3K inhibitor) in combination with trastuzumab in patients with relapsing HER2 overexpressing breast cancer who have previously failed trastuzumab.

ClinicalTrials.gov Identifier	Description
NCT01351597	Evaluate the efficacy and safety of combination chemotherapy with DoceTaxel (Detaxel) and Oxaliplatin (Oxalitin) in recurrent or metastatic breast cancer
NCT01509625	Assess the response to treatment with fulvestrant at a dose of 500 mg/month with a loading dose of 500 mg, in terms of progression free survival, overall survival, and clinical benefit rate, in post-menopausal women with advanced breast cancer and estrogen receptor positive, who were treated with this medicinal product and at said dose after having progressed with a previous anti-estrogen therapy.
NCT01534455	Compare the efficacy and tolerability of two dose-schedules of eribulin (a ketone analog) plus lapatinib in HER2-positive breast cancer, pre-treated with trastuzumab in the adjuvant and/or metastatic setting.
NCT01880385	Evaluating the treatment of bevacizumab in association with pre-operative chemotherapy, followed by surgery, adjuvant chemotherapy and radiotherapy in patients with inflammatory breast cancer.
NCT01881230	Compare the safety and efficacy of nab-paclitaxel in combination with either gemcitabine or carboplatin to the combination of gemcitabine and carboplatin as first line treatment in female subjects with triple negative metastatic breast cancer or metastatic triple negative breast cancer.
NCT02000622	Assess the efficacy and safety of single agent olaparib, a PARP inhibitor, vs standard of care based on physician's choice of capecitabine (that is converted to 5-FU during metabolism), vinorelbine (anti-mitotic drug) or eribulin (a ketone analog) in metastatic breast cancer patients with germline BRCA 1/2 mutations.
NCT02202746	Determine whether lucitanib, a potent tyrosine kinase inhibitor, is safe and effective in the treatment of patients with fibroblast growth factor aberrant metastatic breast cancer.

Table 2. Current clinical trials evaluating therapies for breast cancer

8. Conclusion

Breast cancer continues to be a threat and a challenge to treat. While a lot has been accomplished in the past decade, there is more that can be done. Further understanding of tumor evolution will lead to the eradication and effective prevention of this disease. At the same time delineating the breast oncogenic mechanisms like DNA damage response, conversion of DNA lesions to mutations, etc. will help us target initiating events and further optimize personalized therapies and possibly develop new ones. Therefore we believe that it is the 'DNA' which plays the dominant role and holds the key for effective treatment of the whole phenomenon of breast carcinogenesis.

Abbreviations

5-FU: Fluorouracil

AI: Aromatase inhibitor

AKT: Protein kinase B

ALDH1: Aldehyde dehydrogenase 1

AT: Ataxia telangiectasia

ATM: Ataxia telangiectasia mutated

ATR: Ataxia telangiectasia and Rad3 related

BER: Base excision repair

BRCA1: Breast cancer type 1 susceptibility protein

CDK: Cyclin dependent kinase

CFS: Common fragile sites

Chk1: Checkpoint kinase 1

Chk2: Checkpoint kinase 2

CTC: Circulating tumor cells

CXCR1: C-X-C chemokine receptor type 1

DCIS: Ductal carcinoma in situ

DDR: DNA damage response

EGFR: Epidermal derived growth factor

EMT: Epithelial-to-mesenchymal transition

GCDFP15: Gross cystic disease fluid protein-15

GM-CSF: Granulocyte-macrophage colony-stimulating factor

HER2: Human epidermal growth factor receptor 2

HIF1: Hypoxia-inducible factor 1

HR: Homologous recombination

IL6: Interleukin 6

IL8: Interleukin 8

MMP1: Matrix metalloproteinase-1

MMR: Mismatch repair

MRN: MRE11–RAD50–NBS1

NER: Nucleotide excision repair

NHEJ: Non-homologous end joining

PARP: Poly ADP ribose polymerase

PI3K: Phosphatidylinositol-4,5-bisphosphate 3-kinase

PTEN: Phosphatase and tensin homolog

RT: Radiation therapy

SERM: Selective estrogen receptor modulator

TGFβ: Tumor derived growth factor β

TWIST: Twist-related protein

WRN: Werner syndrome ATP-dependent helicase

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