2. REVIEW OF LITERATURE

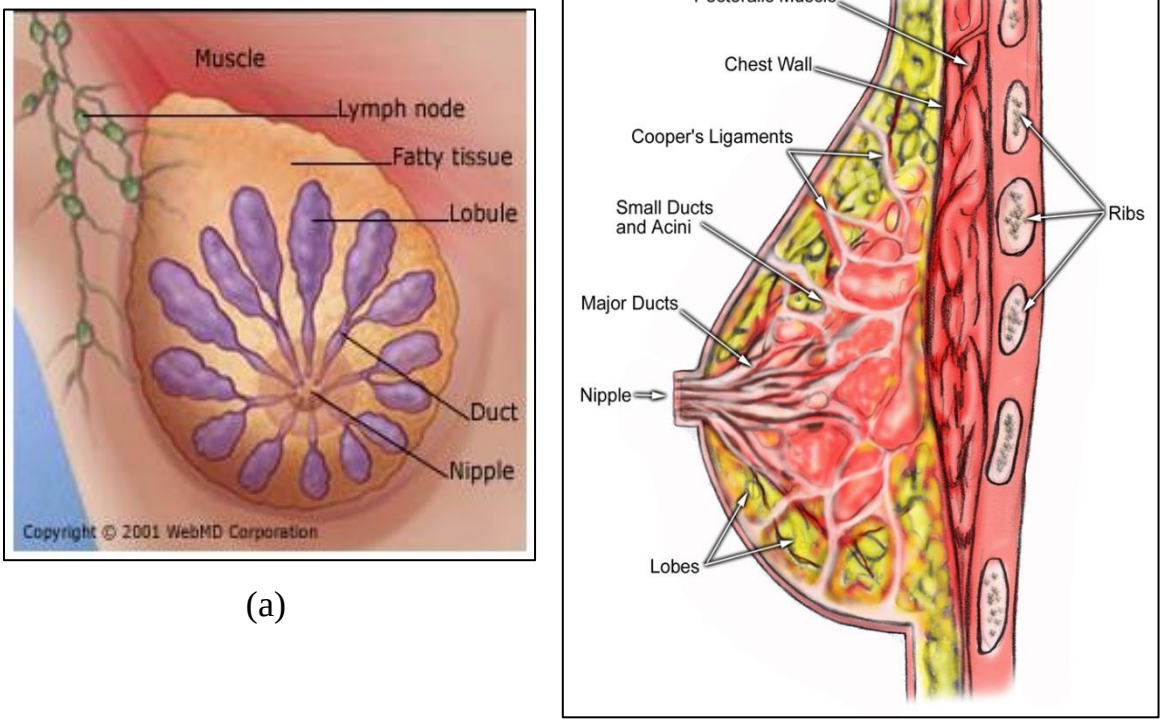
2.1. THE NORMAL BREAST TISSUE

2.1.1. Gross and functional anatomy of breast

Mammals pertaining to class "Mammalia" are differentiated from other creatures by possession of secondary sexual structure called breasts or mammary glands. Mammalia is derived from Latin word "mamma" which means "breasts". Both male and female have similar structure from birth until puberty, where female's mammary glands begin to grow in response to hormones estrogen and progesterone. As such, they are functional only in females and present in rudimentary form in the male. Basically, it is evolved for feeding the offspring by milk, termed lactation.

After puberty, in a period of 3-4 years the female mammary glands drastically changes to develop into fully mature breast. In the grown up female, the breast lies within the superficial fascia on the anterior chest wall. The base of the breast is attached to the chest wall longitudinally from the second or third rib to the sixth rib. In the transverse plane it reaches out from the sternal edge to almost the mid axillary line.[55] The supero-lateral quadrant of the breast extends towards the axilla.[56]

The female breast is composed of epithelial glandular tissue, fibrous connective tissue (stroma) surrounding the glandular tissue and interlobular adipose tissue.[56] The glandular tissue of the breast consists of ducts.[56] Each breast contains 15-25 of lobes, which makes the breast milk and send it to nipples via ducts.[57] (Figure 2.1)



(a) (b)

Figure 2.1: Anatomical organization of human mammary gland
a) Schematic representation of female breast b) Side view of breast with histological structures

During all phases of breast development, stromal-epithelial interaction along with ovarian hormones are very much important.[58] Prolactin, estrogens and progesterone are the most important hormones in all phases.[59] From birth to puberty, the mammary epithelium originating at the nipple remains quiescent[60] (Figure 2.2 A). Under influence of hormones and other factors, during puberty, the ductal epithelium of the mammary gland invades into the mammary fat pad [60, 61] (Figure 2.2 B). The virgin duct contains an outer layer of myoepithelial cells and an inner layer of luminal epithelial cells. The mammary gland is filled with epithelial branching structures (Figure 2.2 C). Although extensive development occurs during puberty, final differentiation happens only at pregnancy. Pregnancy is accompanied by hormonal changes due to which a

large expansion of alveolar cells occurs that mature to milk-secreting acini/alveoli during lactation. The alveoli (inset) expand out from the ducts filling the majority of the fat pad (Figure 2.2 D). Involution proceeds upon weaning through cell death and remodeling of extracellular matrix, resembling to resting adult mammary gland (Figure 2.2 E)

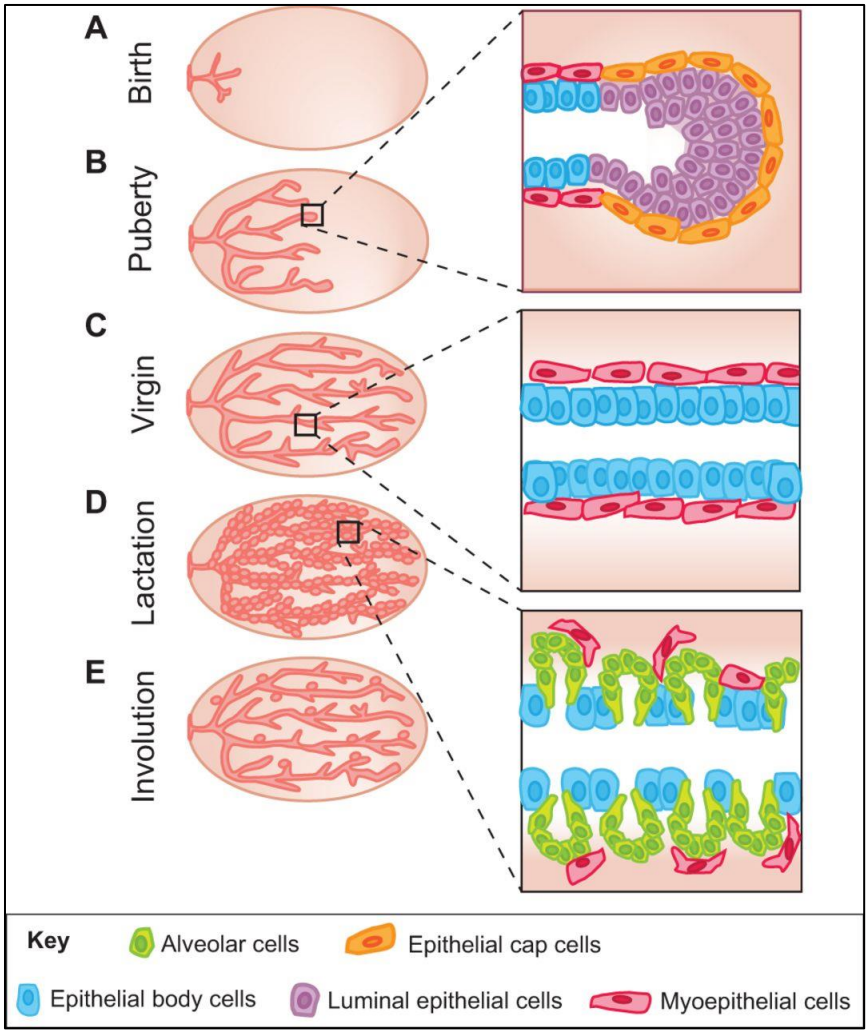


Figure 2. 2: The mammary gland development at different phases with histological structure [62, 63]

2.1.2. Blood and Lymphatic supply to the breast

The arterial blood supply emerges from the axillary artery supplying blood to the breast. The four main arteries are the superior thoracic, the pectoral branch

of the thoraco-acromial artery, the lateral thoracic and the subscapular arteries.[64, 65] The venous drainage predominantly follows the arterial supply.[57] (Figure 2.3)

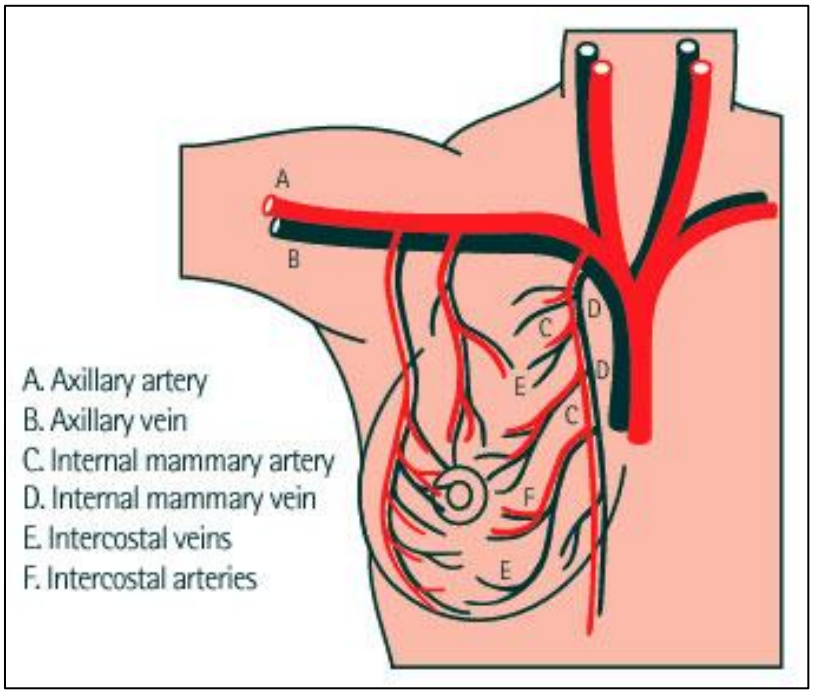


Figure 2. 3: Blood supply to human mammary gland

There is almost a parallel network of lymphatic system to circulatory system. The lymphatic drainage of the breast is much more variable. From a subareolar plexus there are lymphatic vessels draining to the following regions; the contra lateral breast, the internal mammary chain and the ipsilateral axilla.[57] The axilla is the predominant site of lymphatic drainage. There are usually between 20 and 40 nodes in the axilla and these may be grouped into level 1 nodes, below pectoralis minor muscle, level 2 nodes, behind pectoralis minor and level 3 or apical nodes which are between pectoralis minor and the clavicle.[55, 57] (Figure 2.4)

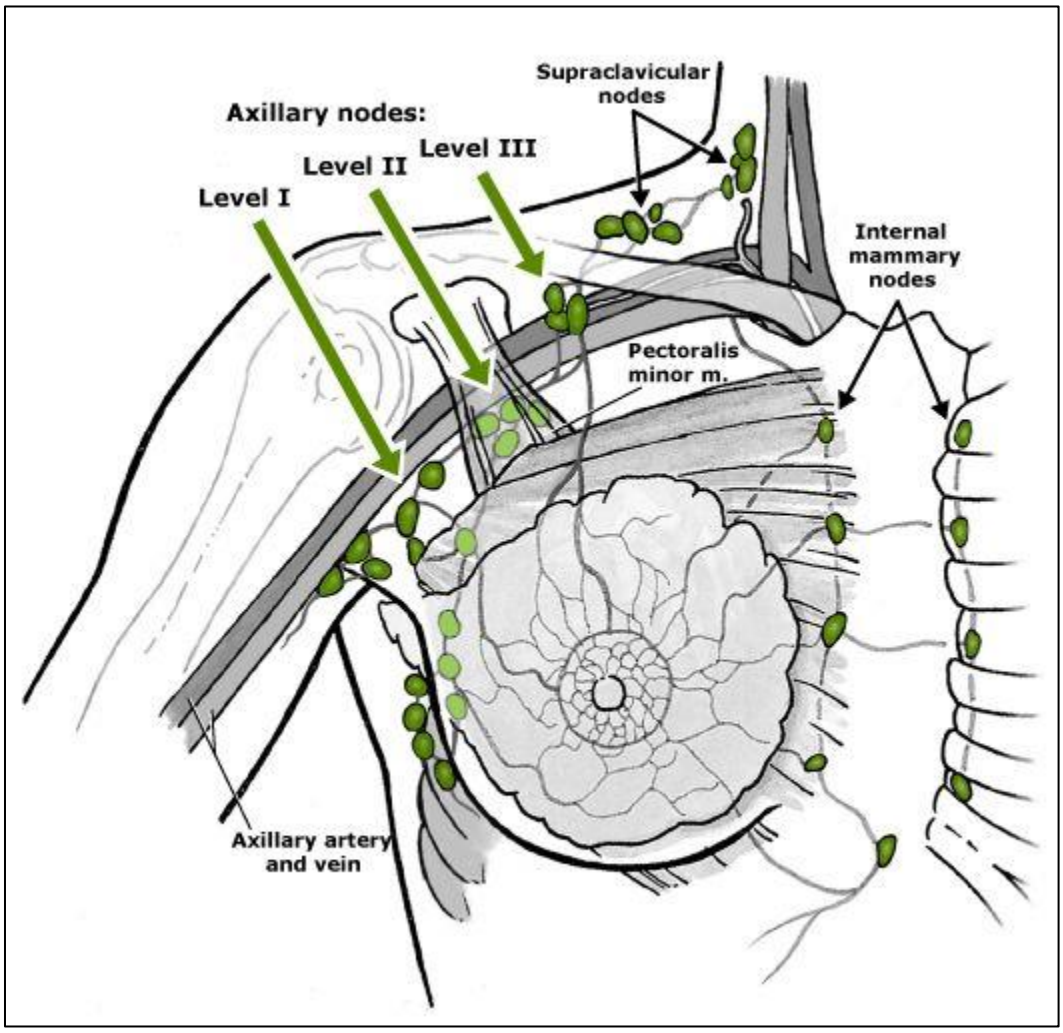


Figure 2. 4: Lymphatic supply in human mammary gland

2.2. BREAST CANCER

Breast cancer is a malignant tumor which originates in the breast cells.[1] A malignant tumor is a group of cancer cells that can invade surrounding tissues or spread (metastasize) to distant areas of the body.[1] It is highly heterogeneous disease from a clinical, genetic and phenotyping perspective occurring almost entirely in women, but men can get it, too.[1]

2.2.1. Types of breast cancer

The simplest classification is based on invasiveness of tumor cells.

- *Carcinoma in-situ*

This is also called non-invasive cancer. The cancer cells have not invaded other tissues except breast. It includes ductal carcinoma *in-situ* (DCIS) and lobular carcinoma *in-situ* (LCIS). In DCIS, cancer cells growth is limited to ducts (Figure 2.5) while in LCIS it is confined to lobules (Figure 2.6). DCIS is heterogeneous disease in terms of histology and molecular attributes.[66] On an average, 75% of DCIS cases express estrogen receptor (ER) and 40% over expresses HER-2 protein.[66] It many times goes unnoticed and progresses to invasive ductal carcinoma (IDC). LCIS is less common than DCIS and rarely diagnosed. Approximately 15-20% females with LCIS develop invasive lobular carcinoma (ILC) and 50% develops contra-lateral breast cancer.[67, 68]

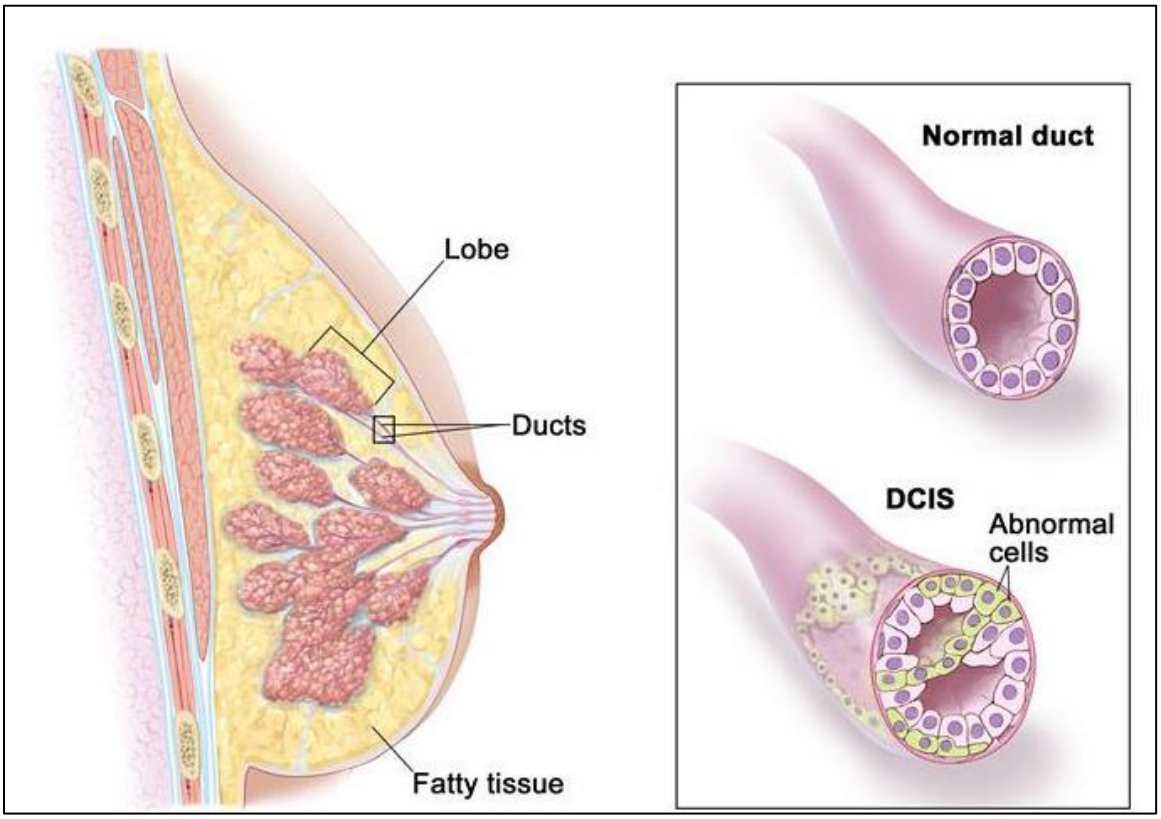


Figure 2. 5: Ductal Carcinoma *in-situ* (DCIS)

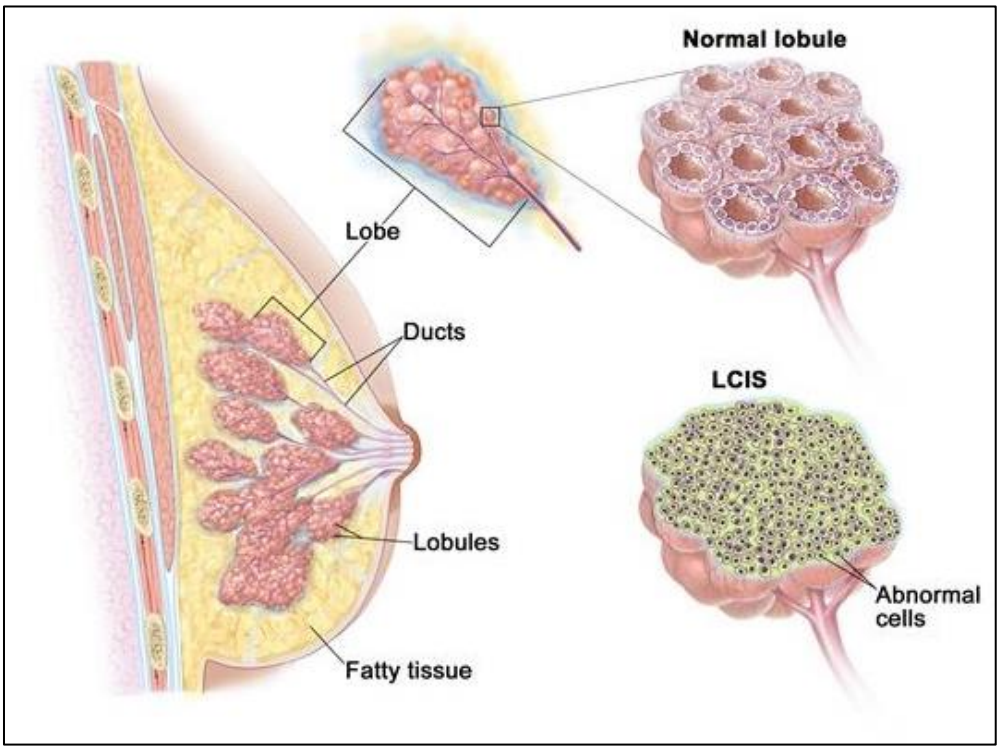
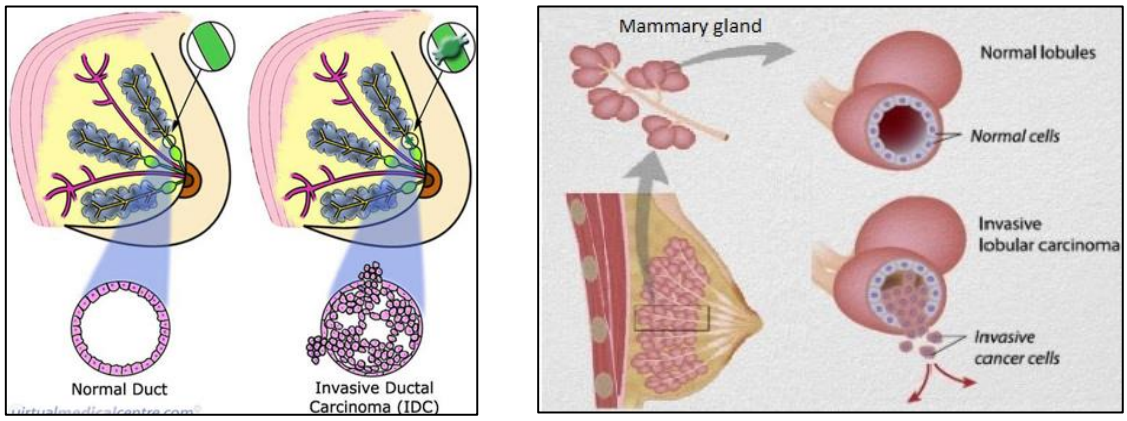


Figure 2. 6: Lobular Carcinoma *in-situ* (LCIS)

- *Invasive Carcinoma*

In invasive breast carcinoma the cancer cells have spread outside the basal lamina, invading the stroma surrounding ducts (IDC) or the lobules (ILC). Around 80% of diagnosed cancer is IDC. ILC accounts for 10-15% of cancer cases.[69] (Figure 2.7)



(a) (b)

Figure 2. 7: Invasive breast carcinoma (a) Invasive ductal carcinoma (b) Invasive lobular carcinoma

2.2.2. Traditional system of classification

- *TNM classification*

The most commonly used staging system is TNM (Tumor, Node, Metastasis); developed by American Joint Committee on Cancer (AJCC) and International Union Against Cancer (AJCC).[1] It was developed to stage different types of cancer based on size of tumor (T), the extent of spread to lymph node (N), and evidence of metastasis (M). (Table 2.1) Once these are assigned, they are combined together with I, II, III or IV stages. Sometimes, they are sub-divided in a, b or c.[1]

Table 2.1: TNM staging of breast cancer

T Tumor size	T1 Size: < 2cm	T2 Size:2-5 cm	T3 Size: > 5cm	T4 Tumor extends to skin or chest wall
N Lymph nodes	N0 No lymph node (LN) involved	N1 Metastasis to ipsilateral, moveable, axillary LNs	N2 Metastasis to ipsilateral, fixed, axillary LNs	N1 Metastasis to infraclavicular/ supraclavicular LNs
M Metastasis	M0 No metastasis		M1 Metastasis	

Stages are as follows:

- Stage 0: Known as "cancer in situ," meaning the cancer has not spread past the ducts or lobules of the breast (the natural boundaries). Also called noninvasive cancer.
- Stage I: The tumor is small and has not spread to the lymph nodes. Tumor is less than or equal to 2 cm.

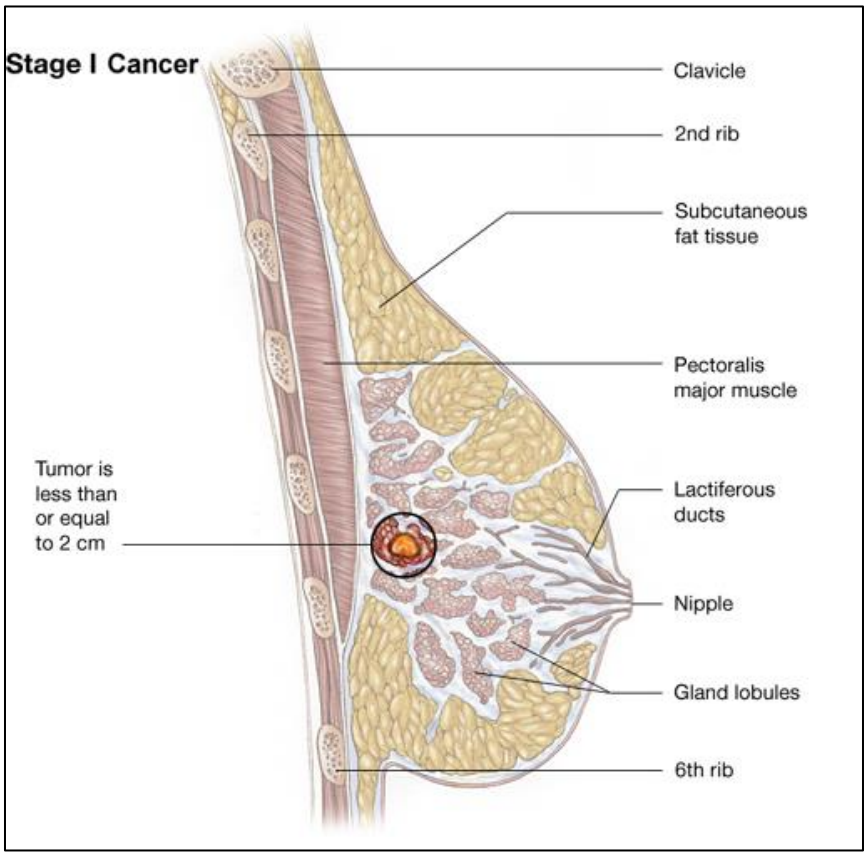


Figure 2. 8: Stage I breast cancer

- Stage II a: A smaller tumor that has spread to the axillary lymph nodes (lymph nodes under the arm), or a medium-sized tumor that has not spread to the axillary lymph nodes. Stage IIa may also describe cancer in the axillary lymph nodes with no evidence of a tumor in the breast.

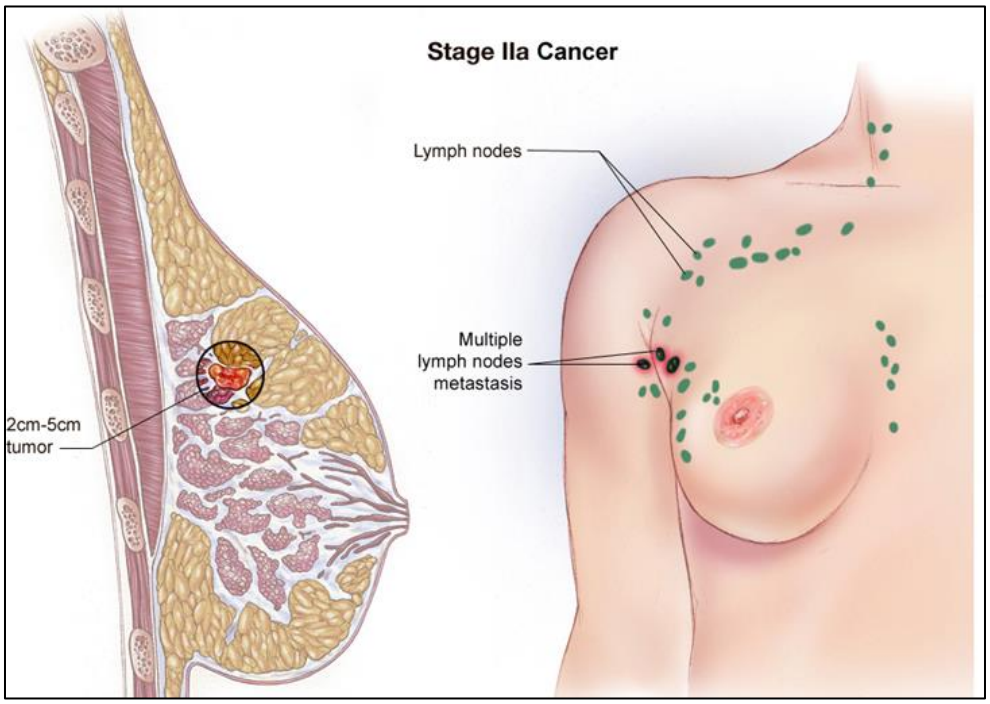


Figure 2. 9: Stage II a breast cancer

- Stage II b: A medium-sized tumor that has spread to the axillary lymph nodes. Stage IIb may also describe a larger tumor that has not spread to the axillary lymph nodes.

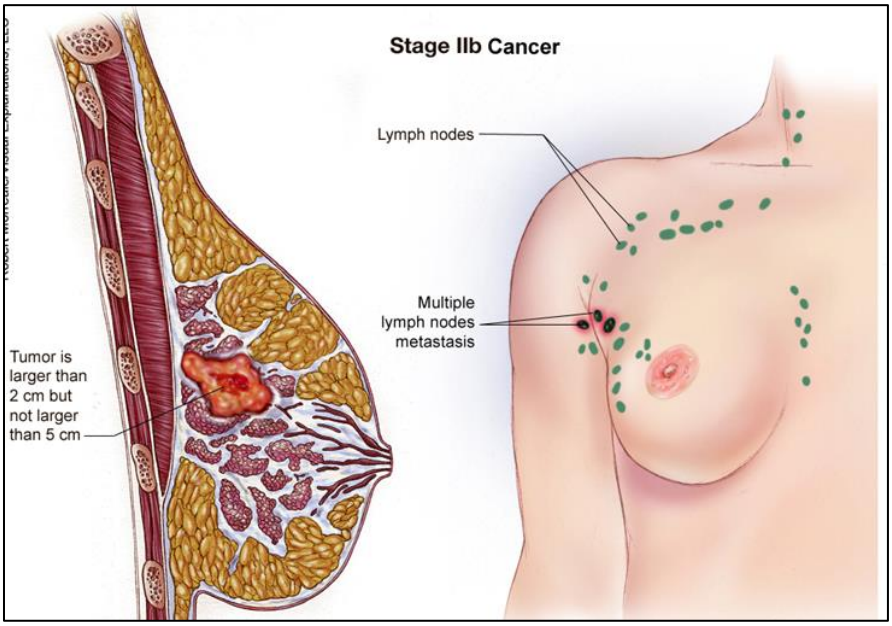


Figure 2. 10: Stage II b breast cancer

- Stage III a: Any size tumor that has spread to the lymph nodes. Tumor is larger than 2 cm but less than 5cm.

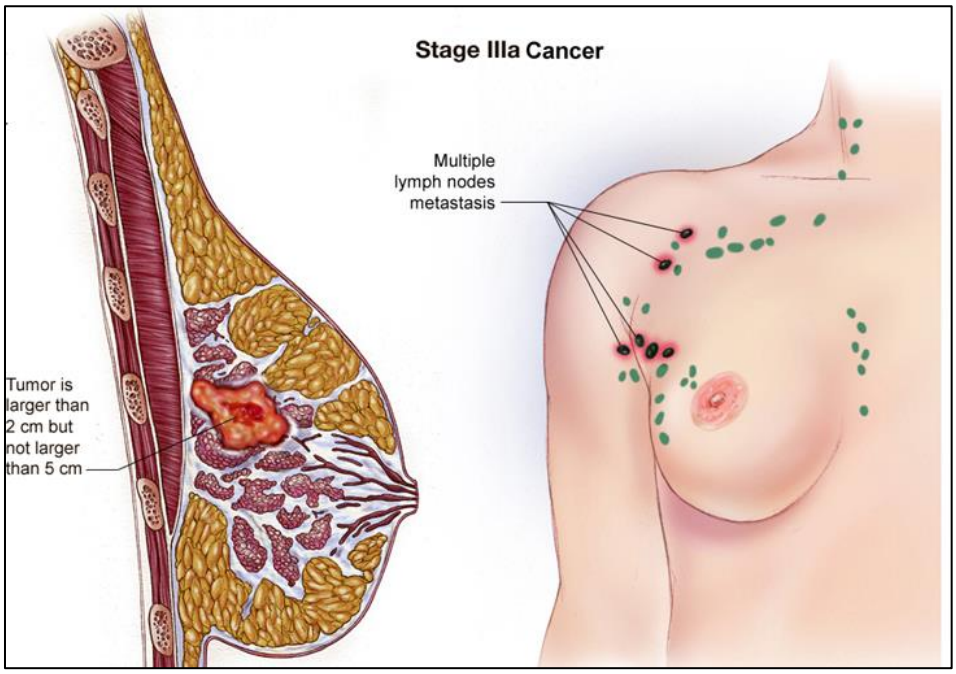


Figure 2. 11: Stage III a breast cancer

- Stage IIIb: Breast cancer has spread to the chest wall, or caused swelling or ulceration of the breast, or is diagnosed as inflammatory breast cancer.

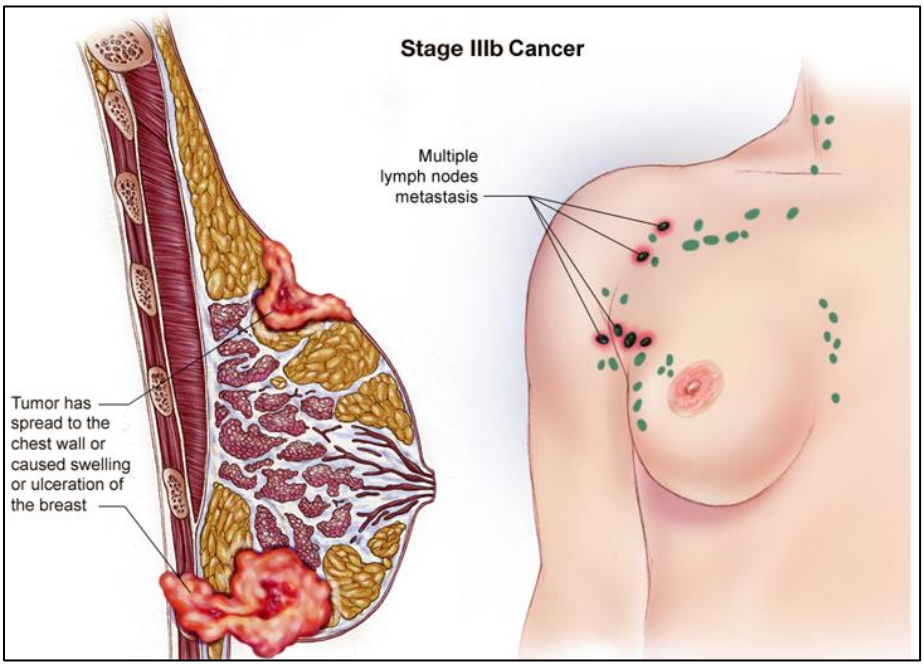


Figure 2. 12: Stage III b breast cancer

- Stage IIIc: Breast cancer has spread to distant lymph nodes but has not spread to distant parts of the body.

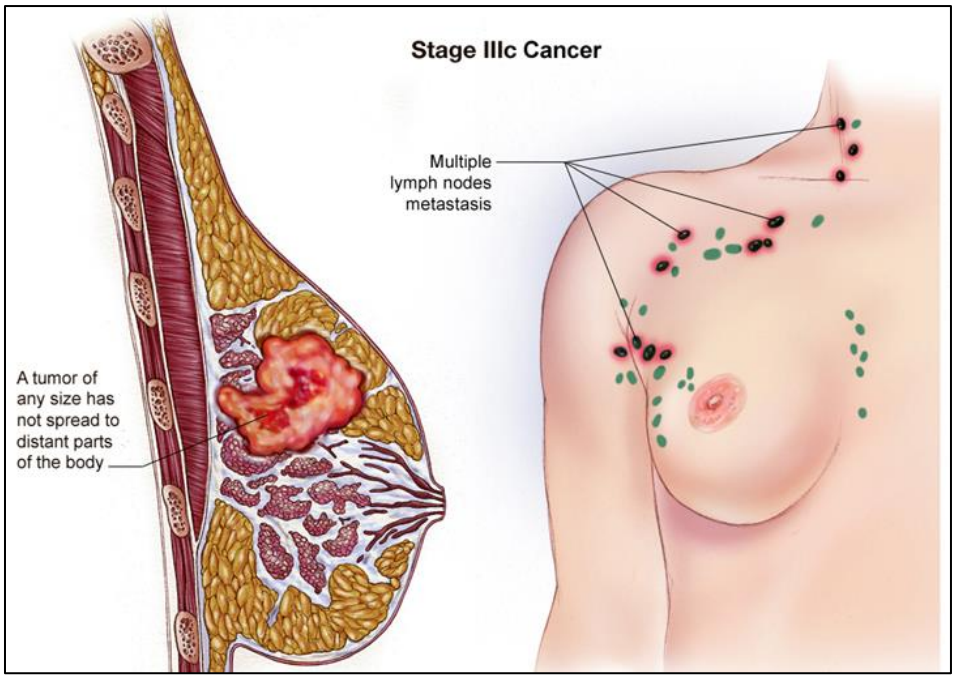


Figure 2. 13: Stage III c breast cancer

- Stage IV: Breast cancer can be any size and has spread to distant sites in the body, usually the bones, lungs or liver, or chest wall.

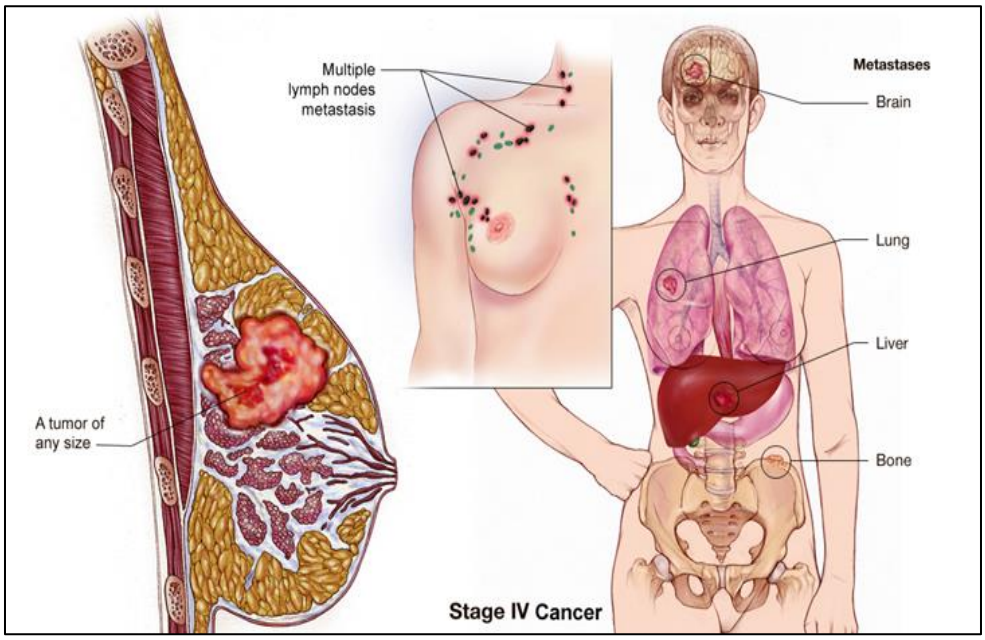


Figure 2. 14: Stage IV breast cancer

Table 2. 2: Staging and TNM classification

Stage Group	Tumour	Nodes	Metastasis
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II A	T0	N1	M0
	T1	N1	M0
	T2	N0	M0
Stage II B	T2	N1	M0
	T3	N0	M0

Stage III A	T0	N2	M0
	T1	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
Stage III B	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
Stage III C	Any T group	N3	M0
Stage IV	Any T group	Any N group	M1

- *Hormone status*

Estrogen receptors (ERs: ERα and ERβ) and Progesterone receptors (PR) are main two fuel steroid hormones which are responsible for gene transcription during cell proliferation and development.[70, 71] ERα is responsible for ductal elongation during puberty[72] while ERβ gets involved in epithelium differentiation.[73] The progesterone receptor is generally involved in formation of lobular- alveolar structures and cellular differentiation in normal breast.[74]

Breast cancers having estrogen receptors are often referred to as *ER-positive* (or ER+) cancers, and those expressing progesterone receptors are called *PR-positive* (or PR+) cancers. If either type of receptor is present, the cancer is said to be *hormone receptor-positive*. They tend to grow more slowly and are much more likely to respond to hormone therapy than breast cancers without these receptors.[75]

- *HER2/neu status*

About 1 of 5 breast cancers have over expression of growth promoting protein called HER2/neu. This leads to cell proliferation with angiogenesis and inhibition of apoptosis. The HER2/neu gene instructs the cells to make this protein.[76] Tumors with increased levels of HER2/neu are referred to as HER2-positive. These cancers tend to grow and spread more aggressively than other breast cancers with poor prognosis.[77]

2.2.3. Newer classification (Molecular classification)

The comprehensive gene expression profiling in breast cancer tumors was carried out by different independent researcher groups using microarray technologies.[78-82] The newer classification is based upon their genetic signature rather than histological phenotype.[83, 84] Recently, a sub-set of 306 gene micro array has been used to distinguish five and possibly 6th subtype of breast cancers.[85] This molecular classification of breast cancer is important in two particular areas. Firstly this classification has important prognostic significances beyond the traditional prognostic indexes[86] and secondly it is now being realized that these subtype divisions are of importance in predicting the response to adjuvant therapies.[86]

Following are the validated subtypes based on gene expression profile:

Luminal (hormone positive) type

- *Luminal A*, predominantly lower grade cancer expressing the highest level of ER α, GATA binding protein A, X-box binding protein 1, Trefoil factor 3, Hepatocyte nuclear factor 3 α and estrogen-regulated LIV-1. It starts in inner lining of mammary ducts. About 30-40% of breast cancer are of this type.[87, 88]
- *Luminal B*, lower expression of the luminal type genes with a higher overall grade and poorer outcome than Luminal A cancers.[87, 89] Females

with luminal B cancer are mainly diagnosed at a younger age than those with luminal A cancer.[89-91] They can be HER 2 positive or negative.

Non-luminal types (low to absent of ER α and other luminal cluster factors.)

- *Triple negative or Basal like subtype,* characterized by low or absence of hormones and HER 2 protein. About 15-20 percent of breast cancers are triple negative/basal-like.[88, 92, 93] They are more common in Black/non-Hispanic; Black/African-American women.[91, 93-95] They are most aggressive and have a poorer prognosis compared to the Luminal subtypes.[87, 90, 95, 96]

2.2.4. Risk factors

- *Gender*

Simply being a female is the main risk factor for developing mammary cancer. Men can develop breast cancer, but chances are near to zero. This is probably because men have less of the female hormones estrogen and progesterone, which can promote breast cancer cell growth.[97]

- *Aging*

Your risk of developing breast cancer increases as you get older. About 1 out of 8 invasive breast cancers are found in women younger than 45, while about 2 of 3 invasive breast cancers are found in women age 55 or older.[2] Among women, the leading cause of cancer death is brain and other nervous system tumors before age 20 years, breast cancer from ages 20 to 59 years, and lung cancer thereafter.[98]

- *Family history of breast cancer*

Blood relatives diagnosed with breast cancer increases the chances.[99] Having one first-degree relative (mother, sister, or daughter) with breast cancer

approximately doubles a woman's risk.[100] Having 2 first-degree relatives increases her risk about 3-fold.[100, 101]

- *Personal history of breast cancer or other cancers*

A woman diagnosed with cancer in one breast has a 3- to 4- fold increased risk of developing a new cancer in the other breast or in another part of the same breast.[102] This is different from a recurrence (return) of the first cancer.[1] A personal history of Hodgkin's disease,[103] ovarian cancer and certain other cancers can also increase the risk of breast cancer.[104]

- *Race and ethnicity*

Generally, white women are more likely to be diagnosed with mammary cancer than African-American women. However, chances of death are greater in African-American women.[1] 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.[1]

- *Pregnancy*

Women with no children or first child after age 30 or 35 have higher breast cancer risk.[105-107] Whether giving birth protects equally against estrogen receptor-positive and estrogen receptor-negative (including triple negative) breast cancers is under study.[108]

- *Menstrual period*

Starting menstrual periods at a young age is linked to a small increase in breast cancer risk.[107-109] For example, women who have their first periods before 12 years of age have about a 20 % higher risk than those who began their periods after 14 years of age.[110]

- *Menopause*

Late menopause increases the risk of breast cancer.[107, 110] Menopause after 55 increases 30% risk than women who have it at age of 45.[110]

- *Birth control*

Studies have found that using oral contraceptives (birth control pills), the risk of breast cancer increases.[111] 20-30% increase chances of breast cancer have been associated with use of birth control pills.[112, 113]

- *Hormone therapy after menopause*

Hormone therapy with estrogen (or in combination with progesterone) are used since many years to relieve menopausal symptoms and osteoporosis.[114, 115] Estrogen plus progestin increases the risk of both developing and dying from breast cancer.[116, 117] Estrogen alone is only used by women who have had a hysterectomy because it increases the risk of uterine cancer.[118] It is usually not given to breast cancer survivors because it may increase the risk of recurrence.[118, 119]

- *Drinking alcohol*

The use of alcohol is linked to an increased risk of breast cancer. The amount of alcohol is directly proportional to increase in risk. In compared with non-drinkers, female who consume one alcoholic drink in a day have a slight increase in risk.[120] 20% risk increases with two to five drinks in a day.[120]

- *Being overweight or obese*

Obese women after menopause increases breast cancer risk.[121] After menopause, the supply of estrogen is from fat tissue. So, more fat tissues will provide more amount of estrogen, thereby, increasing the risk.[122]

- *Genetic risk factors*

About 5% to 10% of breast cancer cases are thought to be hereditary.[123] The most common is mutation in the *BRCA1* and *BRCA2* genes.[124] In healthy cells, they prevent cancer by making proteins that keep the cells from growing abnormally. With *BRCA1* mutations the lifetime risk of breast cancer is as high as 80%.[125] For *BRCA2* mutations the risk is lower, around 45%.[123, 125] They are commonly seen in younger women and more often affect both breasts.[126] Women with these inherited mutations also have an increased risk for developing other cancers, particularly ovarian cancer.[127]

2.2.5. Diagnosis and screening

- Mammography

Mammography has critical part in diagnosing breast cancer. A mammogram is nothing but an x-ray of the breast.[128] A diagnostic mammogram is employed to diagnose symptomatic women or with an abnormal result of screening mammogram.[9, 128] Screening mammograms are utilized for screening of breast disease in asymptomatic women.[129] Screening mammograms capture two views of each breast, while diagnostic mammograms may take more views of the breast.[129] Screening mammograms may reduce the death among women who are 40 years of age or older due to breast cancer.[128, 129] Women older than 40 years should have mammography twice a year.[9, 130]

During mammogram, the breast is squeezed between two plates to flatten and disperse the tissue for few seconds.[131] The entire procedure for a screening mammogram takes about 20 minutes. This procedure produces a black and white image of the breast tissue either on a large sheet of film (film mammography) or as a digital computer image that is read, or interpreted, by a radiologist (digital mammograms).[130, 131]

- *Clinical breast exam*

A trained health care professional will examine breasts for abnormalities in size or shape, or changes in the skin of the breasts or nipple.[130] The special attention will be paid to the shape, texture, location of any lumps, and whether such lumps are attached to the skin or to deeper tissues.[132] The area under both arms will also be examined.[131, 133]

- *Breast awareness and self-exam*

Women above 20 should regularly engage in breast self-exam (BSE).[134] Women should know how their breasts normally look and feel.[135] Literature survey suggests it reduces chances of mortality if done properly.[136, 137] Any abnormalities should be reported and proper clinical diagnosis should be made. Women who examine their breasts should have their technique reviewed during their periodic health exams by their health care professional.

- *Magnetic resonance imaging (MRI)*

MRI uses radio waves and strong magnetic fields. The screening MRI is recommended in combination with yearly mammography for women with high risk of breast cancer.[138] It is not generally recommended as a screening tool by itself, because although it is a sensitive test, it may still miss some cancers that mammograms would detect.[138]

- *Biopsy*

A biopsy is done when any abnormalities are detected with above mentioned techniques. It is the only way to tell if cancer is really present. During a biopsy, a sample of the suspicious area is removed to be looked at under a microscope by pathologist.[139]

There are several types of biopsies, such as fine needle aspiration biopsy, core (large needle) biopsy, and surgical biopsy.[9, 140] In a fine needle aspiration

(FNA) biopsy, an extremely thin and hollow needle attached to a syringe is utilized to aspirate a small mass of tissue from a suspicious area.[140] It can sometimes miss a cancer if the needle is not placed among the cancer cells.[141] In core needle biopsy, a larger needle is used for sampling. It removes a small cylinder (core) of tissue. It is better than FNA as it provide a clear diagnosis with larger part removed.[140]

Usually, needle biopsy serves the purpose but sometimes with the help of surgery all (excisional) or part (incisional) of lump is removed which is called as *surgical biopsy* or an *open biopsy*.[142]

2.2.6. Breast cancer treatment

The treatment modalities mainly include surgery, systemic therapy (chemotherapy, hormonal therapy, targeted therapy) and radiation.[9] The combinations of these modalities are generally used.[9]

- *Surgery*

There are two common types of surgery used in practice: lumpectomy and mastectomy. In lumpectomy, entire lump along with some normal tissue is removed while in mastectomy, a large part or entire breast is removed. The mastectomy can be total mastectomy, modified radical mastectomy or a partial mastectomy. A modified radical mastectomy is used more often as it doesn't remove the chest muscles.[9] For both DCIS and early-stage invasive breast cancer, surgery is recommended.[10]

The chemotherapy can be given before surgery to shrink the larger tumor mass (to opt for lumpectomy) known as neoadjuvant therapy.[143] The chemotherapy given after surgery to eradicate the left over tumor cells (if any) and to reduce the chances of relapse is known as adjuvant therapy.[143] A meta-analysis combining the results of eight studies demonstrated no difference between rates

of breast cancer recurrence or overall survival in women who had neoadjuvant chemotherapy versus those who had adjuvant chemotherapy.[143] Surgery alone can increase the chances of relapse. The side effect mainly include lymphadenopathy.[11, 144]

- *Radiotherapy*

Along with surgery, breast cancer patients might also receive radiotherapy pre- or post-surgery.[1] The most frequently used radiation treatment is external-beam radiation where the machine is outside the body.[15] When radiation is given by placing radioactive material in the tumor, it is called brachytherapy.[15] Radiation therapy can cause side effects like fatigue, redness and/or skin discoloration/hyperpigmentation, swelling of the breast and pain or burning of the skin where the radiation was targeted, sometimes with blistering or peeling.[15]

- *Systemic therapy*

Systemic therapy is treatment taken orally or given intravenously. It include chemotherapy, hormone therapy and targeted therapy.[1] A chemotherapy regimen, or schedule, usually consists of a specific number of cycles given over a set period of time.[1] It may be one drug at a time or combinations of different drugs given at the same time.[1] The drugs include Cyclophosphamide, Methotrexate, Vincristine, Vinblastine, Fluorouracil And Doxorubicin.[9] Hormonal therapy of drugs are antiestrogens (selective estrogen receptor modulators- Tamoxifen, Raloxifen and estrogen antagonist- Fluvestrant); aromatase inhibitors (Anastrozole, Letrozole, Exemestan).[9] The targeted drug therapy mainly target HER2 protein which includes Trastuzumab. The side effects of chemotherapy mainly include fatigue, risk of infection, nausea and vomiting, hair loss, loss of appetite, mouth ulcer and diarrhea.[1] Tamoxifen is associated with risk of endometrial cancer and

thromboembolism.[13] The side effects of aromatase inhibitors include muscle-joint pain, increased risk of osteoporosis and broken bones, hot flashes, vaginal dryness and increased cholesterol levels.[14]

2.2.7. Models to study breast cancer

2.2.7.1. *In-vitro* studies

- *Cell lines*

With the advancement in genomics during last few decades, the new avenues for translational research and direct evaluation of clinical samples have opened. But there is still a requirement for reliable preclinical models to test therapeutic strategies. Human cancer-derived cell lines are the most widely utilized models to recapitulate the science of cancer and to test the hypotheses of efficacy of chemotherapeutic agent. Certain types of manipulations for the genetic and DNA methylation analysis and drug testing are difficult to perform in animals.[145] Cell lines emerge as a feasible alternative to overcome these issues, being at the same time easy to manipulate and molecularly characterize (e.g. genetic and/or epigenetically).[146] The cell lines are exceptional for the fundamental study of the cellular pathways and for disclosing critical genes involved in cancer.[146] In fact, the use of the relevant *in-vitro* model for cancer research is pivotal for the investigation of genetic, epigenetic and cellular pathways,[145] for the study of proliferation deregulation.[147] apoptosis and cancer progression,[147] to define potential molecular markers[148] and for the screening and characterization of cancer therapeutics.[145] The results of the research in cancer cell lines are usually extrapolated to *in-vivo* human tumors.[146]

2.2.7.2. *In- vivo* studies

- *Chemically induced mammary carcinogenesis*

The mammary carcinogenesis can be induced in rodents by intra peritoneal administration of N-methyl N- nitrosourea (MNU) or 7,12-dimethylbenzanthracene (DMBA).[149] DMBA is lipophilic in nature and requires metabolism of it to become carcinogen.[150] MNU is a water soluble direct alkylating carcinogen,[149] and highly specific carcinogen for mammary gland inducing adenocarcinomas which are similar to humans.[151] DMBA is non-specific and deaths are recorded with DMBA due to peritonitis.[149] The induction of mammary carcinogenesis in DMBA model is affected by seasonal variation which is not the case in MNU.[149, 152] MNU induced mammary carcinomas are aggressive and more estrogen dependent.[153, 154] Mammary carcinomas arising from MNU- induced hyperplastic alveolar nodule contain transformed c-Ki-ras proto-oncogene with the present of specific G-35 → A-35 point mutation in codon 12, which results in the substitution of normal glycine with the aspartic acid. [149, 154] With this, amplification of cyclin D1 gene, IGF2, loss of expression of the mitogenic growth factor gene, heparin binding growth factor midkine gene and mutation in the tumor suppressor p53 gene are seen in mammary tumors.[149, 155] Literature survey reveals that at age of 50 days, injecting 50mg/kg b.w (i.p.) MNU to nulliparous Sprague Dawley (SD) female rats induces mammary carcinoma.[156-158] The cancer induced is age-dependent; the rats are most susceptible at the age of 50 days.[151, 159] At age of 35 days, MNU caused acute toxicity and was lethal.[151, 159] The incidence of mammary gland adenocarcinoms are 100% at 50mg/kg MNU.[149, 159] When the intraperitoneal route of administration was compared with subcutaneous or intravenous route of MNU administration, the best route of injecting MNU was found to be intraperitoneal route.[160] It provides ease of administration, quantitative, reproducible results along with decrease in variability of tumor

response to the treatment.[160] The null-parity is of importance as mammary glands of parous rats manifested up-regulation of differentiation-related genes and down-regulation of proliferation-related genes, demonstrating blockage of carcinogen actuated cell proliferation.[151, 161, 162] The studies revealed Sprague Dawley rats are the appropriate species for induction of breast cancer as compared to Wistar rats as tumor incidence in former species is higher than the later one.

- *Cell line induced mammary carcinogenesis*

Two types of model can be developed- Xenograft[163] or Allograft (synegenic).[164] Xenograft involves transplantation of human cancer cell lines or solid tumors in host mice. The host mice are unique as they are immunocompromised.[162] The transplants are called orthotopic, implying that the tumor is placed at the site it is required to grow naturally in the host; for example inducing lung cancer lung tumor cells are placed in lung.[165] An advantage is that it utilizes real human cancer cells so it may be more representative of the properties and mutations of the human cancer. The main drawback of this model system is incompetent immune system, so they don't mimic actual patient situation.[164, 165] In syngeneic model, allografts are immortalized from mice tumor cells, which are engrafted back into same bred. The rejection of transplant is negligible as the cancer tissues and recipient share ancestry. Furthermore, the immune system of host is fully functional so it represents tumor microenvironment more closely to real life situation.[164, 166] However, complexity of human tumors may or may not be depicted.[167]

2.2.8. Newer targets of breast cancer

- *Angiotensins*

Angiotensins have been reported to be involved in cancer pathogenesis. Inhibition of Ang II formation by ACE inhibitors (ACEI), or blockage of its

receptor AT1, can have beneficial effects in cancer suppression. Egami and colleagues described the reduction of tumor growth by ACEI through blockade of angiogenesis.[168] However, Losartan, an angiotensin receptor blocker (ARB), has been reported to increase tumor perfusion, thereby improving chemotherapy outcome by reduction of matrix production and fibroblast density, increasing drug and oxygen delivery. [169] ACEI and ARB also decrease the expression of vascular endothelial growth factor (VEGF) and tissue factors, which correlate with tumor progression.[170] Candesartan, an ARB, significantly reduced transforming growth factor b1 (TGF-b1) expression and suppressed tumor proliferation and stromal fibrosis,[171] and it also significantly inhibited the growth of tumor xenografts in mice and tumor angiogenesis.[168] Ang II can also bind to the AT2 receptor, triggering actions that differ from AT1 receptor. AT2 overexpression in hepatocellular carcinoma (HCC) cell lines and orthotopic tumor grafts led to apoptosis mediated by activation of p38 MAPK, pJNK, caspase-8, and caspase-3, and by inactivation of pp42/44 MAPK (Erk1/2).[172] A preclinical proof-of-concept of AT2 gene delivery through intratracheal administration to lung tumor showed successful tumoral apoptosis.[173]

- *Cyclin D1 and CDK4/6*

The aberrant cell cycle control is a hallmark of cancer[174] and numerous mechanisms are involved in deregulation of the cell cycle checkpoints. The mitogen-activated pathways stimulates autocrine production of cyclin D1 by aberrant expression of growth factors or their receptors.[175] These pathways may also be constitutively active by proteins such as Ras with is frequently overexpressed in breast cancer and also associated with poor prognosis.[176] The cyclin D1 mRNA is overexpressed in over 50% of breast cancers.[177] The ability of these cyclins to stimulate the cyclin-dependent kinases (CDKs) CDK4 and CDK6 is the very well documented mechanism for their oncogenic actions and

provides an attractive therapeutic target.[177] It is worth noting that deregulation of these cell cycle proteins differ according to breast cancer subtype. For example, amplification of cyclin D1 occurs majorly in luminal B breast cancers than in luminal A (58% versus 29%, respectively).[178] Likewise, in luminal B overexpression of CDK4 (25% of luminal B versus 14% of luminal A) is also observed.[178] In contrast, triple negative or basal like breast cancers do not display alterations in cyclin D1 or CDK4/6.[178] Currently, the broad-spectrum CDK inhibitor, flavopiridol, had promising preclinical results in multiple tumor cell types.[179-182]

- *PI3K/AKT/mTOR pathway inhibitors*

The pathway regulates multiple cellular processes to promote cell growth, survival and metastasis.[183-185] This is the most often aberrantly activated pathway in human breast cancer, with 80% alterations in genes coding this pathway.[185] The deregulation of PI3K signaling has been associated with treatment resistance to chemotherapeutic agents, including antiestrogens and trastuzumab.[186-188] Mutations in *PIK3CA* occur more often in luminal A, luminal B, and HER2+ breast cancers (45%, 29%, and 39%, respectively) as compared to basal-like breast cancers (9%).[182] PI3K inhibitors have exhibited clinical activity against advanced solid tumors and metastatic breast cancers.[189, 190] A Phase I study of the PI3K inhibitor, BKM120, in patients with metastatic breast cancer reported partial responses and stable disease in 11% and 50% of patients, respectively.[189] The allosteric mTORC1 inhibitor, Everolimus, also showed efficacy as a single agent in a Phase II placebo-controlled study in breast cancer patients with bone metastases.[191]

- Insulin like growth factor receptor (IGF)

IGF-1 receptors are expressed in 90% of breast cancer.[192] It is found that IGF-1 receptor is potent driver of PI3K pathway. It is highly homologous to insulin

receptor. ATP competitive inhibitors target both IGF ligand and receptor.[192] Combination of Evorlimus combined with IGF-1 receptor antibody, Figitumumab, has shown stable disease in 15 of 18 patients with advanced solid tumors, and partial response in one patient.[193] *In vitro* studies have shown that IGF-1 plays important role in developing resistance to chemotherapy in breast cancer.[194] A Phase I trial of figitumumab in patients with advanced solid tumors showed clinical benefit, with ten of 15 patients experiencing stable disease at the maximum feasible dose of 20 mg/kg.[195, 196]

- *Poly (adenosine diphosphate–ribose) polymerase (PARP)*

It is involved in the recognition and repair of DNA breaks, and PARP works in concert with other proteins including BRCA1 and BRCA2 to repair DNA damage. Preclinical data demonstrate that deficiency in BRCA1/2 or other homologous recombination DNA repair proteins sensitizes cells to PARP inhibition.[197] Therefore, as single agents, PARP inhibitors are likely to be more effective against tumors carrying mutations in genes encoding proteins involved in compensatory DNA repair mechanisms, such as BRCA1/2, as these mutations essentially prime the cells for DNA damage-induced apoptosis.[197, 198] Few developed drugs include Veliparib Niraparib, Olaparib, And Rucaparib.[199] A Phase I trial[200] in breast cancer patients with BRCA1/2 mutations showed that Olaparib, was effective as a monotherapy, achieving responses in 41% of the patients.

- *Histone deacetylase inhibitors (HDACs)*

HDACs modulate ER activity and inhibiting this proteins revert resistance to antiestrogen therapy. [201] [202] In Phase II trials, patients with advanced estrogen positive breast cancer and resistant to aromatase inhibitors demonstrated HDAC inhibitor, entinostat, combined with exemestane improved disease free

survival period.[203] The HDACs inhibitor Vorinostat combined with Tamoxifen showed clinically beneficial in endocrine resistant metastatic cancer patients.[204]

- *Src kinases inhibitors*

The *Src* interacts with steroid hormones, integrins, GPCR and STAT family. Thus how, they involved themselves in variety of oncogenic process.[205] Overexpression of *Src* is strong predictive marker for bone metastasis.[206] Various studies suggested that the high mRNA expression can be correlated to decreased survival.[207-209] *In-vitro* studies of combination of *Src* inhibitors and chemotherapeutic agents have shown tumor cell death.[210] The partial response in Phase I clinical trial was obtained with *Src* inhibitor, Dasatinib, and Paclitaxel in metastatic breast cancer.[211]

- *JAK/STAT pathway inhibitors*

Janus kinases (JAKs) are mediators of cytokine and growth hormone signaling.[212] Activated JAKs phosphorylate STAT proteins, which leads to their translocation. Further, the transcriptions of regulatory genes involved in cell proliferation, differentiation, and apoptosis.[212, 213] Mutation of this proteins is linked to hyperactivation of this pathway resulting in unchecked cell proliferation.[214] Mutations in STAT-1, -3 and -5 leads to elevated levels of cytokines.[215] Preclinical studies demonstrated IL-6/JAK2/STAT3 pathway activation in basal-like breast cancer cells.[216] The clinical trials are underway to prove its efficacy.[192]