INTRODUCTION

The human body is a complex structure which is made up of many types of cells which grow and divide in a controlled way to produce more cells as they are needed to keep the body healthy. When cells become old or damaged, they die and are replaced with new cells. Due to various reasons the genetic material (DNA) of a cell can become damaged or changed, producing mutations that affect normal cell growth and division. When this happens, cells do not die when they should and new cells form when the body does not need them. The extra cells may form a mass of tissue called a tumor which may be benign or malignant (cancerous). Cancer is a natural process where, to put it simply, an overworked and weakened immune system cannot kill it as fast as it is multiplying. Toxin, carcinogens, radiation, even viruses, combined with an unhealthy internal environment, and in conjunction with a weakened immune system, cause more cells to turn cancerous, and allows them to thrive.

Cancer is the leading cause of death in the developed countries and second leading cause of death in developing countries. Cancer was estimated to account for about 7 million deaths (12% of all deaths) worldwide in 2000 (WHO, 2001), only preceded by cardiovascular diseases (30% of all deaths), and by infectious and parasitic diseases (19%). Cancer was also estimated to account for almost 6% of the entire global burden of disease in that same year (WHO, 2001). Pisani et al (1999) have projected a 30% increase in the number of cancer deaths in developed countries, and more than twice this amount (71%), in developing countries, between 1990 and 2010, due to demographic changes alone. Based on the Globocan 2008 estimates, about 12.7 million cancer cases and 7.6 million cancer deaths are estimated to have occurred in
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2008; of these, 56% of the cases and 64% of the deaths occurred in the economically developing world. Breast cancer ranks as commonest cancer in women in both developed and developing regions with around 6,90,000 new cancer cases estimated in each region accounting for 23% (1.38 million) of the total new cancer cases and 14% (458,400) of the total cancer deaths in 2008 according to Globocan 2008 estimates. In India, breast cancer is now the leading cause of cancer in females pushing cervical cancer to the second. According to Globocan 2008 the estimated incidence of breast cancer in India is 22.2% (1,15,251) with mortality of 17.2% (53,592). At Gujarat Cancer & Research Institute a regional cancer centre of Western India, according to hospital based registry of the year 2010, 6942 total new female cancer cases have been registered of which 1460 (21%) were of breast cancer with a mortality rate of 4.1 percent.

Epidemiology of breast cancer:

The distribution of breast cancer varies widely among different groups. Females have much higher risk of breast cancer and Caucasian race has much higher incidence of breast cancer compared to other ethnic groups in the United States. The factors that effect pattern of breast cancer as seen the world and United States have been described.

Race:

Caucasian women have overall higher risk of development of breast cancer compared to African American women. This difference is not very apparent until the menopausal age. The breast cancer incidence in Caucasian women is about twice compared to American Asian, or Hispanic women. Breast cancer risk is very low in Native
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Americans. Even though the incidence of breast cancer is lower in African American women compared to the Caucasian population, the African America population has a higher breast cancer death rate (31.0 per 100,000) compared to Caucasian women or in fact, compared to any other racial or ethnic population in the United States. Biologic and genetic differences in tumors including mutations specific to African American women, the presence of risk factors, access to health system, health behaviors and relatively later stage at the time of diagnosis of disease may contributed to decreased survival of African American women with breast cancer.

Geographical location:
Different countries in the world have varying incidence of breast cancer. There is much as five fold difference in the incidence of breast cancer between the countries that have highest incidence and lowest incidence of breast cancer. The incidence of breast cancer is significantly lower in Japan, Thailand, Nigeria, and India compared to Denmark, New Zealand, U.K. and the United States. In India higher incidence of breast cancer has been observed in southern region as compared to north-east region. The difference in the incidence of breast cancer between these countries and different regions in a country may be related to the difference in dietary habits, cultural differences and the number of pregnancies.

Socio Economic Status:
The incidence of breast cancer is greater in women of higher socio-economic background. The relationship of breast cancer risk with socio-economic factors is most likely related, to the life style differences like age at first childbirth, and number of pregnancies.
Though some of the epidemiological factors are mentioned above there is no one single cause for cancer. Scientists believe that it is the interaction of many factors together that produces cancer. Broadly the factors involved may be genetic, environmental, or constitutional characteristics of the individual.

These factors also effect the increasing incidence of breast in India which may be changed lifestyle and work pattern of women which allows various risk factors such as late age at first childbirth, short duration of breast feeding, early age at menarche, late age at menopause, null parity, family history of breast cancer which are described below to be the causative factors of breast cancer.

**Risk factors of breast cancer:**

There are many risk factors associated with an increased risk of developing breast cancer. However in more than 50% of cases the risk factors are not yet identified. A risk factor does not necessarily cause the disease, but it may make the body less resistant to it.

**Age and Gender:**

Females have high risk of developing breast cancers than males and the risk of breast cancer increases with age. Women progressing towards menopause and having post menopausal status, between 40 to 60 years have high risk of breast cancer. The risk of breast cancer is highly related to hormonal influences, but how this affects the disease is being explored.
First-degree relative with breast cancer:

The risk of breast cancer increases with the number of affected first degree relative (mother, sister, or daughter). However, the majority of cancers occur in women without such a history as only 13% women with breast cancer have one affected relative and only 1% have two or more.

First live birth and Nullparity:

Women with a first full-term pregnancy at younger than 20 years of age have half the risk of nulliparous women or women over the age of 35 at their first birth. It is hypothesized that pregnancy results in terminal differentiation of epithelial cells, removing them from the potential pool of cancer precursors. However, the biologic basis of such differentiation has not been determined.

Breast feeding: The longer the women breast feed the greater is reduction of the risk of breast cancer.

Hormonal factors:

Early menstruation and late menopause: Women who get their periods early (before 12 years) and went through menopause (late after 55 years) are at high risk of developing breast cancer.

Hormone replacement therapy: Postmenopausal hormone replacement therapy slightly increases the risk of breast cancers in the current users. Estrogen and progesterone together increase the risk more than does estrogen alone.
Radiation Exposure:

Women who have been exposed to therapeutic radiation or other types of radiation have a higher rate of breast cancer. Risk increases with younger age and higher radiation doses. For instance, women in their teens and twenties (but not at older age) undergoing mantle radiation for Hodgkin’s disease have a 20% to 30% risk of developing breast cancer 10 to 30 years after treatment.

Carcinoma of the contralateral breast or endometrium:

Increased risk is associated with carcinoma of the contralateral breast or endometrium, probably owing to the shared hormonal risk factors for these tumors.

Diet and Alcohol consumption:

Various items in diet, particularly dietary fat, have been suggested to increase risk, but large studies have failed to find a strong correlation. However, studies do show that moderate or heavy alcohol intake confers an increased risk of breast cancer. Higher estrogen levels and lower folate levels associated with alcohol consumption may be mechanism underlying this association.

Obesity:

There is a decreased risk in obese women younger than 40 years owing to the association with anovulatory cycles and low progesterone levels late in the cycle. There is increased in postmenopausal obese women, which is attributed to synthesis of estrogen in fat depots.
**Exercise:**

Studies have been inconsistent, but some have shown a decreased risk of breast cancer in post-menopausal women.

**Environmental Toxins:**

There is concern that environmental contaminants such as organochlorine pesticides could have estrogenic effects in humans. The possible effect of environmental toxins on breast cancers risk is being intensively investigated. No specific substances have been definitely associated with an increased risk.

**Genetic factors:**

Besides the risk factors mentioned more than 70 percent of breast cancers are observed to have some type of genetic alteration may be due to the effects of carcinogens or genetic abnormalities through error in DNA replication which may be acquired or inherited. Also genetics of cancer pathogenesis, such as DNA methylation, and microRNAs are important. Genetic abnormalities found in cancer typically affect two general classes of genes. Cancer-promoting "oncogenes" are typically activated in cancer cells, giving those cells new properties, such as hyperactive growth and division, protection against programmed cell death, loss of respect for normal tissue boundaries, and the ability to become established in diverse tissue environments. "Tumor suppressor genes" are then inactivated in cancer cells, resulting in the loss of normal functions in those cells,
such as accurate DNA replication, control over the cell cycle, orientation and adhesion within tissues, and interaction with protective cells of the immune system. In hereditary cancers the probability is associated with mutations in BRCA1 and BRCA2 genes and increases if there are multiple affected first-degree relatives. Both act as tumor suppressor genes and it is a loss of function that confers the risk of malignancy. A key function for both appears to be their role in protecting the genome from damage by halting the cell-cycle and promoting DNA damage repair. Genetic susceptibility with other known genes is much less however “cowden syndrome” due to mutation in PTEN gene on chromosome 10 confers a 25% to 50% life time risk of breast cancer in affected women. Mutations may be due loss of heterozygosity (LOH) found in 11% to 41% and studies have been and are being carried out to determine whether the function of other allele is altered (by methylation).

The sporadic breast cancer may be related to above mentioned risk factors and exogenous estrogen. The majority of cancers occur in postmenopausal women overexpress ER as it has two major roles in development of breast cancer, the metabolites of estrogen can cause mutations or generate DNA damaging free radicals or via its hormonal action drive the proliferation of premalignant lesions and cancer. However, other mechanisms also undoubtedly play a role as a significant subset of breast carcinomas are ER negative or occur in women without increased estrogen exposure.

The other mechanism involves complex interactions between hormone receptors and growth factors signaling pathways. One of the important interactions is the cross talk between members of Her family of receptor tyrosine kinases and intracellular
signaling. Activated Her receptors can function to both stimulate and inhibit members of downstream signaling pathways. Henceforth the constellations of the molecular alterations play a major role in the development of breast cancer.

Carcinoma of the breast is frequently studied and reported as different entity as breast cancer in many respects breast cancer is quite diverse and heterogenous and therefore anatomical structure of normal breast and the pathological changes are briefly described.

**Anatomy of normal breast:**

The breast is an important organ of the female reproductive system It is very important to have knowledge about the normal anatomy of breasts. For females, familiarity with breast anatomy will be helpful in recognizing early signs of potential disorders. Breast anatomy is highly complex. Anatomically, breasts are modified sweat glands which produce milk in women. The breasts, forms a conical projection between the subcutaneous tissue of the chest and the greater pectoral muscle. Each breast has 15 to 20 sections, or lobes, that surround the nipple like spokes on a wheel. Inside these lobes are smaller lobes, called lobules. At the end of each lobule are tiny "bulbs" that produce milk. These structures are linked together by small tubes called ducts, which carry milk to the nipples. Each breast has one nipple surrounded by the areola which has several sebaceous glands. In women, the larger mammary glands within the breast produce the milk. They are distributed throughout the breast, with two-thirds of the tissue found within 30 mm of the base of the nipple. These are drained to the nipple by between 4 and 18 lactiferous ducts, where each duct has its own opening.
The network formed by these ducts is complex, like the tangled roots of a tree. It is not always arranged radially, and branches close to the nipple. The ducts near the nipple do not act as milk reservoirs (Figure 1).

Figure 1: Structure of a normal breast (Adapted from pt851.wikidot.com)

The breasts sit over the pectoralis major muscle and usually extend from the level of the 2nd rib. The arterial blood supply to the breasts is derived from the internal thoracic artery, lateral thoracic artery, thoracoacromial artery, and posterior intercostal arteries. The venous drainage of the breast is mainly to the axillary vein, but there is some drainage to the internal thoracic vein and the intercostal veins. Both genders have a large concentration of blood vessels and nerves in their nipples. Each breast also contains blood vessels, as well as vessels that carry a fluid called lymph. Lymph travels throughout the body through a network called the lymphatic system, carrying cells that help the body fight infections. The lymph vessels lead to the lymph nodes (small, bean-shaped glands).

One group of lymph nodes is located in the armpits, above the collarbone and in the chest. If breast cancer has reached these nodes, it may mean that cancer cells have
spread to other parts of the body via the lymphatic system. Lymph nodes are also found in many other parts of the body.

Breast development and function depend on the hormones estrogen and progesterone, which are produced in the ovaries. Estrogen elongates the ducts and causes them to create side branches. Progesterone increases the number and size of the lobules in order to prepare the breast for nourishing a baby.

After ovulation, progesterone makes the breast cells grow and blood vessels enlarge and fill with blood. At this time, the breasts often become engorged with fluid and may be tender and swollen. During each menstrual cycle, breast tissue tends to swell from changes in the body of estrogen and progesterone. The milk glands and ducts enlarge, and in turn, the breasts retain water. During menstruation, breasts may temporarily feel swollen, painful, tender, or lumpy. Physicians recommend that women should practice monthly breast self-exams the week following menstruation when the breasts are least tender to find if any abnormal symptoms are observed. The symptoms are mentioned below (Robins and Cotran, 2010).

Clinical presentation of breast disease:

The most common symptoms reported by women are pain, palpable mass or nipple discharge. In addition women with abnormal findings on mammographic screening require further evaluation but are definition asymptomatic.

Pain is the most common breast symptom may be cyclical with menses and has no pathologic correlation. Painful masses are usually benign, however 10% of breast cancers are presented with pain.
Discrete palpable masses are the second most common breast symptoms. A breast mass usually does not become palpable until it is about 2 cm in diameter. These masses are most common in premenopausal women and become less frequent with age. Only 10% of breast masses in women under age 40 years have proved to be malignant compared to 60% of masses in women over age 50 years. The most commonly encountered lesions are invasive carcinoma, fibroadenoma and cysts.

Nipple discharge is a less common presenting symptom but is of concern when it is spontaneous and unilateral. Milky discharge has not been associated with malignancy. Bloody or serous discharge are most commonly associated with benign lesions but can be due to malignancy. The risk of malignancy with discharge increases with age. Discharge is associated with carcinoma in 7% women younger than 60 years and 30% women older than 60 years.

Based on the clinical presentations and further by histological examination of a tissue biopsy specimen by a pathologist, although the initial indication of malignancy can be symptoms or radiographic imaging abnormalities the tumors are confirmed as benign or malignant.

**Benign breast disorders:**
A wide variety of benign alterations in ducts and lobules are observed in the breast. The changes have been divided into three groups based on subsequent risk of developing breast cancer (Robins and Cotran, 2010).

1. Nonproliferative breast changes
2. Proliferative breast disease
3. Atypical hyperplasia

**Nonproliferative breast changes: (Fibrocystic changes)**

This is a benign morphologic change in the breast and might come into clinical attention when they mimic carcinoma by producing palpable lumps, mammographic densities or calcifications or nipple discharge (Figure 2A).

**Proliferative breast disease:**

This group of disorder is characterized by proliferation of ductal epithelium or stroma without cellular abnormalities suggestive of malignancy. The following entities are included in this category:

*Epithelial hyperplasia:*

In the normal breast only myoepithelial cells and single layer of luminal cells are present above the basement membrane. Epithelial hyperplasia is defined by the presence of more than two cell layers. The proliferating epithelium often including both luminal and myoepithelial cells fills and distends the ducts and the lobules.

*Sclerosing adenosis:*

The number of acini per terminal duct is increased to at least twice the number found in uninvolved lobules. The normal lobular arrangement is maintained. The acini are compressed and distorted in the central portions of the lesions but characteristically dilated at the periphery. Myoepithelial cells are usually prominent. Calcifications are frequently present within the lumens of the acini.
**Complex Sclerosing Lesion (Radical Scar):**

Radial scars are stellate lesions characterized by a central nidus of entrapped glands in a hylanized stroma. The term scars refers to the morphologic appearance as they are not associated with prior trauma or surgery. A more general term is “complex sclerosing lesion” which not only includes radial scars but also related lesions with components of sclerosing, adenosis, papilloma formation and epithelial hyperplasia.

**Papillomas:**

Papillomas are composed of multiple branching fibrovascular cores, each having a connective tissue axis lined by luminal and myoepithelial cells. Growth occurs within a dilated duct. Large duct papillomas are usually solitary and situated in lactiferous sinus of the nipple and small duct papillomas are commonly multiple and located within the ductal system.

**Fibroadenoma:**

This is the most common benign tumor of the female breast occurring at any age within the reproductive period of life somewhat more common before age 30. Fibroadenomas grow on spherical nodules that are usually sharply circumscribed and freely movable in the surrounding breast substance. They vary in size from less than 1 cm in diameter to large tumors that can replace most of the breast. They are grayish white nodules that bulge over the surrounding tissues and often contains slit like spaces. The stroma is usually delicate, cellular and often myxoid resembling intralobular stroma, enclosing glandular and cystic spaces lined by the epithelium. In a study by Dupont et al (1994) only complex fibroadenomas with cysts larger than 0.3 cm conferred a mild increase in the risk of subsequent breast cancer (Figure 2B).
**Proliferative breast disease with atypia:**

Proliferative disease with atypia includes atypical ductal hyperplasia (ADH) and atypical lobular hyperplasia (ALH). ADH is recognized by its histologic resemblance to ductal carcinoma in situ (DCIS) including a monomorphic cell population, regular cell placement and round lumina. ALH refers to proliferation of cells identical to those of lobular carcinoma in situ but the cells do not fill or distend more than 50% of acini within a lobule. ALH can also extend into ducts and this finding is associated with increased risk of developing invasive carcinoma.

![Fibrocystic disease (A) and Fibroadenoma (B)](image)

**Figure 2:** Benign breast diseases

(H&E staining, 400X, Histopathology department, The Gujarat Cancer & Research Institute, 2012)

**The mechanism of carcinogenesis:**

The vast array of histological appearances of proliferative and atypical breast disease as well as carcinomas are the outward manifestations of biologic changes taking place within these lesions and point to the complex and variable pathway to carcinogenesis. The morphologic changes in the breast associated with the smallest increased risk of
cancer are lesions with increased number of epithelial cells (proliferative changes). This suggests that these early changes are related to evasion of growth inhibiting signals, evasion of apoptosis and self-sufficiency in growth signals. The view of oncogenesis focuses on the malignant epithelial cell and does not take into the account the other tissue components. The structure and function of the normal breast requires complex interaction between luminal cells, myoepithelial cells and stromal cells. Abrogation of the basement membrane, increased proliferation, and escape from growth inhibition, angiogenesis and invasion of stroma can be coopted during carcinogenesis by abnormal epithelial cells, stromal cells or both (Figure 3). While the changes described above are accumulating in luminal cells parallel changes also occur due to mutation or epigenetic changes or via abnormal signaling pathways in these other cell types, resulting in the loss of normal cellular interactions and tissue structure (Tlsty et al, 2001). The final step of carcinogenesis, the transition of carcinoma limited by the basement membrane to ducts and lobules carcinoma in situ is least understood. Specific gene functions necessary for invasion have been difficult to identify (Porter et al, 2003). It is possible that this transition is primarily due to loss of basement membrane and tissue integrity caused by abnormal function of myoepithelial and stromal cells rather than to the gain of ability of malignant cells to invade through the basement membrane and into the stroma.

**Classification of breast carcinoma:**

Almost all breast malignancies are adenocarcinomas, all the other types (squamous cell carcinomas, phyllodes tumors, sarcomas and lymphomas) make up fewer than 5% of the total.
The molecular, cellular, and pathological processes that occur in the transformation from healthy tissue to preinvasive lesions, such as ductal carcinoma in situ, to breast cancer.

Carcinomas are divided into in situ carcinomas and invasive carcinomas. Carcinoma in situ refers to a neoplastic population of cells limited to ducts and lobules by the basement membrane. However, carcinoma in situ does not invade into lymphatics and blood vessels and cannot metastasize. Invasive carcinoma has invaded into beyond the basement membrane into stroma. Here the cells might invade into the vasculature and thereby reach regional lymph nodes and distant sites. Even the smallest invasive breast carcinomas have some capacity to metastasize (Robins and Cotran, 2010).

**Carcinoma in situ:**
Carcinoma in situ was originally classified as ductal or lobular on the basis of the resemblance of the involved spaces to ducts and lobules.
**Ductal carcinoma in Situ (DCIS; Intraductal carcinoma)**

Among mammographically detected cancers, almost half are DCIS. DCIS is most frequently presents as mammographic calcifications. DCIS consists of malignant population of cells limited to ducts and lobules by the basement membrane. The myoepithelial cells are preserved although they may be diminished in number. DCIS is a clonal proliferation and usually involves only a single ductal system. However the cells can spread throughout the ducts and lobules and produce extensive lesions involving an entire sector of a breast (Figure 4 A and B).

**Lobular Carcinoma in situ (LCIS)**

Lobular carcinoma in situ is always an incidental finding and not associated with calcifications or a stromal reaction. LCIS is more common in young women, 80% to 90% cases prior to menopause. LCIS and invasive lobular carcinoma are identical and consists of small cells that have oval or round nuclei and do not adhere to one another. Signet ring cells containing mucin are present commonly. LCIS rarely distorts the underlying architecture, and the involved acini remain recognizable as lobules. Women with LCIS develop invasive carcinoma at a frequency similar to that of women with untreated DCIS (Figure 4C).

**Invasive carcinomas:**

In the women not undergoing mammographic screening, invasive carcinoma presents as a palpable mass and by the time the cancer becomes palpable over half the patients
will have axillary lymph node metastases. The invasive carcinoma are of different types as mentioned in the table above.

**Invasive ductal carcinoma:**

Invasive ductal carcinoma or of no special type include the majority of carcinomas (70% to 80%). On gross examination, most carcinomas are firm to hard and have an irregular border. These carcinomas display a wide spectrum of appearances. Well-differentiated tumors consist of tubules lined by minimally atypical cells and can be occasionally difficult to distinguish from benign sclerosing lesions. Others are composed of anastomosing sheets of pleomorphic cells. The majority of invasive ductal carcinoma lie between these two extremes. Most carcinomas induce a marked increase in dense, fibrous desmoplastic stroma and are accompanied by varying amount of DCIS. The grade of DCIS usually correlate with the grade of invasive carcinoma. Carcinomas associated with large amount of DCIS require large excisions with wide margins to reduce local recurrence (Figure 4D).

**Invasive lobular carcinoma:**

Grossly most tumors are firm to hard with an irregular margin. Occasionally, the tissue may feel diffusely thickened and a discrete tumor mass cannot be defined. The histological hallmark of lobular carcinoma is the pattern of single infiltrating tumor cells, often only one cell in width or in loose clusters or sheets. The cells have the same cytologic features as LCIS and lack cohesion without formation of tubules or papillae. Signet ring cells are common. Tumor cells are frequently arranged in concentric rings
surrounding normal ducts. Lobular carcinoma have a different pattern of metastasis compared to other breast cancers (Figure 4E).

**Medullary carcinoma:**

The tumor has a soft, fleshy consistency and is well circumscribed. The carcinoma is characterized by solid syncytium sheets of large cells with vesicular, pleomorphic nuclei, containing a prominent nucleoli and frequent mitoses, a moderate to marked lymphoplasmacytic infiltrate surrounding within the tumors and a pushing nonfilatrvive border. All medullary carcinomas are poorly differentiated. Lymphatic or vascular invasion is not seen (Figure 4F).

**Mucinous (Colloid) carcinoma:**

The tumor is extremely soft and has the consistency and appearance of pale gray-blue gelatin. The tumor cells are seen as clusters and small islands of cells within large lakes of mucin that push into the adjacent stroma (Figure 4G).

**Tubular carcinoma:**

These tumors consists exclusively of well-formed tubules and sometimes mistaken, for benign sclerosing lesions. However, a myoepithelial cell layer is absent and tumor cells are in direct contact with stroma. Cribiform spaces may also be present. Apocrine snouts are typical and calcifications may be present within the lumen. LCIS is frequently present, but this association has not been explained.
Figure 4: Breast Carcinomas

(H&E staining, 400X, Histopathology Department, The Gujarat Cancer & Research Institute, 2012)
**Papillary carcinoma:**

Invasive carcinoma with a papillary architecture are rare and represent 1% or fewer of all invasive cancers. Papillary architecture is more commonly seen in DCIS (Figure 4H).

**Metaplastic carcinoma:**

“Metaplastic carcinoma” includes a variety of rare types of breast cancers including conventional adenocarcinomas with chondroid stroma, squamous cell carcinoma and carcinomas with a prominent spindle cell lesions that might be difficult to distinguish from sarcomas.

**Classification of the tumors:**

The tumors are using the TNM system for the classification of malignant tumors which was developed by Pierre Denoix (France) between the years 1943 and 1952. Breast Cancer is staged using the Tumor, Nodes, Metastases (TNM) system as defined by the American Joint Committee on Cancer (AJCC). Classification by anatomical extent of disease as determined clinically and histopathologically is the one with which the TNM primarily deals. The staging of cancer serves the objectives as:

- Aids the clinician in planning of treatment
- Gives some indication of prognosis
- Assists in the evaluation of results of treatment
- Contributes to the continuing investigation of human cancer
The TNM system for describing the anatomical extent of disease is based on the assessment of three components:

T - The extent of primary tumor

N - The absence or presence and extent of regional lymph node metastasis

M –The absence of presence of distant metastasis.

Breast Cancer is staged using the Tumor, Nodes, Metastases (TNM) system as defined by the American Joint Committee on Cancer (AJCC) and was most recently updated in 2010

**TNM classification**

**Table 1:** Primary tumor

<table>
<thead>
<tr>
<th>N Category</th>
<th>Definition</th>
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</thead>
<tbody>
<tr>
<td>TX</td>
<td>Primary tumor cannot be assessed</td>
</tr>
<tr>
<td>T0</td>
<td>No evidence of primary tumor</td>
</tr>
<tr>
<td>Tis</td>
<td>Carcinoma in situ: intraducal carcinoma, or lobular carcinoma in situ, or</td>
</tr>
<tr>
<td></td>
<td>Paget's disease of nipple with no tumor</td>
</tr>
<tr>
<td>T1</td>
<td>Tumor ≤ 2 cm in greatest dimension</td>
</tr>
<tr>
<td>T1 mi</td>
<td>Tumor ≤0.1cm in greatest dimension.</td>
</tr>
<tr>
<td>T1a</td>
<td>Tumor &gt;0.1 cm but ≤0.5 cm in greatest dimension</td>
</tr>
<tr>
<td>T1b</td>
<td>Tumor &gt;0.5 cm but ≤1 cm in greatest dimension</td>
</tr>
<tr>
<td>T1c</td>
<td>Tumor &gt;1 cm but ≤ 2cm in greatest dimension</td>
</tr>
<tr>
<td>T2</td>
<td>Tumor more than 2 cm but not more than 5 cm in greatest dimensions</td>
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<tr>
<td>T3</td>
<td>Tumor more than 5 cm in greatest dimension</td>
</tr>
<tr>
<td>T4</td>
<td>Tumor of any size with direct extension to chest wall or skin</td>
</tr>
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</table>

**Table 2:** Nodal classifications

<table>
<thead>
<tr>
<th>N Category</th>
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<tbody>
<tr>
<td>NX</td>
<td>Regional lymph nodes cannot be assessed.</td>
</tr>
<tr>
<td>N0</td>
<td>No regional lymphnode metastasis</td>
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<tr>
<td>N1</td>
<td>Metastases to moveable ipsilateral level I, II axillary node(s)</td>
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<tr>
<td>N2</td>
<td>Metastases in ipsilateral axillary nodes that are fixed to one another or</td>
</tr>
<tr>
<td></td>
<td>other strucures</td>
</tr>
<tr>
<td>N3</td>
<td>Metastases in ipsilateral internal mammary lymph nodes</td>
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Table 3: Metastases classification

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<th>M Category</th>
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<tr>
<td>MX</td>
<td>Distant metastasis cannot be assessed</td>
</tr>
<tr>
<td>MO</td>
<td>No distant metastasis</td>
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<tr>
<td>M1</td>
<td>Distant metastasis</td>
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The tables below details how TNM information is translated into a breast cancer stage.

Table 4: Staging of breast cancer

<table>
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<th>Stage</th>
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<th>N Classification</th>
<th>M classification</th>
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<td>M0</td>
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<td>N0</td>
<td>M0</td>
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<td>N1mi</td>
<td>M0</td>
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<td>N1mi</td>
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<td>M0</td>
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<td>N1</td>
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<td>M0</td>
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<tr>
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<td>M0</td>
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<td>N2</td>
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Breast cancers can be treated and some cured, depending on the specific type, location, and stage.

Once diagnosed, breast cancer is usually treated with a combination of surgery, chemotherapy and radiotherapy. As research has progressed treatments are becoming more specific for the malignancy has targeted therapies have largely come into practice.
There has been significant progress in the development of targeted therapy drugs that act specifically on detectable molecular abnormalities in certain tumors, and which minimize damage to normal cells. The prognosis of cancer patients is most influenced by the type of cancer, as well as the stage, or extent of the disease and histologic grading. In addition, the presence of specific molecules or genes involved in various pathways and the alterations within them are important in establishing prognosis, as well as in determining individual treatments.

**Molecular alterations in breast cancer:**

Several hallmarks have been proposed as necessary for tumor development. They include, sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming energy metabolism and avoiding immune destruction. The evolution in the experimental tools that have been thrust into the hands of cancer biologist it has been possible to uncover and dissect the complex. Besides the earlier mentioned risk factors the epithelial malignancies exhibit a wide diversity of molecular aberrations, reflecting the multiple genomic alterations that accumulate during carcinogenesis. Consequently, the behavior of cancers, even those of a seemingly unique diagnosis such as breast cancer can be equally varied. A set of alterations in cell biology and tissue homeostasis are understood to constitute the required phenotypic changes that underlie the development and progression of cancer (Hanahan D, 2000).

Oncogene overexpression via gene amplification is common in human cancer. One of the most amplified gene in human cancer is cyclin D1 seen in early stages of breast
carcinogenesis. Wnt and Notch have also been implicated as oncogenes in human breast cancer. Studies aimed at elucidating the mechanism by which Wnt promotes breast cancer implicated downstream effectors of notch signaling, leading to experiments suggesting a complex interdependence between Wnt and Notch signaling in mammary carcinogenesis (Ayyanan et al, 2006). Another utmost important oncogene overexpressed in breast cancer routinely used for therapeutic implication in Her-2/neu transmembrane tyrosine kinase. It is overexpressed in 15%-35% of breast cancers. Her-2/neu signaling is one of the stimulator of the PI3K/AKT pathway. The pathway therefore begins with interactions of various survival factors such as insulin like growth factors, with their membrane bound tyrosine kinase receptors leading to the activation of AKT, wherein phosphate and tensin homologue (PTEN) is the negative regulator of the pathway. Another important anti-apoptotic pathway is NF-Kappa B wherein the activated NF-kappa B enters the nucleus and binds to specific DNA sequences affecting gene regulation. Her-2/neu signaling can also lead to increase in Vascular endothelial growth factors (VEGF) levels one of the principal mediator of angiogenesis. Increased expression of VEGF and physiologic correlate of increased microvessel density are evident and strategies to counteract VEGF under the study in clinic include VEGF antibodies, VEGF receptor inhibitors.

Signaling pathways that lie a downstream of receptor tyrosine kinase contain a variety of feedback loops that control the amplification and duration of the signal through the pathway. Feedback can have a positive effect, by further enhancing signaling by preceding step, or can be inhibitory and act to shut the pathway down. Members of distinct pathways can also regulate one another in a process called cross talk. The
Ras GTPase, which conventionally stimulates the Raf-MEK-ERK MAP (Mitogen activated protein kinase) pathway can also bind and activate phosphoinositide-3-kinase (PI3K). There are many molecular interconnections between pathways which establish a complex signaling network through which signals can potentially be propagated to influence multiple targets.

Apart from the genetic alterations that has a fundamental role in initiation and progression of human cancer it also now apparent that epigenetic changes may be equally as important in tumor development. The mechanism that controls DNA methylation results in the inactivation of tumors suppressor genes and DNA repair genes leading to conclude that functions of these genes are likely to be lost more frequently by DNA methylation than mutation (Baylin et al, 2000).

The mechanisms of molecular alterations in understanding of cancer have made clear that even tumors of the same pathological classification can have markedly different signaling-pathway profiles and gene-expression patterns. Briefly, in the cell signaling routes, several factors can be activated or inactivated to promote oncogenic proliferation, and more than one gene or epigenetic alteration can be present, including HER2/neu amplification or various mutations in Epidermal growth factor receptor (EGFR), PTEN, PI3K or Ras, illustrating the redundancy of oncogenic events in tumor cells. This redundancy and the oncogenic alterations in other cellular pathways could explain the low reported response rates to therapies and even specific biological therapies in cancer. Effective treatments and use of specific biological therapies will probably be required to decipher these signatures on a patient to-patient basis and the custom design of appropriate treatment strategies by the exploration of
various molecular markers which led us to evaluate Her-2/neu and molecules involved in PI3K/AKT pathway mediated by Her-2/neu signaling PTEN, AKT and mTOR in our set of patients.

**Her-2/neu in breast tumorigenesis:**

Her-2/neu also known as erbb2 or cerbB2 is the human epidermal growth factor 2. Her-2/neu is a member of the epidermal growth factor (EGF) receptor family which consists of four members. EGFR (HER1, erbB1), HER2 (erbB2), HER3 (erbB3), and HER4 (erbB4). The Her-2/neu gene is located on chromosome 17q21 and encodes a 185-kDa protein product which is a transmembrane receptor protein with tyrosine kinase activity (Stern et al, 1986; Akiyama et al, 1986; Fukushige et al, 1986). This receptor tyrosine kinase was found to be amplified in a human breast cancer cell line reported 25 years ago by King et al (1985) and the importance of this amplification in the pathogenesis and progression of human breast cancer was shown by Slamon et al (1987).

The Her-2/neu pathway has been described in systems biology terms as a complex biological network composed of 3 layers: an input layer of membrane receptors and their ligands to trigger the signal coming from outside the cell; a core system processing layer of protein kinases transmitting the signal to the nucleus; and an output layer of transcription factors regulating genes that affect various cellular functions (Figure 5A, Gutierrez, 2011). The genes and gene products regulating the activity of the pathway have been and are being defined. The input layer is composed of 4 membrane receptors/TKs (HER1–4) and their many ligands (Citri et al, 2006).
The Her-2/neu molecule is composed of an extracellular ligand binding domain, an amphipathic transmembrane region and an intracellular tyrosine kinase domain (Figure 5B; Hudelist et al, 2003). Evidence has accumulated that receptor dimerization is essential for receptor activation. Receptor activation requires three variables, a ligand, a receptor, and a dimerization partner (Yarden et al, 2001).

*Figure 5A*: The Her-2/neu pathway (Adapted from Carolina Gutierrez, 2011)

*Figure 5B*: Her-2/neu receptor (Modified from Ross et al, 2004)
After a ligand binds to a receptor, that receptor must interact with another receptor of identical or related structure in a process known as dimerization in order to trigger phosphorylation and activate signaling cascades. Therefore, after ligand binding to an EGFR family member, the receptor can dimerize with various members of the family (EGFR, HER-2, HER-3, or HER-4). It may dimerize with a like member of the family (homodimerization) or it may dimerize with a different member of the family (heterodimerization). The specific tyrosine residues on the intracellular portion of the Her-2/neu receptor that are phosphorylated, and hence the signaling pathways that are activated, depend on the ligand and dimerization partner. The wide variety of ligands and intracellular crosstalk with other pathways allow for significant diversity in signaling. Despite of extensive homology with EGFR no single ligand that binds with high affinity has been identified. Therefore it relies on heterodimerization with another family member or homodimerization with itself when expressed at very high levels to be activated. Infact, it is the preferred dimerization partner of the other family members. Her-2/neu heterodimers are more stable (Tzahar et al, 1996; Roskoski et al, 2004) and their signaling is more potent (Karunagaran et al; 1996) than receptor combinations without Her-2/neu as Her-2/neu has the strongest catalytic kinase activity and Her-2/neu containing heterodimers have the strongest signaling activity (Citri etal, 2006). Her-2/neu can be activated by complexing with other membrane receptors such as insulin–like growth factor receptor I (Nahta et al, 2005). Even estrogen, working via the non genomic activity of estrogen receptor (ER) outside the nucleus has been shown to activate Her-2/neu signaling (Shou et al, 2004). In cell culture, high level overexpression of Her-2/neu even without the addition of activating
ligands can result in basal receptor phosphorylation and activation. High levels can
dimerization domain making it the dimeriztion partner of choice among family
members. The Her2/Her3 dimer is most active in mammals and is the potent
stimulator of PI3K/AKT pathway. Intracyoplasmatic phosphorylated tyrosine residues
of the Her-2/neu molecule function as high-affinity binding sites for SH2 domain
containing proteins, which link the receptor to intracellular signal transduction
processes such as ras-raf- MAPK and the PI3K pathway. Both are believed to be key
elements is cell survival and proliferation (Figure 6, Hudelist et al, 2003). Her-2/neu
gene expression is primarily regulated by two mechanisms. Transcription activation is
responsible for Her-2/neu gene expression in the normal breast tissue (Alroy et al,
1997; Reese et al, 1997) whereas Her-2/neu gene amplification is found in >90% of
the breast cancer cases that have Her-2/neu protein overexpression (Naber et al,

Growth factors and their receptors are known to play critical roles in cell development,
growth and differentiation. Many receptor posses intrinsic Her-2/neu gene amplification
and/or protein overexpression has been identified in invasive breast cancers. Her-
2/neu gene amplification and/or protein overexpression has been identified in10%–
34% of invasive breast cancers. Her-2/neu gene amplification in breast cancer has
been associated with increased cell proliferation, cell motility, tumor invasiveness,
progressive regional and distant metastases, accelerated angiogenesis and reduced
apoptosis (Moaser 2007). When classified by routine clinicopathologic parameters and
compared with Her-2/neu negative tumors, Her-2/neu positive breast cancer is more often of intermediate or high histologic grade (HG), more often lacking estrogen receptors progesterone receptors (PR) (ER and PR negative), and featuring positive lymph node (LN) metastases at presentation (Schechter et al, 1984). In the recent molecular classification of breast cancer, positive HER-2 status does not constitute a unique molecular category and is identified in both the “HER-2” and “luminal” tumor classes. (Perou et al, 2000).

**Figure 6:** The human epidermal growth factor receptor (HER) gene family (Adapted from Ross et al, 2009)
This image depicts the complex crosstalk between members of the HER family of receptor tyrosine kinases and intracellular signaling

Currently, there are at least 107 published studies involving 39,730 patients that have discussed the prognostic significance of HER2 gene amplification (as assessed by
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southern blot, slot blot, polymerase chain reaction (PCR), fluorescent in situ hybridization (FISH) and chromogenic in situ hybridization (CISH), and protein overexpression as analyzed by western blot, immunohistochemistry (IHC), and enzyme-linked immunosorbent assay (ELISA). Of these, 95 (88%) studies showed HER2 gene amplification or protein overexpression in breast cancer as an important predictive factor by either univariate or multivariate analysis. Multivariate analysis was performed on 93 studies of which 68 (73%) showed HER2 as an independent adverse prognostic factor. However, in 13 (12%) studies there was no correlation between prognosis and HER-2/neu status (Ross et al, 2009).

Various methods have been used to measure Her-2-neu and its gene product. These include direct measurement of gene amplification, mRNA level, and protein expression. The most widely studied method is immunohistochemical staining (IHC). The food and drug administration (FDA has approved IHCS for detecting Her-2-neu overexpression an fluorescence in situ hybridization (FISH) for quantifying Her-2-neu gene amplification. At this time, both of these methodologies have been validated as having clinical utility for different clinical purposes.

Her-2/neu status also appears to be predictive for either resistance or sensitivity to different types of chemotherapeutic agents. HER-2 overexpression has also been associated with enhanced response rates to anthracycline-containing chemotherapy regimens in most, but not all, studies (Muss et al, 1994; Hamilton et al, 2000; Di Leo et al, 2001; Petit et al, 2001; Harris et al, 2001; Kim et al, 2009). Because anthracyclines are topoisomerase inhibitors and the topoisomerase II gene is coamplified with HER-2/neu in approximately 35% of HER-2/neu positive breast...
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cancers, it has been suggested that HER-2/neu may be serving as a surrogate marker of anthracycline sensitivity. Her-2/neu was initially associated with the resistance of tumors in patients treated with cyclophosphamide, methotrexate, and 5-fluorouracil adjuvant chemotherapy (Giai et al., 1994), larger follow-up studies failed to demonstrate a lack of benefit in Her-2/neu positive tumors treated with this multidrug regimen (Paik et al., 2000; Menard et al., 2001).

Trastuzumab a monoclonal IgG1 class humanized murine antibody, was developed by the Genentech Corporation to specifically bind the extracellular portion of the HER-2 trans membrane receptor. This antibody therapy was initially targeted specifically for patients with advanced relapsed breast cancer that overexpresses HER-2 protein (Huston et al., 2001). However due to various reasons related to Her-2/neu resistance Lapatinib an orally available small-molecule dual inhibitor of the EGFR and HER-2 tyrosine kinases was approved by FDA in 2007 for use in combination with capecitabine for the treatment of HER-2–positive metastatic breast cancer that has progressed with standard treatment (Geyer et al., 2006; Medina et al., 2008)

Her-2/neu amplified cell line (Zabrecky et al., 1991) and in the serum of nude mice bearing xenografts from Her-2/neu amplified cells (Langton et al., 1991). It was subsequently isolated from pleural effusions and the serum of advanced breast cancer patients (Leitzel et al., 1992). Several studies have shown Her-2/neu extracellular domain (ECD) to be present in roughly 25% of unselected patients. When compared it is found that Her-2/neu is present in primary tumors who shed ECD and also when ECD is shed in metastatic state (Harris et al., 2001). Upon comparison Her-2/neu ECD is generated by proteolytic cleavage of the holo receptor, resulting in the production of
an extracellular soluble fragment of 97-115 KD (HER2 ECD) and a truncated 95-KD intramembrane protein (Zabrecky et al, 1991). In addition, an alternative mechanism of RNA splicing has been described (Scott et al, 1993). The mechanism of cleavage of Her-2/neu is not completely characterized; however, it has been shown that release of Her-2/neu ECD is mediated by metalloproteases. (Esparis et al, 1999; Codony et al, 1999) The functional significance of ECD shedding has not been determined, but in vitro data suggest that deletion of the extracellular carboxy terminus of the molecule enhances the signaling activity and transforming ability of the NH-2 terminally truncated receptor, p95HER2 (Di fiore et al, 1987; Segatto et al 1988). The p95 protein was found to be present and phosphorylated in tumor cell lines (Christianson 1998) and in primary breast tumors. (Molina et al 2001). Though biological role of p95 Her-2/neu is not fully determined but its overexpression has shown to lead to the growth of tumor xenografts in nude mice (Anido et al, 2006). The p95 Her-2/neu protein has kinase activity that is required for tumor growth (Cordony et al, 1999). The biological role for p95 is further suggested by its association with lymph node metastases in HER2-overexpressing breast cancer (Christianson 1998). The in vitro studies have demonstrated that spontaneous proteolytic cleavage of the ECD represents a ligand-dependent activation mechanism of Her-2/neu (Molina et al 2001; Hudelist et al, 2003). Proteolytic cleavage of Her-2/neu ECD might, therefore, result in the generation of a constitutively active transmembrane receptor, thereby contributing to a more aggressive phenotype. p95 Her-2/neu expression was found to be an independent prognosticator in breast cancer and associated with significantly worse outcome (Saez et al, 2006).
Currently there are at least four means of measuring p95. Her-2/neu Intracellular domain (ICD) western blot is the most direct, but requires large fresh frozen sample which is not always readily available. The second is the subtractive method whereby conventional IHC is used to subtract density scores of Her-2/neu ICD from Her-2/neu ECD to infer p95 content from this difference (Pederson et al, 2009). The next method is immunofluorescence method of inferring the presence of p95 Her-2/neu by intracellular localization of the cytoplasmic domain (Sclariti et al, 2007). The Vera tag p95 assay that uses a novel antibody conferring high selectivity for p95 in formalin fixed paraffin embedded tissue sections which is quantified by release of fluorescent tag which is subsequently measured with high sensitivity by capillary electrophoresis. However, the antibody is not yet commercially available (Sperinde et al, 2010). The p95 Her-2/neu measured by these various methods raises the possibility that p95 Her-2/neu distinct from the full length receptor may represent an important target for the therapeutic intervention.

**Her-2/neu Single Nucleotide Polymorphism (SNP):**

As many of the studies indicate HER-2 /neu has an important role in prognosis after a diagnosis of breast cancer, the gene encoding it is a natural target for investigation regarding polymorphisms that might indicate resistance or susceptibility for breast cancer development and also for disease outcome. More than one million single nucleotide polymorphisms (SNP) which may fall within coding sequences of genes, non-coding regions of genes, or in the intergenic regions are available for genotyping and phenotyping studies (Taylor et al, 2001). SNP genotyping have uncovered a
variety of cancer predisposition syndromes based on single and multiple gene variants (Weber et al, 2001).

The only known mechanism of increased HER-2/neu activity in humans is through gene amplification and protein overexpression, experimental studies of rats show that a single missense point mutation (Val664Glu) in the transmembrane domain of the neu proto-oncogene (HER-2 human homologue) that greatly increases its activity and cell transformation properties (Dougall et al, 1994; Bargmann et al 1996). This point mutation has not been identified in cases of human cancer The single nucleotide polymorphism (SNP) in the human HER2 was identified in the transmembrane coding region of the gene at codon 655, encoding either isoleucine (Ile, ATC) or valine (Val, C) (Paik et al 2000). A study by Xie et al (2000) suggested that a germ-line missense variant in the transmembrane region of the HER-2/neu gene may influence breast cancer risk. Specifically, the authors found a 40% increase in risk among women with a single base pair (bp) variation (Ile655Val) within the coding region of the Her-2 gene. The effect was present exclusively among younger women (≤45 years of age), and they concluded that the HER-2 variant may be an important susceptibility marker. However none of the studies have determined if this polymorphism affects the ability of Her-2/neu to transform cells, and/or affects its tyrosine kinase activity. The relationship between the Val allele and breast cancer suggests that this polymorphism may be functionally important (McKay et al, 2002). The results of Cowdin et al (2001) suggested that women with germ-line Valine genotypes were more likely to develop localized disease and less likely or slower to progress to high-stage breast cancer than women with Isoleucine homozygous
genotypes. This increase in risk for low stage breast cancer without progression is like
the pattern of diagnosis seen for estrogen replacement therapy and endometrial
cancer. They hypothesized that this relationship could occur if the germ-line variant
initially increases cellular proliferation, while subsequently decreasing the likelihood
that the HER-2/neu gene will undergo amplification or protein overexpression. It is
possible that a single base change in the transmembrane region of the HER-2 gene
may be adequate to alter the binding site of the protein receptor given that a missense
mutation in the same domain of the rat neu gene alters the tyrosine kinase
phosphorylation site. The actual relationship between the HER-2lle655Val variant and
HER-2 somatic activation is yet to be explored. To better understand this relationship,
the authors evaluated the presence of HER-2 amplification among cases genotyped
for the lle655Val variant and stated that when measured in combination, the germ-line
and somatic variants may provide more information on breast cancer progression and
treatment response than either alone.

The lowest frequency of Valine genotypes was found among African-American
women, who consistently present with an overall higher stage of breast cancer than
white women (Moormeier 1996; Lyman et al 1997). Although there are other reasons
for the racial/ethnic variation in breast cancer rates, differential expression of the
Valine genotype may serve as one example of a genetic marker that may contribute to
understanding of breast cancer progression.
PI3K/PTEN/AKT/mTOR pathway:

The transformation of normal mammary epithelial cells into cancer cells involves a multistep process with alterations in signal transduction pathways that confer important survival and growth advantages to malignant cells (Hanahan et al., 2000). The major pathways involved in signal transduction include the Ras/mitogen-activated protein kinase pathway, the phosphatidylinositol3 kinase PI3K/AKT pathway, the Janus kinase/signal transducer and activator of transcription pathway, and the phospholipase C pathway. These pathways including the PI3K/AKT signaling pathway activated by Her-2/neu over expression affect cell proliferation, survival, motility, and adhesion. The PTEN/PI3K/AKT pathway also plays a major role in a variety of cellular processes mentioned above in both normal and tumor cells. Genetic aberrations found at different levels, either with activation of oncogenes or inactivation of tumor suppressors, make this pathway one of the most commonly disrupted in human breast cancer. As part of the growth factor receptor (GFR) signaling, the PI3K pathway is a key mediator of cell metabolism and cell growth that is affected by genetic aberrancies at different levels, becoming a crucial pathway for cancer development and representing a therapeutic target against breast cancer (Sherr 2000; Hoeflich et al., 2009; Samuels et al. 2006). Signaling through the PI3K/PTEN/AKT/mTOR pathway is responsible for balancing cell survival and apoptosis (Nicholoson et al., 2002; Bellacosa et al., 2005). The signal is initiated by growth factors and hormones that bind receptor tyrosine kinases such as (EGFR, vascular endothelial growth factor receptor (VEGFR), and platelet-derived growth factor receptor (PDGFR) (Schlessinger 2000).

Understanding the principal effector mechanisms of the PI3Ks and the cross talk with
other oncogenic signaling pathways has been the focus of extensive research to
develop drugs with clinical efficacy (Agarwal et al, 2010).

PI3K belongs to a large family of PI3K-related kinases. PI3Ks are heterodimers with
separate regulatory (p85) and catalytic (p110) subunits. A large numbers of the
plasma membrane receptors, in particular those with tyrosine kinase (TK) activity, can
activate class I PI3Ks and lead to receptor activation and autophosphorylation on
tyrosine residues. PI3K catalyzes the synthesis of the membrane phospholipid PI
(3,4,5) P3 from PI (3,4) P2 which PI3K phosphorylates phosphoinositol lipids on the
D3 position of the inositol ring generating PtdIns-3-phosphates (PtdIns-3,4-P2, and
PtdIns-3,4,5-P3; Fruman & Cantley 2002). The p110s are encoded by the PI3KCA
gene and are regulated upstream by growth factor binding to tyrosine kinases
receptors and G protein-coupled receptors. Activated RAS protein can interact with
p110 and also activate class IA PI3Ks. The generation of the second messenger 3,4,5-
PIP₃ by class IA PI3Ks plays a key role in downstream signaling by several effectors
proteins including the serine/threonine kinase AKT and PDK1 (phosphoinositide-
dependent kinase 1; Bellacosa et al, 1991). The membrane colocalization of both
PDK1 and AKT through their pleckstrin homology domains results in phosphorylation
at Thr308 and partial activation of AKT kinase. The phosphorylation of Ser473 by
PDK2 generates complete activation of AKT (Wang et al 2000). It can effectively
recruit AKT to the plasma membrane by direct interaction with AKT pleckstrin
homology domain (Frenso et al, 2004). Growth factor stimulation of PI3K activity leads
to AKT activation. The tumor suppressor PTEN antagonizes PI3K activity by
converting the second messenger PIP3 to its inactive state PIP2 and
dephosphorylation results in AKT inhibition. The fundamental in vivo role of PTEN therefore appears to be inhibition of PI3K-dependent activation of AKT (Li et al. 1997; Li DM et al., 1997). The lack of its negative regulatory action causes the activation of the PI3K pathway through the phosphorylation of AKT (Carnero et al., 2008). After recruitment to the membrane, AKT is phosphorylated and consequently activated, by PDK at serine and threonine residues which in turn phosphorylates multiple downstream proteins. Proteins phosphorylated by activated AKT promote cell survival and control essential cellular processes, such as glucose metabolism and cell proliferation. Some proteins phosphorylated by AKT are Bad, caspase 9, Ikappa-B kinase, FKHR, MDM2, etc (Nicholson et al., 2002). Most importantly, AKT can exert effects on cell metabolism and growth through activation of the protein kinase mTOR ‘mammalian target of rapamycin’ (Figure 7). AKT relieves the negative regulation of mTOR mediated by the tumor-suppressor proteins: TSC1 and TSC2 (tuberous sclerosis complex proteins; Inoki et al., 2002; Potter et al. 2002). Activation of mTOR plays a key role in the activation of protein synthesis contributing to the pathogenesis of multiple tumor types. Phosphorylation of TSC2 by AKT inactivates the GTP hydrolysis of the small GTP-binding protein Rheb (ras homologue enriched in the brain), permitting Rheb to remain in the GTP-bound state. Rheb-GTP binds and activates the mTOR kinase domain (Li et al., 2004). The proline-rich AKT substrate (PRAS40) is also a negative regulator of mTOR and it is inactivated by AKT phosphorylation (Oshiro et al., 2007, Wang et al. 2007).
These findings expose the fundamental role of AKT in the mTOR activation by growth factors in that AKT inactivates two negative regulators of mTOR (Gibbons et al, 2009). The TSC1/2 complex is also regulated by the LKB1-AMPK (AMP-dependent kinase) and MAPK pathways. The convergence of these signals through the TSC1/2 complex allows mTOR to control cell growth and proliferation based on the availability of nutrients and energy source. In regulating the initiation and elongation steps, mTOR controls the overall rate of protein synthesis. mTOR exists in two multi protein complexes: mTOR complexes 1 and 2 (mTORC1 and mTORC2). mTORC1 activation controls protein synthesis by phosphorylating two translational regulatory proteins: eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4EBP1) and p70 ribosomal
protein S6 kinase (S6K1). The activation of S6K and 4EBP1 promotes translation initiation for protein synthesis (Brunn et al, 1997; Gingras et al, 1999; Dorrello et al, 2006, Wullschleger et al 2006). The activation of mTORC2 complex remains poorly understood; it appears to be through growth factors in an AKT-independent manner (Huang et al, 2008). mTORC2 phosphorylates AKT at Ser473 (Sarbassov et al, 2005) leading AKT activation toward the Forkhead transcription factor FOXO and the apoptosis regulator BAD. The capacity of mTOR to regulate protein synthesis explains in part how the tumor-promoting functions of deregulated mTOR may be distributed among multiple targets (Browne et al, 2004; Proud 2009).

The role of PI3K/AKT pathway in breast tumorigenesis:
Inappropriate co-option of PI3K signaling is one of the most frequent occurrences in human cancer. The pathway has been found to be activated in a variety of malignancy including breast, colorectal, ovarian, pancreas, brain, endometrium, and other tumor type. Next to p53 pathway this pathway is considered to have maximum genetic alterations in cancer and shows that aberration in this pathway are causes of cell transformation and PI3K pathway inhibition causes tumor progression (Bayascas et al, 2005; Chen et al 2006). More than 70% of breast tumors have molecular alterations in at least one component of the pathway (Lopez et al, 2010). Clinical pathological data of (Zhou et al, 2004) suggested that over expression of ErbB2 leads to increased p-mTOR and p-4EBPI levels in cultured breast cells. Therefore the AKT/mTOR pathway plays a crucial role during development and progression of breast malignancy. The phosphorylation of AKT, mTOR and 4EBPI increase progressively in breast cancer in association with overexpression of ErbB2. This observation indicates the utility of this pathway in predicting the prognosis of patients with breast cancer, especially those
treated with inhibitors. Loss of PTEN, PIK3CA mutations, and mutations or other aberrations at the level of PDK1, AKT1, AKT2, and p70S6kinase are some of the known mechanisms that activate the pathway. PIK3CA mutations may contribute to carcinogenesis through both AKT-dependent and AKT-independent mechanism. When the AKT-dependent signal is compromised, PIK3CA mutations may transduce an AKT-independent signal that engages PDK1 and SGK3 (Vasudevan et al, 2009). AKT appears to function as a survival factor during breast tumorigenesis. Activation of P13K/AKT pathway causes phosphorylation of Bad, leading to modulation of cellular apoptosis. In tumors cells loss of PTEN function is one of the genetic alteration used by tumor cells to activate the PI3K pathway.

The frequency and type of PI3K pathway aberrations vary among the different breast cancer subtypes (Lopez et al, 2010). Each molecular alteration may have a different clinical impact depending on the breast cancer molecular background, the presence of other aberrations, and the treatments received. The genetic heterogeneity of breast cancer and likely different cell origin for each tumor subtype make necessary an independent analysis of the PI3K pathway aberrations by tumor subtype.

The PTEN/PI3K/AKT pathway is also instrumental in epithelial mesenchymal transitions (EMT) and in angiogenesis during tumorigenesis. It plays a pivotal role in increasing invasion and migration and in promoting the EMT program of breast tumor cells.

From the integrated analysis of the PI3K-Akt pathway, one observes immediately that this is not a linear growth pathway like the Ras/Raf/MEK pathway. Instead, the pathway is sandwiched with pathway activators between pathway inhibitors. Hence,
the cancer cells take advantage of the complexity of this pathway to deregulate it at multiple levels in a combinatorial fashion (Figure 9).

**Figure 8:** PTEN/AKT/mTOR pathway in breast cancer (Adapted from Wolfram E et al, 2007)

From the integrated analysis of the PI3K-Akt pathway, one observes immediately that this is not a linear growth pathway like the Ras/Raf/MEK pathway. Instead, the pathway is sandwiched with pathway activators between pathway inhibitors. Hence, the cancer cells take advantage of the complexity of this pathway to deregulate it at multiple levels in a combinatorial fashion (Figure 9).

The common activation of the PI3K pathway in breast cancer has led to the development of compounds targeting the effector mechanisms of the pathway including selective and pan-PI3K/pan-AKT inhibitors, rapamycin analogs for mTOR inhibition, and TOR-catalytic subunit inhibitors.
Figure 9: Nonlinear signaling through the PI3K-Akt pathway (Modified from Georgescu et al, 2011)

 Taken together, the pathway is a promising potential target for cancer chemotherapy. Indeed, many companies and academic laboratories have initiated a variety of approaches to inhibit the pathway at different points. Essentially, PI3Ks, PDK1, AKT and mTOR are heavily targeted for therapy in different ways. These proteins are kinases, which are very "druggable" targets a priori, and, according to the "addiction hypothesis", cancer cells with the activated pathway will be more dependent on its activity for their survival (Carnero A, 2010).
PTEN:

PTEN is also known as MMAC and TEPI cloned and mapped to cytoband 10q23.3 is a tumor suppressor gene that encodes a dual specificity phosphatase with lipid and protein phosphatase activity. In two instances groups specifically searching either glioma or breast cancer happened on the same gene and both the names are acceptable (Li J et al, 1997; Li DM et al, Steck et al, 1997). Germline mutations constitute a minor fraction of the alterations in tumor suppressor genes that contribute the pathogenesis of human tumors and are instead useful for the discovery of somatic mutations in sporadic non familial tumors.

The protein encoded by the gene is a phosphatase-an enzyme that facilitates the removal of phosphate group from macromolecules (dephosphorylation). These lipids, phosphatidylinositol 3,4,5-triphosphate (PIP3) and phosphatidylinositol 3,4 biophosphate are produced during cellular signaling events by the action of the lipid kinase phosphoinositide 3 kinase (PI3K) (Rameh et al, 1999). Thus, an elegant on-off switch has been deposits a phosphate group on the D3 position of the inositol ring and is turned off when PTEN removes the phosphate group from the same position. The discovery of PTEN's lipid phosphatase activity and its ability to act as on “off” switch for the PI3K signalling, suggested that PTEN functioned as a tumor suppressor by directly antagonizing the activity of the PI3K signaling pathway (Maehama et al, 1998). PTEN is frequently inactivated by mutation with loss of heterozygosity (LOH) in a number of cancers including breast, brain, prostate, and uterine cancer. PTEN can also be inactivated by other mechanisms in somatic cancers, including promoter methylation (Khan et al, 2004; Weincke et al, 2007, Gallardo A 2012) micro-RNA
interference (Kim et al, 2010) phosphorylation (Silva et al, 2008) and delocalization from the plasma membrane (Molina et al, 2010).

Due to its high frequency of inactivation in somatic cancer, PTEN ranks as the second most mutated tumor suppressor gene after p53. Similarly to p53 and other bona fide tumor suppressors, germline mutations in PTEN gene cause Cowden syndrome characterized by intestinal hamartomas, mucocutaneous lesions, macrocephaly, fibrocystic disease, and increased risk for developing breast, thyroid, and endometrial cancer.

Fifteen years after its discovery as a tumor suppressor, (Li J et al, 1997; Li DM et al, Steck et al, 1997) PTEN reveals itself as a highly regulated tumor suppressor that behaves differently in different types of tumors. In some tumors, such as glioblastoma in which 10q chromosome deletion is present in 70% of cases (Furnari et al, 2007; Ohghaki et al, 2007) mutation with LOH of PTEN eliminates both alleles and therefore completely eliminates its expression. This situation conforms to Knudson’s 2-hit hypothesis for a tumor suppressor in which the complete gene elimination is required for tumor growth (Knudson 1971). In other types of tumors, PTEN shutdown is not complete. Mutations of one allele, transcriptional repression, epigenetic or posttranslational mechanisms, all of which would achieve partial inactivation of PTEN, and a combination of these mechanisms are also possible, leading to a continuum of lower than normal levels of functional PTEN in tumors. These mechanisms of reduced expression would exemplify a haplo insufficient tumor suppressor mode for PTEN, and examples for such a behavior are found in both Cowden syndrome, in which not all tumors show LOH, and in most somatic cancers. The fact that the levels of PTEN
inversely correlate with tumorigenesis and AKT activation has been experimentally proven in mice with hypomorphic and hypermorphic PTEN alleles (Alimonti et al, 2010). It appears that even a small reduction of PTEN levels confers growth advantage to tumor cells, but the higher the reduction is, the more rapidly the tumor develops. This result explains why cancer cells target one or more of the plethora of mechanisms regulating PTEN levels and activity. In the PI3K-Akt pathway, coexisting PI3K and PTEN mutations are present. As these mutations are likely to occur sequentially, they might represent an alternative mechanism of eliminating both alleles of PTEN.

The rate of hemizygous inactivation in the 10q23 region significantly exceeds the rate of mutation of the remaining allele. The tumor suppressor paradigm characteristically calls for loss of both functional copies of the gene and indeed many but not all tumor suppressor genes must undergo biallelic inactivation to sustain a true loss of function effect. The discordance between the rate of LOH and the rate of mutation of the second allele has led some to suggest that a second tumor suppressor gene is harbored in the 10q23 region. The difference could be due to technical inability to detect second mutational events or due to epigenetic alterations in the gene, mRNA or protein leading to a true loss of function.

Promoter hypermetylation is the most common epigenetic mechanism for the loss of gene expression. The most widely studies epigenetic modification is the cytosine methylation of DNA within the CpG dinucleotide (Jones and Baylin, 2007). The CpG dinucleotides are not equally distributed throughout the genome, but are found in short GC-rich DNA stretches known as CpG islands found preferentially in the promoter
regions of genes. In cancer, the methylation landscape is profoundly distorted. Human tumors undergo a global overall loss of DNA methylation, also acquire hypermethylation at specific promoters. The underlying mechanism that causes these changes are unknown, but there is a suggestion that at least a subset of DNA methylation changes occur in early tumor development and may initiate carcinogenesis. Therefore methylated genomic DNA has several properties that make it an attractive potential biomarker in cancer. The hypermethylation of most genes is rarely found in healthy individuals and majority of the methylation changes detected in cancer cells are acquired during neoplastic development and therefore are specific to cancer (Lopez et al, 2009).

PTEN methylation has been extensively studied in cancer (Garcia et al, 2004; Khan et al, 2004; Sadeq et al, 2011; Gallardo A, 2012). The various studies groups have reported PTEN methylation at a variable range. Also PTEN protein has been extensively studied by Immunohistochemistry by various study groups (Feilotter et al, 1999; Bose et al, 1999; Perren et al, 1999; Depowskii et al 2000, Bose et al, 2002; Lee et al, 2004; Tsutsui et al, 2005; Wang et al, 2011; Gallardo et al, 2012). Some studies have demonstrated a direct relation of PTEN loss with progression of breast cancer. Therefore, the study of PTEN an important molecule of the pathway may be helpful in the development of ideal therapies with meaningful clinical efficacy. The development of medications with multi target properties and the identification of potent drug combinations are expected to generate results in the management of breast tumors driven by multiple oncogenic pathways and to overcome resistance by feedback mechanisms.
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AKT:

AKT/PKB (protein kinase B) intricately regulates many cellular functions such as cell growth and proliferation, cell survival, apoptosis, energy metabolism, and resistance to anticancer therapeutics. AKT/protein kinase b (PKB) is a serine/threonine kinase and was identified by Tsichilis et al as a pro survival protein in a PI3K dependent manner (Bellacosa et al 1991). In Mammals AKT consists of three isoforms, Akt1,Akt2, and Akt3. Although these three isoforms are encoded by separate genes, they share a common NH2-terminal pleckstrin homology (PH) domain, a catalytic domain in the middle, and a COOH terminus (Kumar et al 2005; Nicholoson et al, 2002). The identity of the overall amino acid sequence of the three isoforms is very high (80%) however, the COOH terminus and the PH-linker region are more diverse (Kumar et al 2005). PI3K can effectively recruit AKT to the plasma membrane by direct interaction with the AKT PH domain (Frenso et al, 2004). Subsequently AKT is phosphorylated at Thr308 by PDK1 and at Ser473 via autophosphorylation or other kinases to reach maximal activation (Brazil et al 2004). Once phosphorylated and activated, AKT is transferred to several subcellular locations where it can phosphorylate its various pathways. AKT plays a critical role in cell survival by interacting negatively with apoptosis promoting proteins like BAD, MDM2, NF-KappaB, Caspase-9 and Forkhead, and with proteins involved in cell proliferation (p21, p27), cell growth (mTOR), cell motility and invasion (GSK-3beta). These proteins also play critical roles in tumorigenesis and metastasis. Three AKT isoforms are encoded by distinct genes localized on different chromosomes. They have similar structure with approximately 80% amino acid identity but their expression patterns and biological activities differ. In normal physiology,
AKT1 is involved in placental development and maintenance, AKT2 in glucose metabolism, adipogenesis and AKT3 plays a role in postnatal development of brain. The study of Kikegaard et al (2005) showed that high levels of cytoplasmic AKT2 and not AKT1 and AKT3 were associated with improved survival showing the different functions of AKT isoforms.

AKT signaling is modulated by enzymes such as MDM2, alpha6beta4 intergrin, tumors suppressor PTEN which are often mutated in human cancers and increase growth and cell survival of tumor cells (Nakatani et al, 1999; Jones et al 1991; Li et al 1997, Gao et al 2005). The increased role of AKT in breast cancer is sustained by observation that constitutive activation of AKT pathway are seen in situ breast carcinoma and invasive cancers, it plays an important role in conferring resistance to anti-estrogens and in chemoresistance as well as radioresistance for breast cancer.

In a study Moulder (2010) suggested that triple negative cancers have high level of AKT activation as compared to non-triple negative cancers. However, Aleskandarany et al (2011) showed that higher proportions of luminal tumours were pAkt positive relative to triple negative/basal subtypes. Activation of AKT and its prognostic value in breast cancers have been reported (Perez et al, 2002; Zhou et al, 2000; Schmitz et al, 2004). The biological effects of HER-2/neu over expression are mediated via several intracellular pathways regulating important downstream substrates including MAP kinase, PI3K and AKT (Hung et al, 1999). Studies on breast cancer cell lines have shown a crosstalk between Her-2/neu overexpression and activation of the AKT signaling pathway (Muthuswamy et al, 1999; Ahmad et al 1999). Few investigations have therefore revealed AKT as a downstream target of Her-2/neu signaling (Altiok et
al, 1999; Liu et al 1999) and dimerization of HER-2/HER-3 has been shown to be connected to PI3K with subsequent phosphorylation of AKT (Hellyer et al, 2001, Figure 6). In a study by Baccus et al (2002) AKT activation was observed in Her-2/neu positive breast cancer and may be responsible for higher tumor aggressiveness by increased resistance to stress-induced apoptosis. AKT activation by Her-2/neu overexpression has also been reported in various studies (Zhou et al, 2000; Zhou et al, 2001; Stoica et al, 2003 (A); Stoica et al, 2003 (B); Campbell et al, 2001). A significant positive correlation between pAKT and Her-2/neu was demonstrated which may result from activation of AKT via Her-2/neu overexpression (Bacus et al, 2002; Schmitz et al, 2004; Tokunaga et al, 2006). Also Cicecans et al (2005) concluded that AKT activation was associated with tumor proliferation and poor prognosis, particularly in the subset of patients with ErbB2-overexpressing tumors.

The another major mechanism of AKT activation is through loss of function in tumor suppressor gene PTEN (Figure 8). The studies have demonstrated AKT activation through PTEN abnormalities such as reduced PTEN expression, PTEN mutation in breast cancer. Reduced PTEN expression could result in increased activity of PI3K/AKT mediated anti-apoptotic pathway. Another key target of AKT is mTOR which is involved in a variety of functions including transcriptional and translational. Zhou et al (2004) reported that expression of mTOR, phosphorylated AKT and 4E-BPI increased progressively as proliferation and invasion increased in breast cancer.

In light of homeostasis of breast epithelial cells in controlling proliferation and apoptosis and AKT appears to function as a survival factor during breast tumorigenesis and also
epithelial Mesenchymal transition (EMT) as PI3K/AKT is instrumental in EMT and in angiogenesis during tumorigenesis.

As AKT can be activated by a variety of factors thus drugs designing drug specific to AKT protein kinase may sound theoretically appropriate but there are many practical problems. The study by Tokunaga et al (2006) suggested that pAkt (phosphorylated AKT) may be a useful predictor of resistance to endocrine therapy for breast cancer, while also suggesting that the inhibition of AKT may increase the efficacy of endocrine therapy. In a review article by Steelman et al (2008) searched for altered expression of the pathway in various malignancy concluded that this pathway is frequently aberrantly regulated in various cancers and targeting this pathway with small molecule inhibitors and may result in novel, more effective anticancer therapies. Spears et al (2012) stated AKT activation is associated with poor outcome in endocrine-treated breast cancer wherein high levels of pAKT1 were associated with reduced disease free survival (DFS). Using the proximity ligation assay they measured levels of AKT1 and AKT2 in paraffin embedded tissue section and suggested that AKT1 drives progression in early breast cancer. Andre et al (2007) suggested that pAKT was not predictive for the efficacy of anthracycline-based adjuvant chemotherapy.

In a study by Zhou et al (2004) patients whose tumors had higher pAKT, pmTOR, or p4E-BP1 levels tended to have shorter DFS, on the other hand, patients whose tumors had lower AKT, mTOR, or 4E-BP1 phosphorylation were more likely to be free of recurrence and further stated that they may be benefited from mTOR inhibitors. Some studies have reported that AKT activation can mediate anthracycline resistance (Liang et al, 2006; Knuefermann et al, 2003; Lee et al, 2006). In a study by Andre et al (2007)
the predictive value of pAKT expression in patients receiving adjuvant anthracycline-based chemotherapy was evaluated where pAKT expression was not predictive for anthracyline efficacy and therefore did not support the use of AKT and possibly mTOR inhibitors to modulate anthracycline resistance in breast carcinoma. Nevertheless, some investigators have reported that doxorubicin induces a secondary AKT activation that could lead to either antiapoptotic signaling or secondary tamoxifen resistance. However, some of the AKT inhibitors are being tested in clinical trials. Perifosine is a phospholipid derivative of alkylphosphocholine, and appears to inhibit not only AKT-mediated signaling but MAPK and JNK pathways as well (Ruiter et al, 2003; Hideshima et al, 2006; Gills et al, 2009). In several preclinical models, the agent demonstrated substantial activity. RX-0201 represents an antisense oligonucleotide to mRNA encoding AKT1. In in vitro models, culture with AKT1 at nanomolar concentrations resulted in growth inhibition of various human cancer cell lines (Yoon et al, 2009). ErPC is structurally related to perifosine and is currently in preclinical development. Like perifosine, the agent appears to inhibit AKT, but also impacts other signaling pathways most prominently, Raf-MEK-ERK (Handrick et al, 2006). GSK690693 was noted to inhibit all isoforms of AKT at nanomolar concentrations (Rhodes et al, 2007). With a spectrum of new agents directed at inhibiting AKT, it will be critical to determine where these agents fit into existing management paradigms. Studies assessing the safety and efficacy of AKT inhibitors combined with either traditional cytotoxic agents or other targeted therapies are therefore of prime importance these may allow novel AKT inhibitors to complement existing treatments.
**mTOR:**

mTOR, the downstream regulator of AKT, is a member of PI3K kinase-related family and a highly conserved protein belonging to the PtdIns3K-related kinase family (PIKK family) of serine/threonine protein kinases that includes ataxia-telangiectasia mutated (ATM), ataxia telangiectasia, and Rad3-related and DNA-dependent protein kinase. mTOR is a 289 kDa protein and has two different protein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (Wullschleger et al., 2006). mTORC1 is a rapamycin-sensitive complex defined by its interaction with the accessory protein raptor (regulatory-associated protein of mTOR) mLST8, Deptor, and PRAS40 (Kim et al., 2002; Loewith et al., 2002; Sarbassov et al., 2004) and is regulated by oxygen levels, amino acids, energy, and growth factors (Dufour et al., 2011). mTOR raptor coordinates growth-promoting signals from nutrient and energy availability, particularly from amino acids, and relays them to downstream targets (Hay et al., 2004). When activated, mTORC1 participates in translation initiation and protein synthesis by phosphorylating S6K1 and 4E-BP1 (Zoncu et al., 2011). One target is the translational regulator S6K1, which mTOR–raptor phosphorylates in coordination with the PDK1 protein kinase (Figure 10). Through its interaction with S6K1, raptor directs mTOR to phosphorylate Thr389 in the hydrophobic motif of S6K1 in a rapamycin-sensitive manner, whereas the PDK1 kinase phosphorylates Thr229 in the activation loop (Fingar et al., 2004). mTOR–raptor can also phosphorylate and inhibit the eIF4E-binding proteins (4E-BP), which are a family of negative regulators of eukaryotic translation-initiation factor 4E (eIF4E)-dependent translation (Hay et al., 2004). Studies implicate both S6K1 and eIF4E in cellular transformation (Mamame et al., 2004; Barlund et al., 2000). In addition, one major consequence of mTORC1 inhibition is the
downregulation of several mRNA coding for proteins implicated in the G1-S phase progression arrest and accounts in part for the anti-proliferative properties of mTOR inhibitors (Easton et al, 2006). Other less-characterized roles for the rapamycin-sensitive mTOR complex in ribosome biogenesis, transcription, cytoskeletal rearrangements and autophagy have also been described (Hay et al, 2004; Fingar et al 2004). mTORC2 consists of Rictor, mTOR, mLST8, Protor, Deptor as well as mSin1 and is regulated by growth factors. Downstream effectors of mTORC2 are AKT which regulates cell proliferation and survival, and PKC alpha, which controls cytoskeletal organization (Alessi et al, 2009). mTORC2 interaction with rictor (rapamycin-insensitive companion of mTOR) (Kim et al, 2002; Loewith et al, 2002; Sarbassov et al, 2004) also activates SGK1, however the functional significance of this activation needs to be further characterized (Figure 10).

![Figure 10: mTOR and its two complexes, mTORC1 and mTORC2](Adapted from Dufour et al, 2011)

The molecular mechanisms that regulate the activation of mTORC1 have been extensively studied. As described earlier the phosphatidylinositol-3 kinase (PI3K)/Akt signaling pathway has been identified as a major mediator of growth factors-induced
mTORC1 activation (Figure 11, Zoncu et al, 2011; Guba et al, 2002; Wullschleger et al, 2006).

Figure 11: Growth factors mediated mTORC1 and mTORC2 activation

(Adapted from Dufour et al, 2011)

Following stimulation with growth factors, PI3K is activated and catalyzes the formation of phosphoinositol-3,4,5-tri-phosphate (PIP3) resulting in the recruitment to the plasma membrane and to the activation of AKT. In turn, AKT inactivates TSC2, a large protein that is part of the TSC1-TSC2 complex. Inactivation of the TSC1/2 complex leads to the activation of the small GTPase Rheb which stimulates the kinase activity of mTORC1 (Zoncu et al, 2011). In parallel to the PI3K/AKT axis, growth factors also stimulate mTORC1 activity through the Mek/Erk signaling pathway (Ma et al, 2005). Phosphorylation of TSC2 by Erk or phosphorylation of raptor by p90RSK, a downstream effector of Erk, have both been proposed to explain the activation of mTORC1 by Erk signaling pathway (Carriere et al, 2008).
Previous studies have implicated the role of phosphorylated mTOR (pmTOR) in breast cancer pathogenesis. Zhou and co-workers have examined 165 breast cancers with specific antibody for pmTOR using immunohistochemistry, cell culture and western blot techniques which was associated with poor disease free survival (Zhou et al 2004). The group observed that the expression of pmTOR protein is increased from normal breast epithelium to hyperplasia and abnormal hyperplasia to tumor invasion. The study of Bose et al (2006) showed pmTOR overexpression in DCIS cases and in invasive carcinomas overexpressing mTOR showed a three times greater risk for recurrence. Herberger et al (2007) found that overall survival was significantly shorter for patients with p-mTOR-positive tumors of the biliary tract, as compared with that of patients with pmTOR-negative tumors. In another study, which examined the relationship between AKT (upstream of mTOR) and 4E-BP1 (downstream of mTOR) in primary breast tumors and their distant metastasis, it was revealed that most primary breast tumors and metastatic tumors expressed pAKT. Similarly, most of the primary and metastatic tumors were also positive for p-4EBP1 (Akcakanat et al 2008). In a study by Chen et al (2009) in hepatocellular carcinoma (HCC) pmTOR expression was detected in the cytoplasm of the malignant cells in of HCC. However, all adjacent non-cancerous liver tissues were negative for pmTOR staining. In a study by Dakuen et al (2012) by immunohistochemistry pmTOR was associated with better overall survival. In a study by Walsh et al (2012) using immunohistochemistry, mTOR and pmTOR were measured in TNBCs and non-TNBCs. The finding was that nuclear pmTOR was found more frequently in triple-negative than non triple-negative cancers suggesting
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that mTOR may play a more important role in the progression of TNBC compared to non-TNBC.

Since mTOR plays a central role in cell growth and proliferation and since it is frequently over activated in tumor cells, mTOR represents an ideal target in cancer therapy. The effect of mTOR inhibition in clinical trials has been extensively studied using rapalogs that specifically inhibit mTORC1. Notably, recent studies have shown that only certain functions of mTORC1 are targeted by rapalogs (Thoreen et al, 2009). In contrast to mTORC1, mTORC2 is insensitive to short-term exposure of rapalogs. However, depending on the cell type, prolonged exposure to rapalogs also inhibits mTORC2 (Sarbassov et al, 2006). mTORC2 is insensitive to rapamycin has led to the rapid development of inhibitors able to block both mTORC1 and mTORC2. It was speculated that blocking both complexes would produce stronger anticancer effects than rapalogs. Indeed, mTORC2 exerts an emerging role in cancer development and has been shown to participate in prostate and colon cancer progression (Dufour et al, 2011). Several such inhibitors have been developed and characterized. Activation of Mek/Erk signaling pathway following exposure of cancer cells to ATP-competitive inhibitors of mTOR has also been reported even with dual PI3K/mTOR inhibitors (Dufour et al, 2011). This finding suggests that combining Mek and ATP-competitive inhibitors of mTOR may have additive anticancer effects. (Dufour et al, 2011) and their toxicity and efficacy are being tested in clinical trials. So the new therapeutic strategies which have proved their efficacies in experimental models and are being evaluated in ongoing clinical trials and so that they may further be implemented in patient care.
Great studies have been and are being made in understanding of the diverse roles that PTEN/PI3K AKT/mTOR signaling plays in cancer initiation, progression, and maintenance.

Novel therapeutics targeting different components of this pathway are demonstrating efficacy in an array of human cancer types in preclinical studies, and these drugs are being carried forward into clinical trials. There is growing preclinical evidence that some genetically defined cancer subtypes maybe the most sensitive to single-agent PI3K pathway inhibitors. However, it remains to be determined whether these sensitive cancers will demonstrate stable disease or tumor shrinkage in response to single agent therapeutics with a large number of studies.
AIM:

The aim of the present study is to determine role of Her-2/neu in breast cancer by evaluation of Her-2/neu protein, and Her-2/neu gene Ile655Val SNP. Further, its role in downstream PTEN/PI3K/AKT signalling pathway by evaluation of molecules of this pathway PTEN, AKT and mTOR.

SPECIFIC OBJECTIVES:

Chapter I included study of Her-2/neu protein (Section 1A) and Her-2/neu Ile655Val (Section -1B) SNP in breast cancer.

The main aim was to detect truncated form of Her-2/neu (p95 Her-2/neu) protein in breast cancer.

a) To evaluate protein expression of membranous Her-2/neu internal domain, cytoplasmic Her-2/neu internal domain and membranous Her-2/neu external domain by immunohistochemistry and confirmation of cytoplasmic form of Her-2/neu internal domain as truncated Her-2/neu.

b) To correlate expression of membranous Her-2/neu internal domain, cytoplasmic Her-2/neu internal domain and membranous Her-2/neu external domain with clinicopathological parameters such as age, menopausal status, tumor size, lymph node (LN) status, disease stage, histological grade (HG), nuclear grade (NG), lymphatic permeation, vascular permeation, Bloom Richardson (BR) score, Estrogen Receptor (ER) and Progestrone Receptor (PR), disease status and treatment offered.
c) To intercorrelate membranous Her-2/neu internal domain, cytoplasmic Her-2/neu internal domain and membranous Her-2/neu external domain expression.

d) To evaluate these markers in patients with benign breast diseases and to compare with patients with breast cancer.

Her-2/neu gene Ile655Val Single Nucleotide Polymorphism (SNP):

a) To evaluate SNP in transmembrane coding region at codon 655Her-2/neu gene, encoding either isoleucine or valine (Ile655Val).

b) To correlate Ile655Val SNP with clinicopathological parameters such as age, menopausal status, tumor size, LN status, disease stage, HG, NG, lymphatic permeation, vascular permeation, BR score, ER and PR, disease status and treatment offered as well as according to molecular subtypes.

c) To intercorrelate Her-2/neu Ile655Val SNP with protein expression of membranous Her-2/neu internal domain, cytoplasmic Her-2/neu internal domain and membranous Her-2/neu external domain.

d) To evaluate Her-2/neu Ile655Val SNP in patients with benign breast diseases and to compare with breast cancer patients.

Chapter II (Section-II) included study of molecules involved in PTEN/PI3K/AKT pathway mediated by Her-2/neu signalling

a) To evaluate PTEN methylation and protein expression of PTEN, AKT and mTOR by immunohistochemistry.
b) To correlate PTEN, AKT and mTOR with clinicopathological parameters such as age, menopausal status, tumor size, LN status, disease stage, HG, NG, lymphatic permeation, vascular permeation, BR score, ER and PR, disease status and treatment offered as well as according to molecular subtypes.

c) To intercorrelate PTEN, AKT and mTOR and also with Her-2/neu protein membranous Her-2/neu internal domain, cytoplasmic Her-2/neu internal domain and membranous Her-2/neu external domain protein and Her-2/neu Ile655Val SNP.

d) To evaluate PTEN, AKT and mTOR in patients with benign breast diseases and to compare with breast cancer patients.