INTRODUCTION

Cancer is a progressively debilitating disease which is characterized by a persistent, abnormal and relatively autonomous proliferation of cells. It is the result of a permanent cellular defect that is passed on to the cell progeny. The defect is induced by single or combination of several factors and once developed, usually becomes independent of them.

Breast cancer is one of the major health problems confronting female population around the world. Carcinoma of the breast can be defined as “a solid tumour in which the epithelial cells proliferate at high rate, causing necrosis of surrounding tissues”. As they infiltrate at a high rate, the malignant cells metastasize to regions like lungs, bone, liver, brain and meninges. Cancer is fast catching up with coronary heart disease, and is emerging to be the leading killer disease, not just because of limited treatment options, but also due to the magnitude of damage caused by the disease and the treatment (Hershman et al., 2006).

EPIDEMIOLOGY

Chronic diseases such as cancer and other non-communicable diseases are fast replacing communicable diseases in India and other developing countries. The burden of cancer is still increasing worldwide despite advances in diagnosis and treatment. It is widely held that 80-90% of human cancers may be attributable to environmental and life style factors. Cancer prevalence in India is estimated to be around 2.5 million, with over 8,00,000 new cases and 5,50,000 deaths occurring each year due to this disease in the country (Yeole, 2003). The common sites for cancer in Indian population are oral cavity, lungs, oesophagus and stomach in males and cervix, breast and oral cavity among females. Over
70% of the cases report for diagnostic and treatment services in advanced stages of the disease, resulting in poor survival and high mortality rates (Nandakumar, 1996). The disease is associated with a lot of fear and stigma in the country. From the population-based registries in India covering 28-30 million populations from different parts of the country, the age adjusted incidence rates vary from 44 to 122 per 100,000 population in males and 52 to 128 per 100,000 females. Cancer incidence is higher in females compared to males (Yeole, 2004).

CLASSIFICATION OF TUMOURS

Benign tumours

- Non-invasive and remain localised
- Slow growth rate
- Close histological resemblance to parent tissue

Malignant tumours

- Invasive and thus capable of spreading directly or by metastasis
- Relatively rapid growth rate
- Variable histological resemblance to the parent tissue

Classification of Malignant tumours

Carcinoma - Tumour of endodermal or ectodermal origin (skin, epithelial lining of internal organs and glands).
Leukemia - Non-solid tumours of cells of the hematopoietic lineage
Lymphoma - Solid tumours of cells of the hematopoietic lineage
Sarcoma - Tumour of mesodermal origin (bone, fat, cartilage)
CARCINOGENESIS

Carcinogenesis is the process initiated due to genetic mutations induced by physical or chemical agents. Conceptually, this process can be divided into three distinct stages: initiation, promotion and progression (Hennings et al., 1993). Initiation involves an irreversible genetic change, usually a mutation in a single gene. Promotion is generally associated with increased proliferation of initiated cells, which increases the population of initiated cells. Progression is the accumulation of more genetic mutations that lead to the acquisition of the malignant or invasive phenotype. In the best-characterized model of chemical carcinogenesis, the mouse skin model, initiation is an irreversible event that occurs when genotoxic chemical or its reactive metabolite, causes a DNA mutation in a critical growth-controlling gene such as Ha-ras (Nelson et al., 1992).

Outwardly, initiated cells seem normal. However, they remain susceptible to promotion and further neoplastic development indefinitely. DNA mutations that occur in initiated cells can confer growth advantages, which allow them to evolve and/or grow faster bypassing normal cellular growth controls. The different types of mutations that can occur include point mutations, deletions, insertions, chromosomal translocations, inversion and amplifications. Three important steps involved in initiation are carcinogen metabolism, DNA repair and cell proliferation. Many chemical agents must be metabolically activated before they become carcinogenic. Most carcinogens or their active metabolites are strong electrophiles and bind to DNA to form adducts that must be removed by DNA repair mechanisms. Failure to repair chemical adducts, followed by cell proliferation, results in permanent alterations or mutation(s) in
the genome that can lead to oncogene activation or inactivation of tumour suppressor genes.

Promotion is a reversible process in which chemical agents stimulate proliferation of initiated cells. Typically, promoting agents are non-genotoxic, which are unable to form DNA adducts or cause DNA damage but are able to stimulate cell proliferation. Hence, exposure to tumour promoting agents results in rapid growth of the initiated cells and the eventual formation of non-invasive tumours. In the mouse skin tumourigenesis model, application of a single dose of an initiating agent does not usually result in tumour formation.

Progression refers to the process of acquiring additional mutations that lead to malignancy and metastasis. Many initiating agents can also lead to tumour progression, which lends strong support for the notion that further mutations are needed for cells to acquire the phenotypic characteristics of malignant tumour cells. Chemicals are converted into positively charged metabolites that bind to negatively charged groups on molecules like proteins and nucleic acids. This results in the formation of DNA adducts which, if not repaired, lead to mutations (Minamoto et al., 1999). The result of these mutations enables the tumours to grow, invade surrounding tissue and metastasize (Figure 1).

SEQUENTIAL INTERRELATED STEPS IN CANCER

A series of sequential interrelated cascade consists of steps which are rate limiting, since a failure at any of the steps aborts the process. The outcome of the process is dependent on both the intrinsic properties of the tumour cells and the responses of the host; the balance of these interactions can vary among different patients. In principle, the steps or events in the pathogenesis of a
Figure 1. Tumour formation and progression to metastasis

Source: www.albany.edu/cancergenomics/faculty/jaguirre/research.html
metastasis are similar in all tumours. The process of metastasis consists of sequential linked steps. Metastatic cells must complete all of these steps if a clinically relevant lesion is to develop. If a disseminating tumour cell fails to survive any of these steps, it will not produce metastasis. The major steps in the formation of a metastasis are as follows:

**Transformation**

Transformation of normal cells into tumour cells and their growth after the initial transforming event. Growth of neoplastic cells must be progressive, with nutrients for the expanding tumour mass initially supplied by simple diffusion.

**Extensive vascularization**

Vascularization, that is, angiogenesis must occur if a tumour mass is to exceed 1 mm in diameter. The production and secretion of proangiogenic factors by tumour cells and host cells play a major role in establishing a capillary network from the surrounding host tissue. Why is this important?

**Local invasion**

Local invasion of the host stroma by some tumour cells occurs by several parallel mechanisms. Thin-walled venous, like lymphatic channels, offer very little resistance to penetration by tumour cells and provide the most common routes for tumour cell entry into the circulation.
Detachment and embolization

Detachment and embolization of single tumour cells or aggregates occurs next, with the vast majority of circulating tumour cells being rapidly destroyed. Once the tumour cells have survived the circulation, they must arrest in the capillary beds of distant organs, by adhering either to capillary endothelial cells or to subendothelial basement membrane, which may be exposed.

Extravasation

Extravasation occurs next, probably by mechanisms similar to those operative during invasion.

Proliferation

Proliferation within the organ completes the metastatic process. To continue growing beyond the size of 0.2 mm in diameter, the micro metastasis must develop a vascular network and evade destruction by host defenses. Then, the cells can invade blood vessels, enter into circulation and produce additional metastases.

TUMOUR DORMANCY

After surgical removal, radiotherapy and/or chemotherapy, there may be no clinically detectable tumour remaining in a patient. This does not mean that the tumour has been completely eradicated, however, as minute deposits can evade detection by even the most sophisticated imaging techniques. These occult tumour foci can remain clinically dormant for perhaps several years before their regrowth causes signs and symptoms. For this reason, it is virtually impossible to speak of a cancer patient as being 'cured', and prognosis can be given only in
terms of the probability of survival or the length of the disease-free interval. The prognostic information derived from tumour type, grade and stage is used to predict the patient's chances of surviving, say 5 years (Currie and Currie, 1982).

**BREAST CANCER**

Breast cancer is not just one disease, but rather is a general term used to describe a number of different types of cancers that occur in the breast. Breast cancer starts in the epithelium and proliferate at a high rate causing necrosis of surrounding tissues. As they infiltrate at a high rate, the malignant cells metastasize to regions like lungs, bone, liver, adrenals, brain and even meninges (Velicer et al., 2004). Breast cancer is an area of tissue that has an abnormal and uncontrolled growth of cells. It can either be malignant (cancerous) or benign.

**EPIDEMIOLOGY**

In a worldwide population of 6 billion, in the year 2000, approximately 10 million cancers were diagnosed and there were an estimated 6.2 million cancer deaths. Whereas the universality of cancer incidence and mortality is established, the burden of cancer by type or organ site is distributed unequally between developing and industrialized nations. Cancers in which infectious agents are causal disproportionately affect populations in developing countries (Schottenfeld and Beebe-Dimmer, 2005). The burden of breast cancer worldwide in both developed and developing countries is increasing and evidence suggests that unless action is taken, it will continue to grow for the foreseeable future (Parkin and Fernández, 2006).
INCIDENCE

Breast cancer incidence and mortality vary considerably. In general, the incidence is high in developed regions of the world and low in developing regions; the range of mortality rates is much less (approximately 6–23 per 100,000) because of the more favorable survival of breast cancer in (high-incidence) developed regions. The age-adjusted incidence is low in most Asian countries, although world-standardized rates are greater than 50 per 100,000 in Manila, Philippines, and in Karachi, Pakistan. Rates in Singapore, particularly among the Chinese population, are also relatively high for the region. Rising incidence has been observed in India and Singapore (Yeole and Kurkure, 2003).

Figure-2 depicts the International comparison of age adjusted incidence rates with that of India with respect to breast cancer (females) per 100,000. According to a population-based study, the observed survival rate of breast cancer in Chennai, India is 48 % Vs 80 % in US (Gajalakshmi, 1997). As discussed earlier, the obvious reason for the low survival rate in India is due to the presentation, diagnosis and treatment at the advanced stage of the disease (Nandakumar, 1996). The incidence of breast cancer is increasing almost everywhere (Figure-3). This unfavorable trend is due to increase in risk factors (Parkin and Fernández, 2006).

Death rates from breast cancer in older women (age above 50 Years) continuous to be higher than the death rates in younger women (below 50 Years) (Breast Cancer Facts and Figures 2005-2006). A further problem is that approximately 60 % of patients with cancer are aged 65 years or more and are in postmenopausal state (Yancik, 2000). One in nine women develops breast cancer at some time in their lifetime and it mostly affects women over 50 years. In India, it shows mixed incidence pattern with breast cancer being second to
Figure 2. International Comparisons of Age Adjusted Incidence Rates with that of PBCRs under NCRP (ICMR, India) BREAST (Females) Cancer

PCBR : Population Based Cancer Registries
NCRP : National cancer Registry Programme
Figure 3  Breast cancer incidence rates worldwide

Rates are age-standardized (world standard) rates (per 100,000)

Source: Gl. OBOCAN 2002
cancer of the cervix in rural areas, however, in cities like Mumbai, New Delhi, Trivandrum and Coimbatore, the incidence of breast cancer has crossed that of cervix. The incidence of breast cancer in India ranges from 8.8 to 100,000 at Barshi to 28.6 to 100,000 at Mumbai. In Trivandrum, the age-adjusted-rate is 31.7 to 100,000 for the urban population and 16.5 to 100,000 for rural population (Pandey et al., 2005). A district wise minimum age adjusted incidence rate of breast cancer (females) per 100,000 in India is depicted in figure-4.

An estimated 178,480 new cases of invasive breast cancer are expected to occur among women in the US during 2007. After continuously increasing for more than two decades, female breast cancer incidence rates leveled off from 2001-2003. About 2,030 new cases of breast cancer are expected in men and 62,030 new cases of in situ (in addition to Invasive breast cancer) breast cancer are expected to occur among women in 2007. Moreover an estimated 40,910 breast cancer deaths (40,460 women, 450 men) are expected in 2007. Breast cancer ranks second among cancer deaths in women (after cervix cancer). Death rates from breast cancer have steadily decreased in women since 1990, with larger decreases in women younger than 50 (a decrease of 3.3 % per year) than in those 50 years and above (2.0 % per year). These decreases are due to a combination of earlier detection and improved treatment (Cancer Facts and Figures 2007). In India, the total number of breast cancer cases diagnosed per annum has been projected to increase from 60,000 to 80,000 by the year 2001 (Murthy et al., 1990) and will exceed 1,000,000 by 2010 (Ferlay et al., 2001). Although the overall incidence rate of breast cancer in Indian women is not as high as that in Western countries (23.5 vs. 90.7), the incidence of early-onset breast cancer cases (< 40 years) does not show significant variation between populations world wide, ranging from 12-33 cases per 100,000 women (Parkin et al., 1997). This suggests that a greater proportion of all breast cancer is due to
Figure 4  Districtwise Minimum Age Adjusted Incidence Rate of Breast Cancer (Females) Per 100,000 in India
early-onset disease in the Indian population than in Western populations, where the main burden of breast cancer is due to post-menopausal disease.

**Causative factors: Etiology / Carcinogens / Risk**

- Increasing age – risk doubles between age 45 and 65
- Previous cancer of the breast in the same patient
- Family history: A first degree blood relative with history of any cancer - in particular a woman with breast, ovary or endometrial cancer
- Genetics: BRCA 1 and BRCA 2 gene mutant patients are at very high risk and 1 in 20 women with breast cancer has the genes mutated (Palma et al., 2006)
- Early menarche with late menopause, nulliparity, first pregnancy after age of 30, obesity, breast augmentation, use of oral contraceptives and hormone replacement therapy (HRT) are some of the less significant but proven risk factors for the breast cancer.

**BREAST CANCER TYPES**

The breast is comprised mainly of fatty tissue. Within this tissue is a network of lobes, which are made up of many tiny lobules that contain milk glands. Tiny ducts connect the glands, lobules and lobes and carry the milk from the lobes to the nipple, located in the middle of the areola. Blood and lymph vessels run throughout the breast; the blood nourishes the cells and the lymph drains the waste. Breast cancers usually start in cells that line the milk ducts (ductal cancer) or milk producing lobes (lobular cancer). A few other rare forms of breast cancer also exist. 15% to 20% of breast cancers fall into the
category of noninvasive cancer or carcinoma in situ - tiny growths that have not spread across the wall of the milk ducts or lobules. More advanced cancers are called "invasive". They have spread beyond the ducts and lobules.

**Structure of the Breast**

Breast cancers grow at different rates, but some oncologists estimate that the average tumour doubles in size every 100 days. Since cancers start with one irregular cell, even with this doubling time, they may not be palpable for years. Mammography can find tumours that are too small to be felt, but even so, the tumours have probably been growing for years before they are large enough to be visible on a mammogram.

Breast cancer cells migrate to the lymph nodes under the arm (axillary), in the neck (cervical), or those just below the collarbone (supra-clavicular). The most common sites of metastasis, or spread, of breast cancer are skin, distant lymph nodes, bone, lung, and liver.
Ductal Carcinoma *in situ*

Ductal carcinoma *in situ* (DCIS) is an early stage cancer. It is noninvasive, which means it has not spread beyond the milk ducts to other parts of the breast, to the lymph nodes in the under arm, or to other parts of the body. Several types of DCIS exist. If not removed, some may change, developing into an invasive cancer. Others may never progress to this stage. Ductal carcinoma in situ is highly curable.

Lobular carcinoma *in situ* (LCIS)

Lobular carcinoma *in situ* (LCIS) is a noninvasive growth limited to the milk lobules of the breast. According to the National Cancer Institute, however, it is a warning of increased cancer risk. Women with LCIS have about a 1% risk of developing invasive breast cancer in either breast per year. At 20 years, this risk is about 18%.

Invasive Ductal Carcinoma

In more advanced stages, breast cancer cells cross the lining of the milk duct or lobule, and begin to invade, or infiltrate adjacent tissues. In this stage, the cancer is called "infiltrating cancer". Invasive ductal carcinoma (also known as infiltrating ductal carcinoma) is the most common kind of invasive breast cancer, and the most common type of breast cancer overall. More than half of all cases are of this type.
Other Types of Breast Cancer

Medullary carcinoma

This is a type of invasive ductal carcinoma which appears well confined, but often has infiltrated the lymph nodes. This cancer may grow large, but has a better than average prognosis.

Mucinous carcinoma

This is a type invasive ductal carcinoma which produces a gelatinous-like tumour. These cancers have a very good prognosis.

Tubular carcinoma

This is a type of cancer which produces many small glands and tubules that closely resemble normal mammary ductules.

Invasive lobular carcinoma

This cancer arises at the ends of the ducts or in the lobules and may cause widespread breast thickening rather than a specific lump. The prognosis is better than average.

Paget's disease

This is a very rare type of breast cancer. It appears as an itchy rash around the nipple and areola. It should not be mistaken for a benign skin condition such as eczema or contact dermatitis.
Inflammatory carcinoma

This is the most serious breast cancer. The skin over the breast becomes very inflamed and swollen because the skin lymph vessels are blocked by cancer. While the prognosis has improved considerably with new treatments, this cancer still has the least favorable prognosis.

GRADING AND STAGING OF BREAST CANCER

Each cancer is unique, and the combination of treatment options is practically endless. To help determine who should get what treatment, cancer specialists rely on staging - a system that places the cancer into a certain group.

TNM

In simplified form, staging is based on: the size of the tumour; presence of cancer cells in the lymph nodes; and metastases, or spread, to other organs. This is the so-called TNM -- tumour, node, metastases -- staging system.

Stages of Breast Cancer

There are several staging systems in use today. Here is one of them:

**Stage 0** : Ductal or Lobular carcinoma *in situ*, or Paget's disease of the nipple.

**Stage 1** : Tumour is 2 centimeters (3/4 inch) or smaller. Axillary lymph nodes are negative and there is no evidence of distant metastases.

**Stage II** : Tumour is larger than 2 and up to 5 centimeters (3/4 to 2 inches) in size. Axillary lymph nodes may or may not
be positive for cancer. (If tumour is smaller than 2 centimeters and lymph nodes are positive, cancer is considered Stage II.)

**Stage III**: Tumour is larger than 5 centimeters and axillary lymph nodes are positive. Tumour may extend into the pectoral muscle or into the skin of the breast, but there are no distant metastases.

**Stage IV**: If metastasis to other organs has occurred, cancer is considered Stage IV regardless of the size of the tumour in the breast or number of axillary lymph nodes affected.

**Therapy for Breast cancer**

Treatment for breast cancer is by combinatorial mode, usually followed as surgery (mastectomy) followed by chemotherapy and radiation therapy. This is followed by adjuvant hormonal therapy usually Tamoxifen (10 mg twice a day), a non-steroidal anti-estrogen for 5 years for estrogen receptor (ER) positive carcinomas. However based on age, stage of the disease and the condition of the patient, the above protocol is frequently altered.

**Chemotherapy**

Cyclophosphamide, Methotrexate and 5-Fluorouracil (CMF) or 5-Fluorouracil, Adriamycin and Cyclophosphamide (FAC) are the usually used chemotherapeutic agents in combination. Taxols and Etoposide form the alternate drug of choice in case of resistant tumours.
Hormonal Therapy

There are two types of endocrine therapy used for ER positive breast cancer. Anti-estrogens (Tamoxifen and Ralofexine) and Aromatase inhibitors (Anastrozole and Letrozole), inhibit aromatase, the enzyme that catalyzes the conversion of androstenedione to estrone. AIs are used to postmenopausal breast cancer patients.

Immuno-therapy

Trastuzumab (Herceptin®) is a monoclonal antibody produced against oncoprotein HER2-neu to inhibit tumour growth. Use of primed immune cells such as dendritic cells and lymphocytes are at the experimental stages.

Tamoxifen - In Breast Cancer Treatment

Breast cancer treatment and prognosis are based on the histological type, degree of differentiation, clinical stage and population of hormone receptors. Postmenopausal women account for approximately two-thirds of all breast cancers. Hormonal therapy for breast cancer for postmenopausal women includes Tamoxifen administration, in combination with chemotherapy or alone (Jaiyesimi et al., 1995).

Tamoxifen is a widely used drug that is known to prevent breast cancer growth. When breast cancer first arises, the cancer cells often continue to require estrogen to grow, just as their normal counterparts do. The requirement for estrogen provides an unparalleled opportunity to control the growth of the tumour. Tamoxifen acts by opposing the action of estrogens and is therefore termed an antiestrogenic. This property means that Tamoxifen will arrest estrogen-dependent breast cancer growth while other treatments, such as surgery
and chemotherapy, can be tried. Tamoxifen treatment for breast cancer has proven very successful.

Properties

Tamoxifen, code number ICI 46,474, is the trans-isomer of 1-[4-(2-dimethylamino-ethoxy)phenyl]-1,2 diphenyl-1-butene. The drug is sold as the monocitrate salt using the trade name “Nolvadex” in most countries. The molecular formula is C₆₀H₇₀NO.C₄H₈O₇ and the molecular weight is 563.6. Tamoxifen citrate is a fine, white, odourless, crystalline powder, slightly soluble in water and soluble in ethanol, methanol and acetone. The melting point of the free base is 96°-98°C and of the citrate is 140°-142°C. Tamoxifen can be converted into its anthracene derivative, on exposure to light, particularly to UV light.

Pharmacologic and Pharmacokinetic Properties

The compound administered to patients is trans-tamoxifen (as the citrate salt), because this isomer has higher affinity for estrogen receptors than the cis isomer (Langan Fahey et al., 1994). These receptors are nuclear transcription factors present in normal breast and other tissues and in 60 to 70 percent of breast cancers. The trans-tamoxifen has not only antiestrogenic but also estrogenic properties, depending on the species, tissue, and gene (Osborne et al., 1996). Drugs such as Tamoxifen are more properly referred to as selective estrogen receptor modulators, because of their multiple activities (Horwitz et al., 1996). The molecular basis for these properties is poorly understood, but the estrogen-agonist activity of Tamoxifen may explain its favorable effects on bone and serum lipid concentrations and its ability to stimulate the endometrium. Its
estrogen-antagonist activity in breast tissue accounts for its ability to inhibit tumour growth.

The major metabolites of Tamoxifen in humans are N-desmethyltamoxifen and trans-4-hydroxytamoxifen (Figure 5); the affinity of the latter for estrogen receptors is equivalent to that of 17β-estradiol (Buckley and Goa, 1989). The dimethylaminoethoxy side chain and the trans configuration are crucial for the antiestrogenic activity of Tamoxifen (Langan Fahey et al., 1994; Buckley and Goa, 1989); more highly estrogenic cis metabolites and metabolites without the side chain have been found in breast tumours, but their importance is unclear (Wiebe et al., 1992).

Tamoxifen is absorbed readily after oral administration. The serum half-lives of Tamoxifen and its major metabolites range from 7 to 14 days, permitting once-daily administration (Lien et al., 1991). The usual dosage is 20 mg per day. In long-term treatment, the steady-state concentrations of Tamoxifen and its metabolites in serum remain constant for as long as 10 years; reduced bioavailability is not a cause of acquired resistance to the drug (Langan-Fahey et al., 1990). Tamoxifen can be detected in serum for several weeks and in tumour tissue for several months after treatment is discontinued. As a result, for several months after Tamoxifen treatment is stopped, ligand-binding assays of estrogen receptors in tumour tissue can give false negative results because of receptor occupancy by the drug (Encarnacion et al., 1993). Tamoxifen undergoes extensive metabolism in the liver and is excreted predominantly in the feces.

**Mechanism of Action**

The antitumour effects of Tamoxifen are thought to be due to its antiestrogenic activity, mediated by competitive inhibition of estrogen binding to
Figure 5 Metabolism of Tamoxifen in animals and Humans
estrogen receptors (Osborne et al., 1996). As a consequence, Tamoxifen inhibits
the expression of estrogen-regulated genes, including growth factors and
angiogenic factors secreted by the tumour that may stimulate growth by autocrine
or paracrine mechanisms. The net result is a block in the G1 phase of the cell
cycle and a slowing of cell proliferation. Tumours may then regress because of
this altered balance between cell proliferation and ongoing cell loss. Tamoxifen
may also directly induce programmed cell death, apoptosis (Ellis et al., 1997).
This dynamic equilibrium of cell rejuvenation and death may ultimately be the
target for Tamoxifen. Moreover, Tamoxifen also expresses its antiproliferative
effect directed through other mechanisms such as inhibition of protein kinase C
(O' Brian et al., 1985) and its binding to calmodulin, a protein that plays a role in
DNA synthesis (Lam, 1984). An overview of Tamoxifen and its antiproliferative
actions are illustrated in Fig 6. and Fig 7 respectively (Wiseman et al., 1994 a,b).

Biochemical aspects of Tamoxifen

Apart from its antineoplastic activity and apoptotic activity, Tamoxifen
serves as an anti atherosclerotic agent (Thangaraju et al., 1994) and also offers
protection against bone metastasis (Love et al., 1992).

Side Effects

Cancer of the lining of the uterus (endometrial cancer) is more
common in women taking Tamoxifen. Women using Tamoxifen
should have yearly pelvic examinations and should be evaluated
further if they experience any abnormal uterine bleeding.
Tamoxifen produces overgrowth of the lining of the uterus
(endometrial hyperplasia), which can be a precancerous change.
Figure 7 An Overview of Tamoxifen

History
Emerged from a 1950s oral contraceptive programme

Pharmacology
Oestrogen antagonist and partial and full agonist

Toxicity
Low toxicity metabolism - related liver cancer in rats not shown in humans

Apoptosis

Chemistry
Triphenylethylene trans isomer

Current status
Used in the treatment of breast cancer and may be useful in the prevention of this disease

Side effects
Side effects of tamoxifen used in prevention of breast cancer?

Bad: risk of endometrial cancer, liver cancer and retinopathy

Good: Protection against cardiovascular disease and osteoporosis

Mechanisms of anticancer action
Oestrogen receptor antagonist
Enzyme inhibitor modulator of growth factors and membranes

Membrane mediated actions

Antioxidant
Cardioprotective action

Anti-MDR action
Anticandida and antiviral action
- Adjuvant Tamoxifen therapy also promotes hepatocarcinoma by forming DNA adduct of rat liver DNA.
- Tamoxifen causes an increased risk of blood clots in the legs (deep vein thrombosis) and the lungs (pulmonary emboli). Changes in the clotting factors have been reported in patients receiving Tamoxifen.
- Tamoxifen may also cause a small increase in the risk of stroke.
- Ovarian cysts occur more frequently in women who are taking Tamoxifen.
- Tamoxifen use causes an increased risk of cataract formation and the need for surgery for cataracts.
- Side effects of Tamoxifen may include hot flushes and vaginal discharge or irritation.
- Tamoxifen should not be used during pregnancy or breast-feeding.

Tamoxifen-induced Biochemical Derangements And Combinatorial Therapy

The introduction of Tamoxifen (TAM), a non-steroidal anti-estrogen in the early 1970's represented a landmark in the treatment of breast cancer (Clemons et al., 2002). Since 1990, death rates from breast cancer have decreased by over 25%, and this is at least a part due to adjuvant treatment with Tamoxifen (Rutqvist et al., 1995). Apart from its use in adjuvant treatment protocol, Tamoxifen has also shown to reduce the risk of contralateral breast cancers in carriers of BRCA 1 or BRCA 2 genes and in advance metastatic breast cancers (Narod et al., 2000).
Chemo, hormone and radiation therapies are accompanied by various deleterious side effects and biochemical alterations. Though Tamoxifen does not produce visible side effects as Chemo or radiation therapy (as nausea, vomiting, active infection, hair loss, etc...), its prolonged administration for a period of 5 years causes severe biochemical derangements such as hypertriglyceridemia, hypercalcemia, deep vein thrombosis in legs, increased risk of stroke and red blood cell hemolysis resulting in hemolytic anemia (Decensi et al., 2005 and Cruz Silva et al., 2001). Due to its estrogen-like action on liver and endometrium, there is an increased risk of hepatic and endometrial carcinoma.

Formulating the right combination of vitamins is of paramount importance in cancer therapy. This fact is supported by global epidemiological surveys, which indicate better prognosis for patients receiving combinatorial therapy than those receiving mono drug therapies (Marchioli, 1999). Combinatorial therapy (CT) of Coenzyme Q₁₀ (Co Q₁₀), Niacin and Riboflavin with Tamoxifen was tried in experimental animals, with satisfactory results from our laboratory (Perumal et al., 2005). This current study is an extension of the above work to find if the Combinational Therapy could ameliorate the biochemical derangements of sole Tamoxifen therapy in postmenopausal women with breast cancer.

**Riboflavin**

Riboflavin, also known as Vitamin B₂ or yellow enzyme, is an easily absorbed, water-soluble micronutrient with a key role in maintaining human health. Like the other B vitamins, it supports energy production by aiding in the metabolism of fats, carbohydrates and proteins. Vitamin B₂ is also required for red blood cell formation and respiration, antibody production and for regulating
human growth and reproduction. It is essential for healthy skin, nails, hair growth and general good health, including regulating thyroid activity.

![Structure of Riboflavin](image)

**Sources**

Lean meats, eggs, legumes, nuts, green leafy vegetables, dairy products and milk provide riboflavin in the diet. Breads and cereals are often fortified with riboflavin. Because riboflavin is destroyed by exposure to light, foods with riboflavin should not be stored in glass containers that are exposed to light.

**Pharmacokinetics**

In food, riboflavin is found mainly in the form of flavin mononucleotide (FMN, riboflavin-5'-phosphate) and flavin adenine dinucleotide (FAD). Riboflavin is used for food fortification. Riboflavin and riboflavin-5' -phosphate are the principal nutritional supplement forms of riboflavin, with riboflavin being the major form. Coenzyme forms of riboflavin (FAD, FMN) that are not covalently bound to proteins are released from proteins in the acid environment.
of the stomach. Covalently bound forms of riboflavin (e.g., in mitochondrial succinate dehydrogenase) are released from the proteins they are bound to following proteolysis. FAD and FMN are converted to riboflavin in the small intestine via the action of pyrophosphatase and phosphatase, respectively. Riboflavin is mainly absorbed in the proximal small intestine by a saturable transport system. The rate of absorption increases when riboflavin is ingested with food. The presence of bile salts appears to facilitate absorption of riboflavin. The maximal amount of riboflavin that is absorbed from a single oral dose appears to be about 27 milligrams. The amount of absorption of riboflavin-5-phosphate and FAD appears to be very low. During the process of absorption, riboflavin, in part, appears to be converted to FMN which is either used by the enterocytes for their metabolic requirements, or converted back to riboflavin for further processing. Riboflavin is transported via the portal circulation to the liver and by the systemic circulation to the various tissues of the body.

A large percentage of serum riboflavin is carried by immunoglobulins. Some serum riboflavin is carried by albumin. Riboflavin is transported into cells via facilitated diffusion at physiological concentrations, and by passive diffusion at higher concentrations. Within cells, riboflavin is converted to FMN via flavokinase. FMN is converted to FAD via FAD synthetase. FAD is the predominant form of riboflavin in tissues.

Very little riboflavin is stored in tissues. Riboflavin in excess of body requirements is excreted mainly by the kidneys. A number of riboflavin metabolites are also found in the urine, including 7-hydromethylriboflavin, 8-hydroxymethylriboflavin, 8 alpha-sulfonylriboflavin, 5'-riboflaviny1 peptide, 10-hydroxyethylflavin, lumiflavin, 10-formylmethylflavin and carboxymethylflavins. A
significant percentage of large intakes of riboflavin - greater than 30 milligrams in a single dose are excreted in the feces.

**Pharmacology and Biochemical functions**

Living organisms derive most of their energy from oxidation-reduction (redox) reactions, which are processes involving the transfer of electrons. Flavin coenzymes participate in redox reactions in numerous metabolic pathways (Imada, 2003). FAD is part of the electron transport (respiratory) chain, which is pivotal to energy production. In conjunction with cytochrome P_{450}, flavins also participate in the metabolism of drugs and toxins. Riboflavin is an antioxidant that works in conjunction with glutathione reductase which is an FAD - dependent enzyme that participates in the redox cycle of glutathione. Riboflavin is involved in regenerating glutathione, the main cellular protector against free radical damage. Riboflavin may also have anti-atherosclerotic activity secondary to its antioxidant action. It protects against lipid peroxidation and oxidation of low-density lipoprotein (LDL). Oxidized-LDL is thought to be a key etiological factor in the pathophysiology of atherosclerosis. Riboflavin deficiency has been associated with an increased incidence of esophageal cancer in certain parts of the world. Riboflavin supplementation has been found to reduce the incidence of esophageal cancer (Siassi and Ghadirian, 2005). The mechanism of the possible anticarcinogenic activity of riboflavin is also unclear.

**Niacin**

Niacin (vitamin B\textsubscript{3}) is a water-soluble vitamin required by all living cells. Niacin is the common name for both nicotinic acid and niacinamide (also called nicotinamide).
Food Sources

Good sources of niacin include yeast, meat, poultry, fish (e.g., tuna, salmon), cereals (especially fortified cereals), legumes, and seeds. Milk, green leafy vegetables, coffee and tea also provide some niacin (Jacob and Swenseid 1996). In plants, especially mature cereal grains like corn and wheat, niacin may be bound to sugar molecules in the form of glycosides, which significantly decrease niacin bioavailability (Gregory, 1998). The recommended dietary allowance is 6.6 mg per 1,000 calories, but no less than 15-20 mg per day.

![Structure of nicotinic acid](Image)

![Structure of nicotinamide](Image)

Pharmacokinetics

Both nicotinic acid and nicotinamide are efficiently absorbed from the stomach and small intestine. At low amounts, absorption is mediated by sodium-dependent facilitated diffusion. Passive diffusion is the principal mechanism of absorption at higher doses. Doses of up to three to four grams of nicotinic acid and niacinamide are almost completely absorbed. They are transported via the portal circulation to the liver and via the systemic circulation to the various tissues of the body. Nicotinic acid and nicotinamide enter most cells by passive diffusion and enter erythrocytes by facilitated transport.
Nicotinic acid and nicotinamide are metabolized through different pathways. Nicotinic acid is not directly metabolized to nicotinamide. It undergoes a number of metabolic steps to yield NAD⁺ which in turn can be converted to nicotinamide. Nicotinamide can be directly converted to nicotinic acid. In the liver, the principal catabolic product of high doses of nicotinic acid is the glycine conjugate of nicotinic acid called nicotinuric acid. The principal catabolic products of nicotinamide are N'-methyl nicotinamide, N'-methyl-5-carboxamide-2-pyridone, N'-methyl-5-carboxamide-4-pyridone and nicotinamide-N-oxide.

High doses of nicotinic acid are excreted in the urine as unchanged nicotinic acid and the glycine conjugate of nicotinic acid, nicotinuric acid. High doses of nicotinamide are excreted in the urine as unchanged nicotinamide, N'-methyl nicotinamide, N'-methyl-5-carboxamide-2-pyridone, N'-methyl-5-carboxamide-4-pyridone and nicotinamide-N-oxide.

**Pharmacology and Biochemical functions**

Niacin, via its metabolites, is involved in a wide range of biological processes, including the production of energy, the synthesis of fatty acids, cholesterol and steroids, signal transduction, the regulation of gene expression and the maintenance of genomic integrity (Virag and Szabo, 2002). Nicotinic acid, in pharmacological doses, is used as an antihyperlipidemic agent. It decreases blood cholesterol, LDL-cholesterol and triglyceride levels and raise HDL ("good") cholesterol levels. Through the cholesterol lowering effects, niacin protects against cardiovascular disease (Mortaza et al., 1997). Nicotinic acid significantly reduces the risk of death among those who have previously had heart attacks. Studies suggest that niacin may be a chemo-preventive compound.
that cannot cure cancer, but may help prevent it. NAD is important in modulating ADP-ribose polymer metabolism (Jacobson, 1993), cyclic ADP-ribose synthesis and stress response proteins, such as p53, following DNA damage; therefore understanding how NAD metabolism is regulated in the human has important implications in developing both prevention and treatment strategies in carcinogenesis (Jacobson et al., 1999; Ames, 2001).

**Coenzyme Q₁₀**

Coenzyme Q₁₀ (CoQ₁₀) belongs to a family of compounds known as ubiquinones. It is a lipid soluble quinone with a chemical structure similar to vitamin K. Most of CoQ₁₀ is synthesized by the cell and is mainly localized in the mitochondria and the nucleus (Ernster and Dallner, 1995). Ubiquinone contains a functional group known as a benzoquinone. Ubiquinones are fat-soluble molecules with anywhere from 1 to 12 isoprene (5-carbon) units. The ubiquinone found in humans, ubidecaquinone or coenzyme Q₁₀, has a "tail" of 10 isoprene units (a total of 50 carbons) attached to its benzoquinone "head" (Crane, 2001).

**Structure of Coenzyme Q₁₀**

![Diagram of Coenzyme Q₁₀ and related compounds](image-url)
Coenzyme Q can exist in three oxidation states: the fully reduced ubiquinol form (CoQH₂), the radical semiquinone intermediate (CoQH⁺), and the fully oxidized ubiquinone form (CoQ).

Sources

Coenzyme Q₁₀ is synthesized in most human tissues. Rich sources of dietary coenzyme Q₁₀ include mainly meat, poultry, and fish. Other relatively rich sources include soybean and canola oils and nuts. Fruits, vegetables, eggs and dairy products are moderate sources of coenzyme Q₁₀ (Mattila and Kumpulainen, 2001).

Pharmacokinetics

The total body content of CoQ has been estimated to be approximately 0.5-1.5 g, with the highest concentrations in heart, liver, adrenal, spleen, kidney and pancreas (Linn et al., 1959). Within cells, CoQ is largely located in the nucleus and in mitochondria and it is the protein bound form of CoQ that appears to be the active form of ubiquinone in the cell. Following oral administration of CoQ peak plasma levels are attained 5-10 hours later and the mean plasma half-life is 34 hrs (Tomono et al., 1986). Absorption is facilitated if the CoQ is administered along with lipid-carriers such as vegetable oil or Vitamin E. Once absorbed, CoQ is sequestered by chylomicrons and deposited largely in the liver as part of very low density lipoproteins (VLDL). Excretion of this fat-soluble compound is largely via the biliary tract and more than 50% of a dose is eventually eliminated in the feces.
Pharmacology and Biochemical functions

CoQ₁₀, due to the involvement in ATP [cellular energy] synthesis, affects the function of all cells in the body, making it essential for the health of all human tissues and organs. CoQ₁₀ particularly effects the cells that are the most metabolically active: heart, immune system, gingiva and gastric mucosa. It is believed by proponents that cancer patients lack CoQ₁₀ in their blood and few studies have shown low plasma CoQ₁₀ levels as an independent prognostic factor for disease progression (Oytun Portakal et al., 2000; Rusciani et al., 2006). There has been some evidence that CoQ₁₀ has efficacy when used in combination with other cancer treatments to ameliorate harmful side effects of those treatments. Folkers et al. (1993) reported several case histories of cancer patients with prolonged survival on therapy with CoQ₁₀. In line with the above findings supplementation with CoQ₁₀ can cause complete regression of tumours in advanced breast cancer, including one patient with numerous metastases to the liver (Lockwood, 1994). Mechanisms in cancer include immune system enhancement and antioxidant activity (Crane, 2007). Cardiac toxicity of the anthracyclines may be ameliorated by using low doses or concomitant treatment with CoQ₁₀ (Conklin, 2005).

Biochemical Changes in Cancer

Lipid and Lipoprotein Changes

Abnormal lipid synthesis or defective degradation of lipids is implicated in pathological conditions like diabetes, atherosclerosis and cancer (Ray and Husain, 2001). The exact mechanism by which lipid and lipoprotein contribute to carcinogenesis is unclear. However, reports suggest that lipid peroxidation products, malondialdehyde may cross-link proteins and DNA on the same and
opposite strands (Summerfield and Tappel, 1983). Earlier studies have reported that lipids might primarily affect gonads and subsequently higher estradiol secretion could influence the development of malignancies in the mammary glands and lymphoid system (Szepsenwol, 1966). Tamoxifen's effect on plasma lipids and lipoproteins, one of our earlier studies along with others has indicated that Tamoxifen increases VLDL-C levels resulting in hypertriglyceridemia (Thangaraju et al., 1994; Liu and Yang, 2003). High triglyceride levels are proved to be independent and statistically significant risk factor for the development of cardiovascular complications (Austin et al., 1998).

**Lipid Peroxidation and Antioxidant Defenses**

During last two decades, a considerable attention has been focused on the involvement of free radicals in various diseases. ROS are produced by a number of processes *in vivo* and are highly reactive and toxic. An imbalance between the production and detoxification of ROS results in oxidative stress. ROS has been implicated in the pathogenesis of certain diseases, including cancer. ROS reacts with polyunsaturated fatty acids to induce the release of toxic and reactive aldehyde metabolites such as malondialdehyde (MDA), one of the end products of lipid peroxidation (LPO). MDA may be involved in tumour promotion because it can interact with functional groups of a variety of cellular compounds (Vaca, 1998). However, biological system has evolved an array of enzymatic and non-enzymatic antioxidant defense mechanisms to combat the deleterious effects of ROS. Superoxide dismutase (SOD) catalyses the dismutation of the $O_2^-$ into $H_2O_2$. $H_2O_2$ is metabolized by catalase and glutathione peroxidase (GPx) is reduced into water and molecular oxygen. GPx reduces $H_2O_2$ and organic peroxides while oxidizing glutathione (GSH).
Oxidized glutathione. GSSH is reduced back to GSH by glutathione reductase (GRx) in the presence of NADPH (Bakan. 2003).

GLYCOPROTEINS

Glycoproteins play a key role in mediating cell surface function, such as cell-cell recognition, cellular adhesion, antigenicity, intracellular processing of proteins, cell activation and ability of cancer cells to metastasis (Sell, 1990) hence, they may be considered as tumour markers. Elevated levels of serum hexose, hexosamine and sialic acid have been observed in breast cancer condition (Romppanen. 1997). Sialic acid is reported as a sensitive marker in cancer condition and is useful in diagnosis, assessment of prognosis and staging of the breast carcinoma (Patel et al., 1990). Sialic acid levels have also correlated with the tumour burden, degree of metastasis and recurrence (Wang, 2002).

MARKER ENZYMES

Cathespin-D is an ubiquitous aspartyl endoprotease which is involved in normal protein degradation within lysosomes. Montcourrier et al. (1990) have hypothesized that the breast cancer patients make an abnormally processed cathepsin-D, which is secreted rather than transported to the lysosome. An increased expression of cathepsin-D was observed in malignant breast tissue when compared with normal breast tissue and has been reported to be an independent prognostic factor (Lah et al., 1995). LDH is a tetrameric enzyme, recognized as a potential tumour marker in assessing the progression of the proliferating malignant cell (Subhash et al., 1993). The elevated activity of LDH may be due to the over production by the tumour cell (Helmes et al., 1998), or it may be due to the release of isoenzymes form destroyed tissues (Mirmomeri
et al., 1979). Any disturbances or strains rendered to the liver cells break the membrane and the cell organelles leading to the leak of the liver enzymes (ALP, AST and ALT) into the circulation. Thus, these marker enzymes under cancer condition talks about the stage and prognosis of the disease.

**CANCER MAKERS**

Detecting and / or monitoring changes in circulating tumour markers might assist in evaluating cancer risk, diagnosis, prognosis, response to treatment, or recurrence. CEA and CA 15-3 are the classical tumour-associated antigens frequently used tumour markers in breast malignancies (Molina, 1998). CA 15-3 is a breast -associated antigen encoded by the MUC-1 gene. In mammary gland, MUC-1 has a relative molecular mass of 250-500 kDa and contains approximately 50% carbohydrates by weight. CEA is also a glycoprotein of about 180-200 kDa molecular weight. CEA and CA 15-3 have been found to play an important role as adhesion molecules in cancer cells and their presence in tumour cell membrane has been considered to be responsible in increasing the tumour cell dissemination (Yoshioka, 1998). Their usefulness in early diagnosis of relapse and follow-up are well accepted (Kumpulainen, 2002).

**HEMATOLOGICAL PARAMETERS**

Tamoxifen induces multiple cellular adverse effects, including changes in normal discoid shape of erythrocytes with formation of stomatocytes, meaning that the drug may insert in the inner leaflet of the erythrocyte membrane. Tamoxifen changes the lipid-protein interaction in the bi-layer and the framework of erythrocyte cytoskeleton protein and plasma membrane causing hemolytic anemia (Cruz Silva, 2001). Tumour anemia is a common symptom in cancer patients, particular in those receiving chemotherapy (Skillings, 1993).
Anemia in cancer patient could lead to impaired tumour oxygenation with subsequent radio-and chemo-resistance (Bush, 1986).

**Membrane ATPases**

The normal erythrocyte's internal composition of sodium, potassium, calcium, magnesium etc., is maintained by energy requiring mechanisms mediated by adenosine triphosphatases, which are membrane bound. The membrane regulation of cellular cation homeostasis by providing a permeability barrier and the proper milieu for various transport pathways (Ranganathan Rao, 1980).

A number of membrane proteins, which are involved in solute transport, have been investigated in great details which includes, sodium-potassium ATPase; magnesium ATPase, etc. Sodium-potassium ATPase or sodium-potassium pump is essential for the transport of glucose and aminoacids. It hydrolyses adenosine triphosphate (ATP), only when sodium and potassium ions are present in addition to magnesium ions, which are tightly bound to the membrane. Translocation of sodium and potassium ions by this phosphatases is coupled with the hydrolysis of ATP. It maintains high concentration of potassium and low concentration of sodium inside the cell (Ranganathan Rao, 1980). Various factors are responsibe for the altered legels of ATPases. Among, lipid peroxidation; membrane fluidity; and extra cellular calcium are important determinant factors.

**Cancer and Mineral Metabolism:**

Tissues undergoing neoplastic transformations may affect calcium and magnesium metabolism in several ways. Malignancy may occur in glands, that
normally synthesize hormones such as parathyroid or thyroid glands, thus leading to the consequences, expected from supra physiological dosages of the respective hormones. Neoplastic involvements of non-endocrine organs might also lead to production of substances that are capable of influencing calcium metabolism (Albright. 1941). Generally, mammary carcinoma is associated with hypercalcemia in the properties of 30-40 % patients (Hickey et al., 1981), chiefly due to bone metastases, which develops steeply in breast cancer progressions. The disturbances in calcium metabolism is due to differential involvement of calcium regulatory hormone called calcitonin (Sherwood et al., 1967).

**DNA Damage**

![in human cell?](image)

The amount of DNA fragmentation is significantly increased in cancer. The single-cell gel electrophoresis technique is used to access the DNA strand breakage in a single cell. It is based on the alkaline lysis of the labile DNA at sites of damage (Speit, 2005). The unwound, relaxed DNA is able to migrate out of the cell during electrophoresis and can be visualized by ethidium bromide staining. Cells that have accumulated DNA damage appear, as fluorescent comet with tails of DNA fragments, where as normal, undamaged DNA does not migrate far from the cell origin. The role of DNA damage/repair in breast cancer susceptibility has been well documented (Smith et al., 2003).