Breast Cancer - Diagnosis and Treatment Prolonging Life: A Review

Susmitha Kasina, Hemant K.S. Yadav* and H.G. Shivakumar

Department of Pharmaceutics, JSS College of Pharmacy, JSS University, Mysore, Karnataka – 570015, India

Abstract: Breast cancer is a malignant tumour that starts either in the ducts or lobules, this can be generally differentiated as either in situ or invasive (in filtering) type. It is expected that in 2014 every 1 in 8 women are likely to develop invasive breast cancer during their lifetime when compared to a decade back where an average of 1 in 10 was seen. With this increase, breast cancer alone, roughly accounts for 25 to 30% of new cancer cases this year. Despite such diagnostic statistics, there are millions of survivors across the globe and this increasing rate can be attributed to the tremendous increase in advances in treatment and also early diagnosis. New drug delivery carriers like nanoparticles, liposomes, monoclonal antibodies, etc. are being used to improve the efficacy of therapy and for site specific delivery to reduce side effects. As a result of the constant effort by researchers, today the commercial market has a range of products apart from the conventional dosage forms like Herceptin (trastuzumab), a monoclonal antibody; the others in this class are Pertuzumab (Perjeta), Kadcyla (ado-trastuzumab emtansine) used for targeted delivery; Myocet (doxorubicin), a liposomal formulation and Paclitaxel nanoparticles all these are available as injections *via* intravenous route or infusion in few cases. There are yet certain other promising technologies like magnetic nanoparticle hyperthermia and cMethDNA assay a very assuring method to monitor recurrence of breast cancer by a simple blood test. This review will focus on the description of disease, diagnosis, current treatment therapies and ongoing research to provide better facilities.

Keywords: Breast cancer, diagnosis, advancements, treatment, trials.

1. INTRODUCTION

Breast cancer is the second most common cancer in the world with around 1.7 million new cases diagnosed in 2012 [1]. The estimated new cases of breast cancer in 2014 (females) in the United States is 232,670 [2], in Australia 15,270 [3] and about 50,000 in the United Kingdom. It is said that 1 in 8 women would be diagnosed with breast cancer during their life time in 2014 [1] and the increase in risk increases with age.

Breast cancer is a malignant tumour occurring in breast cells. This tumour starts either from the lobules, the milk producing glands or ducts, the passages to drain milk; rarely do they develop from the stromal cells which are the connective tissue of breast [4]. The types of breast cancer are broadly classified into invasive and non-invasive types. The breast cancer which starts in ducts and/or lobules of breasts and remains in the same site is called non-invasive breast cancer. The tumours which spread to other tissues of breast breaking through the ducts or glands where they originated are called infiltration or invasive type of breast cancer. Sometimes these tumours travel through the reticuloendothelial system to other parts of the body and are called metastatic tumours [3, 4].

*Address correspondence to this author at the Dept. of Pharmaceutics, JSS College of Pharmacy, JSS University, S.S Nagar, Mysore-570 015, India; Tel: +91-9886112637; Fax: 0821-2548359; E-mail: haisunny2@yahoo.co.in

Breast cancer can be histologically classified as follows [5]:

1. Ductular Carcinoma
   - *In situ*
   - Invasive
   - Tubular Carcinoma of the Breast
   - Medullary Carcinoma of the Breast
   - Mucinous Carcinoma of the Breast
   - Papillary Carcinoma of the Breast
   - Cribriform Carcinoma of the Breast
2. Lobular Carcinoma
   - *In situ*
   - Invasive [6]
3. Inflammatory Breast Cancer
4. Paget's Disease of Nipple
5. Phyllodes Tumours of Breast [7]
6. Recurrent and Metastatic Breast Cancer
7. Male Breast Cancer

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2. SIGNS AND SYMPTOMS

The earliest stages of breast cancer generally shows no symptoms but as the tumour progresses there can be signs. The detection can generally be from a lump or mass in the breast or in armpit, which maybe painless or with pain. Other signs include [8, 9]:

- Change in the size and shape of breast
- Redness, swelling or increase in temperature as seen in inflammatory breast cancer.
- A change in nipple either retraction, dimpling or ulceration. Scaling is seen in Paget's disease.
- Nipple discharge other than milk like clear, watery or serous or bloody fluid [10].

3. RISK FACTORS CAUSING BREAST CANCER

Risk factor is the chance of getting the disease. Presence of one or more risk factors doesn't mean that women will be attacked by the disease, except for the fact that the person is a woman and she ages every year no other factor has explainable effect. With the changes in lifestyle there will be a change in the effect of risk factor, this is said so because sometimes women with no risk factor procure the disease [11].

The most common risk factors are:

1. Women are 100 times more prone to breast cancer than men.

2. The chance of acquiring the disease increases with increase in age as shown in Table **1**.

Table 1: Risk of Developing Breast Cancer in Women from 2008 to 2010 [2]

Age interval (years)	Probability (%) of developing invasive breast cancer during that interval
Birth to 49	1.9 (1 in 53)
50 to 59	2.3 (1 in 43)
60 to 69	3.5 (1 in 29)
70 and older	6.5 (1 in 15)
Birth to death	12.3 (1 in 8)

3. Genetic Factors: Women having a first degree relative with breast cancer increases the risk by 1.5 to 3 times. More the number of first degree relatives affected more will be the risk. Women with cancer in one breast have higher risk of developing in another breast or recurrence in the same breast.

The BRCA1 gene on chromosome 17 and BRCA2 gene on chromosome 13 increase the risk of breast cancer. These genes account for about 30% of inherited breast cancer [12]. Other genes associated with breast cancer are TP53, CHK2, PTEN, ATM etc. [13].

It has been found that women with dense breast tissue that is, has more glands and less fat have higher risk (2 to 6 times) than women of same age with less dense breasts. Dense tissue can also make detection of deformities in the mammogram difficult for the doctors [14].

4. Endocrine Factors: Early menstruation that is before the age of 12 years and late menopause, after 55 years increases the risk of developing breast cancer. Nulliparous women or women with late first pregnancy (after 30 years) have a higher risk of developing breast cancer. The period between onset of menstruation and first pregnancy is called "Window of initiation" which is the time where the unbalanced hormones react with highly responsive breast tissue and hence the overall risk increases [15].

Postmenopausal Hormone Replacement Therapy is contradicted by doctors in women with familial history of breast cancer. Although it was found that oestrogen alone doesn't increase the risk [16] when oestrogen and progesterone are given as a combination incidence of breast cancer was higher; these were diagnosed at an advanced stage [17].

A consistent relationship between oral contraceptives and breast cancer was not seen. However, data have been obtained for use of oral contraceptives containing low hormone doses and in newer formulations that their usage has no risk of breast cancer [18].

5. Environmental and Life style factors: Dietary fat intake has a relationship with steroid hormone metabolism and this relation is more prominent in postmenopausal women. A low-fat diet is linked to lower oestrogen levels, as levels of oestrogen influence growth of breast cells, it influences tumour growth. Some food products which have a heterocyclic amine ring like oestrogen mimic the hormones action on

oestrogen receptor [19]. In case of obese women with upper body obesity increases the risk of breast cancer compared to overall obesity [15]. Alcohol is the only dietary factor which showed a dose-response relationship with breast cancer. Irrespective of the type of alcohol consumed, increase in alcohol consumption increases the risk [15].

4. PREVENTION OF BREAST CANCER

Some changes in lifestyle probably can help reduce the risk of breast cancer. A few suggestions are:

i Since alcohol and breast cancer have a close link restricting the consumption of alcohol would help decreasing the risk.

ii Being obese and particularly gaining weight after menopause increases the risk. Hence, exercise and weight maintenance in accordance to one's height becomes important.

iii Studies show that women who have a low fat diet have a lower risk of breast cancer apart from maintaining their weight.

iv Early detection of breast cancer can be the easiest way to prevent it. Annual mammograms are recommended for women above the age of 40 years [11].

5. SCREENING AND DIAGNOSIS

The term **screening** refers to testing asymptomatic individuals for detecting the disease. Four types of screenings are used in case of breast cancer, they are:

a. Mammograms

b. Clinical breast examination

c. Breast self-examination

d. Magnetic Resonance Imaging (MRI)

Mammograms: A mammogram is an X-ray of the breast. Two types of mammograms are taken depending on the purpose 1) Screening mammogram is taken to detect any abnormalities in asymptomatic individuals 2) Diagnostic mammogram is taken to check the abnormal result of screening mammogram.

A mammogram is taken by pressing the breast between two plates to spread and flatten the tissue. This pressure lasts for a few seconds and the image is recorded. The plates are then repositioned to take another image. The entire process takes about 20 minutes. Although painful it is effective in specifically detecting the type of tumour [11].

Clinical breast examination: Examination of area under the arms and breast by a doctor, medical practitioner, nurse or any such qualified health expert is called clinical breast examination [11].

Breast self-examination: It is said that every woman must know how her breast would look and feel normally, so that once a month they examine themselves to prevent breast cancer. This must be done when breasts are not tender or swollen so that any abnormality can be reported to a doctor. 70% of breast cancers can be detected by self-examination and then there is 98% survival rate when diagnosed within 5 years. Since 8 out of 10 lumps observed through self-examination are not cancerous, there is no need to panic [11].

Magnetic Resonance Imaging (MRI): MRI scans use magnets and radio waves to produce detailed cross-sectional images. A person has to lie on a flat platform with head facing downwards in an enclosed space. Each breast has an opening avoiding the need to apply pressure. The contrast material is injected into vein for better imaging. This takes a long time, often up to an hour [11].

Of all the available screening techniques, MRI scan is the most sensitive technique where 79.5% of invasive breast cancers were detected. Although its specificity is less compared to mammograms, its overall discriminating capacity is higher than mammograms [20].

Diagnosis is defined as the process of evaluating or reviewing a lab report to determine the nature and cause of injury. After the screening, if any abnormality is detected either in a mammogram or MRI or if a lump is felt in the breast, then a biopsy is done. The need for performing a biopsy could be eliminated if it's found that the abnormality in the mammogram is due to a cyst and not solid tumour, this can be detected using a breast ultrasound.

Breast Ultrasound: Ultrasound uses sound waves for outlining the body. The echoes of these waves are collected and image is created on screen. Breast ultrasound is performed by applying gel on the spot which was detected abnormal in a mammogram and a

small microphone like instrument is placed to collect waves. This eliminates the need to do biopsy if it's found that the abnormality is due to a cyst (fluid sac) and not a solid tumour [11].

Biopsy: Biopsy refers to removal and examination of living tissue for precise diagnosis. So a biopsy is done as final confirmation of presence of tumour; that is, when all other tests show positive results. Different types of biopsies are performed on breast [11, 21]:

1. Fine needle aspiration (FNA) biopsy: It uses a very fine, hollow needle (thinner than one used for blood tests) to remove fluid or cysts from the lump. The needle is guided by feeling the lump or using an ultrasound. As the sample removed is very less the results may not be clear sometimes.

2. Core needle biopsy: The needle used for this test is larger than FNA biopsy, hence can remove more tissue and clearer results can be obtained.

3. Vacuum Assisted Biopsy: A small cut is made after numbing the skin and a probe is inserted through the cut. A piece of tissue is sucked using special systems like Mammotome or ATEC (Automated Tissue Excision and Collection). Many samples are taken from the same incision. More tissue is removed in this biopsy.

4. Surgical biopsy: In this biopsy the whole lump is removed surgically sometimes along with normal cells and is analysed under a microscope. Rarely this procedure is followed.

5. Lymph node biopsy: After examination of armpit region if an enlarged or swollen lymph nodes are felt then they are biopsied to check for tumour. Along with chest biopsy lymph node biopsy is also performed.

Lab Exams of Breast Tissue

After determining whether the tissue removed during biopsy is cancerous or benign, further tests are done if it's cancerous. These tests help in indicating the stage of disease, the quickness of the growth and also the most effective treatment can be determined. Lab tests are conducted for the following [21]:

1. Breast cancer grade: This test helps determine the prognosis of disease. The growth is scaled from 1 to 3 where lower number indicates slower growth and higher number indicates faster growth.

2. Hormone receptor status: Hormone receptors for oestrogen and progesterone occur in some breast cells which help aid in growth of the cells. These biopsied tissue is studied for the presence or absence of these receptors and is mentioned as hormone receptor positive or specifically ER-positive, PR-positive. The positive cells grow slowly and they respond to hormone therapy during therapy. 2 out of 3 breast cancers have at least one receptor.

3. HER2/neu status: HER2/neu is a protein and 1 out of 5 tumours have excess of this protein. When this protein is in excess it is called HER2/neu-positive. These cancers grow very fast unless the treatment targets this receptor.

4. Tests of gene patterns: Recurrence of cancer and need for additional treatment can be decided by studying number of gene patterns simultaneously. Two tests are available now namely, Oncotype DX [22] and MammaPrint [23].

6. TREATMENT OF BREAST CANCER

Several factors like stage and biological characteristic of disease; age, preference and general health of patient; risks and benefits with treatment are considered before deciding the treatment for cancer [21]. Various types of treatments available for breast cancer are:

1. Local therapy:
   a. Surgery
   b. Radiation therapy
2. Systemic therapy:
   a. Chemotherapy
   b. Hormone therapy
   c. Targeted therapy (Biological therapy) [24].

Apart from this adjuvant therapy or neoadjuvant therapy are given to patients. The additional treatment given to patients who have no detectable cancer after a surgery is called adjuvant therapy. The aim of adjuvant therapy is to prevent recurrence of cancer by killing any

cancer cells present but remain undiagnosed and asymptomatic. Some patients undergo chemotherapy or hormone therapy before a surgery, this is called neoadjuvant therapy. The goal of neoadjuvant therapy is to shrink the tumour size and make the surgery less extensive [24].

Surgery

Most women undergo some type of surgery either in the breast or lymph nodes depending on the stage of breast cancer. Surgery is combined with any of the other systemic therapies or radiation therapy to assure that cancer cells are completely removed [21]. Mastectomy and breast-conserving surgery are performed on patients with breast cancer.

Breast-conserving surgery (BCS) involves the removal of a part of the breast tissue along with a rim of normal tissue. This is also called as partial (or segmental) mastectomy, lumpectomy or quandrantectomy. Depending on the size of the tissue removed the shape of the breast will change and this can be corrected by reconstruction with an implant or tissue from any other part of the body [11, 21].

Mastectomy involves removal of the entire breast. If the breast alone without any lymph nodes is removed then it's called simple or total mastectomy. Removal of the entire breast along with lymph nodes under the arm, but not the breast muscle is called modified radical mastectomy and when breast muscle is also removed it's called radical mastectomy. Radical mastectomy is rarely performed as the spread of cancer cells to the breast muscle is uncommon [11, 21].

In mastectomy or BCS generally lymph nodes are also removed to check for the spread of cancer. It is considered that if cancerous cells are present in the lymph nodes, then the disease is spread systemically and further treatment can be decided. Removal of lymph nodes can be done in two ways:

1. Axillary lymph node dissection: This involves removal of 20 to 40 lymph nodes at the same time of the mastectomy or BCS or can be performed as a separate surgery. It was the traditional way to check the spread of disease, now it is seldom used [11, 21].

2. Sentinel lymph node biopsy: This test is a way to know the spread of cancer without removing so many lymph nodes as the above case. For this biopsy, a radioactive substance or a blue dye is injected near the tumour. This is carried by the lymphatic system and the first lymph nodes, which receive the substance are the sentinel lymph nodes. These nodes are directly connected to the tumour and hence probability of spread of cancer can occur from here. If these nodes don't contain cancer cells then further lymph node surgery isn't required [11, 21].

Disadvantages: The disadvantage of surgery is lymphedema, which is due to the removal of lymph nodes rather than due to the removal of breast tissue. Axillary lymph node dissection causes greater lymphedema than sentinel lymph biopsy, but the extent of the benefit of biopsy is yet to be determined [25].

Radiation Therapy

Radiation therapy uses high energy beams or particles to kill cancerous cells. Radiation therapy is generally given after the BCS, mastectomy or even in conjugation with other therapies like chemotherapy, targeted therapy, etc., and infrequently it is used to diminish the tumours before surgery [21]. Radiation therapy may be externally or internally administered.

External beam radiation is exposing the breasts wholly to the rays. It is done five days a week for 5 to 6 weeks and shortening this period due to advancement is called accelerated breast irradiation [11].

Internal radiation therapy is called brachytherapy, which uses radioactive material sealed in needles, wires or catheters. They are inserted into the tumour or near the tumour. The most commonly used internal therapy is intracavitary brachytherapy and is only a five days treatment.

Disadvantages: Swelling and heaviness of the breast along with sunburn like skin is the common side effect in case of external irradiation. Redness, infection and breakdown of area fat tissue are seen in internal irradiation. Weakening of ribs and fractures are the side effects of radiation therapy [11].

Chemotherapy

Chemotherapy is the use of drugs to inhibit and kill cancer cells. These drugs are administered orally or as injections. Since they enter the blood stream, they kill cancer cells, if any present systemically. But the disadvantage of this therapy is the death of normal cells along with cancer cells, causing side effects like

Table 2: Commonly Used Drug Combinations

FEC – Fluorouracil 500 mg/m^2 IV, day 1 Epirubicin 100 mg/m^2 IV, day 1 Cyclophosphamide 500 mg/m^2 IV, day 1 Repeat cycle every 21 days for 6 cycles.	**TAC (BCIRG 001)** – Docetaxel 75 mg/m^2 IV, day 1 Doxorubicin 50 mg/m^2 IV, day 1 Cyclophosphamide 500 mg/m^2 IV, day 1(Doxorubicin should be given first) Repeat cycles every 21 – 28 days for 6 cycles.
AC - Doxorubicin 60 mg/m^2 IV, day 1 Cyclophosphamide 600 mg/m^2 IV, day 1 Repeat cycles every 21 days for 4 cycles. **AC followed by Paclitaxel (CALGB 9344)** - after completion of above cycle Paclitaxel 175 mg/m^2 IV over 3 hours. Repeat cycles every 21 days for 4 cycles.	**CAF** – Cyclophosphamide 600 mg/m^2 IV, day 1 Doxorubicin 60 mg/m^2 IV, day 1 Fluorouracil 600 mg/m^2 IV, day 1 Repeat cycles every 21 – 28 days for 6 cycles.
CMF – Cyclophosphamide 100 mg/m^2 per day orally, days 1 – 14 Methotrexate 40 mg/m^2 IV, day 1 and 8 Fluorouracil 600 mg/m^2 IV, day 1 and 8 Repeat cycles for every 28 days for 6 cycles. Oral cyclophosphamide can be replaced by 600 mg/m^2 IV, day 1.	**FAC –** Fluorouracil 500 mg/m^2 IV, day 1 and 4 Doxorubicin 50 mg/m^2 IV, IV continuous infusion over 72 hrs Cyclophosphamide 500 mg/m^2 IV, day 1 Repeat cycles every 21 – 28 days for 6 cycles.

loss of hair, nausea, higher risk of infection and stopping of menstrual cycle [11]. It is considered that a combination of drugs is more effective than using a single drug. Chemotherapy will be most effective when the dosage regimen is followed strictly [21].

Commonly used combinations and their doses are given in Table **2** [15].

Hormone Therapy

Hormones act as chemical messengers in the body. Oestrogen and progesterone aid in the growth of breast cells [26]. The growth of hormone receptor-positive cells, a type of breast cells can be regulated by either blocking these hormones or by lowering their levels. Hormone therapy focuses on this aspect of cancer cells atopsosis.

Oestrogen blockers are most commonly used in hormone therapy. Drugs like Tamoxifen are used commonly up to five years after surgery. In postmenopausal women, Aromatase Inhibitors (AIs) are used to decrease the production of oestrogens because after menopause, fat produces oestrogen and not ovaries; hence these drugs inhibit the conversion of fats to oestrogens. In premenopausal women drugs which inhibit ovaries will affect estrogen production also hence luteinizing hormone releasing hormone analogues (LHRH analogues) are used. LHRH analogues use can be replaced by surgical removal of ovaries too [11].

Targeted Therapy or Biological Therapy

Drugs used in this therapy act specifically on the tumour or use body's immune system to act on the cancer cells to kill them [21]. HER2/neu protein is the drug target for targeted therapy; other targets are still under consideration. Monoclonal antibodies like Trastuzumab, Pertuzumab along with many others are commonly used for targeting. Apart from these, drugs targeting bones are also used as breast cancer easily spreads to the bones and causes damage. A Bisphosphonate class of drugs is most commonly used for treating breast cancer spread to bones, apart from it denosumab is also used commonly [11].

7. FDA APPROVED DRUGS FOR BREAST CANCER

The following is list of drugs approved by FDA for treatment of breast cancer [27]:

- Paclitaxel
- Methotrexate
- Doxorubicin hydrochloride
- Fluorouracil
- Everolimus
- Anastrazole
- Capecitabine
- Cyclophosphamide
- Epirubicin hydrochloride
- Exemestane
- Fulvestrant
- Gemcitabine hydrochloride
- Goserelin acetate

- Trastuzumab
- Ixabepilone
- Lipatinid ditosylate
- Letrozole
- Megestrol acetate
- Pertusumab
- Pamidronate disodium
- Toremifine
- Tamoxifen citrate

Apart from these drugs some other drugs and their combinations are under study for their effectiveness. For example a study included 55 women with advanced breast cancer and were treated using a combination of Adriamycin (40 mg/m^2 IV, 1 day) and cyclophosphamide (200 mg/m^2/day orally for 4 days on days 3-6). This combination proved to be safe and effective in outpatients with advanced breast cancer [28]. Many other such studies have been performed to determine the best suited regimen for various stages of breast cancer.

8. ADVANCEMENTS IN THE AREA OF DIAGNOSIS AND THERAPY

8.1. Approvals by FDA

The drugs approved by FDA have been prepared in novel drug delivery systems so that the efficacy of the drug is increased and side effects are decreased. The recent additions by FDA to approved drugs for use in breast cancer in their order of decent up to 2010 are:

- Approval of doxorubicin hydrochloride liposomal injection (October, 2013).
- Approval for the use of Pertuzumab injection (PERJETA, Genentech, Inc.) in combination with trastuzumab and docetaxel for neoadjuvant therapy in various stages of breast cancer (September, 2013).
- Approval for use of ado-trastuzumab emtansine (KADCYLA for injection, Genentech, Inc.), as a single agent for the treatment of patients with HER2-positive, metastatic breast cancer who previously received trastuzumab and a taxane, separately or in combination (February, 2013).
- Approval of Everolimus tablets (Afinitor®, Novartis Pharmaceuticals Corporation) for the postmenopausal women treatment with advanced hormone receptor-positive, HER2-negative breast cancer in combination with exemestane, after failure of treatment with letrozole or anastrozole (July, 2012).
- Approval of Pertuzumab injection (PERJETA, Genentech, Inc.) for use in combination with trastuzumab and docetaxel for the treatment of patients with HER2-positive metastatic breast cancer who did not receive prior anti-HER2 therapy or chemotherapy for metastatic disease. Pertuzumab is a humanized recombinant monoclonal antibody which targets the extracellular dimerization domain (Subdomain II) of HER2, and hence blocks the ligand-dependent heterodimerization of HER2 with other HER family members (June, 2012).
- Granted approval of using Eribulin Mesylate (Halaven Injection, Eisai Inc.) in treating patients with metastatic breast cancer who previously received an anthracycline and a taxane in either in adjuvant or metastatic stage, and using at least two chemotherapeutic agents for the treatment of the metastatic disease (November, 2010).
- Granted accelerated approval to Tykerb (lapatinib) tablets to be used in combination with letrozole tablets for the treating postmenopausal women with hormone receptor positive metastatic breast cancer along with over-expression of HER2 receptor for whom hormonal therapy is indicated (January, 2010) [29].

8.2. Clinical Trials

Many drugs have been modified chemically or the delivery system is modified to generate efficient therapy, clinical trials of these drugs in various stages along with new combination of drugs for different stages of breast cancer are going on. An attempt to summarize these trials is made here in Table **3** [30].

8.3. Novel Drug Carriers for Treatment and Diagnosis of Breast Cancer

Nanoparticles

Quantum dots are fluorescent nanoparticles, which are used in biomedical diagnosis, the fluorescence is

Table 3: Ongoing Clinical Trials for Treatment of Breast Cancer

Trial Phase	Drug or drug combination	Need for study	Stage of breast cancer
I	Mifepristone and Eribulin (Havalen®)	Decide the dose of Mifepristone and to determine its effect with Eribulin.	Advanced triple negative breast cancer.
II	Dacomitinib	Evaluation of safety, efficacy and CNS pharmacokinetics	HER2+ breast cancer patients with progressive brain metastasis
I - II	KD01, investigational drug	Determination of safety, efficacy and maximum tolerated dose	HER2+ breast cancer patients with progressive brain metastasis in patients treated with Herceptin
I	AMP-514, a monoclonal antibody targeting PD-1	Evaluation of safety, efficacy and pharmacokinetics	Solid tumour
III	Docetaxel (Taxotere®), Carboplatin (Paraplatin®), Trastuzumab (Herceptin®), Pertuzumab (Perjeta®), (TCHP), Goserlin acetate and AIs	Effect of TCHP treatment alone or with AIs as neoadjuvant therapy.	ER+, HER+ Stage I-III tumours.
Pilot study	Propranolol Hydrochloride	Effect of drug on growth of breast cells.	Locally recurrent or metastatic breast cancer.
II/III	Abraxane, Gemzar and Paraplain	Comparing safety and efficacy of combinations.	Triple negative metastatic breast cancer.
II	Cisplatin, Doxorubicin (Andriamycin®) and Cyclophosphamide	Efficacy of cisplatin in treating breast cancer.	BRCA+ women and men with newly diagnosed breast cancer.
I-II	Azacitidine (Vidaza) and Nab-Paclitaxel (Abraxane®)	Safety and effectiveness of combination	Advanced or metastatic HER2-negative breast cancer.

measured to detect tumours. These quantum dots are conjugated with antibodies in breast cancer to target the tumour for specific diagnosis. Carbon nanotubes are being used as delivery systems and also sentinel lymph node visualization. Albumin bound nanoparticles of paclitaxel [31-33] and polymeric nanoparticles of Tamoxifene offer advantages of an overall decrease in toxic effect [34, 35].

Magnetic Nanoparticles

Magnetic nanoparticles have varied applications in breast cancer like imaging, sentinel lymph node biopsy, drug delivery and magnetic hyperthermia as shown in Table **4** [36]. The preparations include use of magnetic iron oxides Fe_3O_4 (magnetite) and γ-Fe_2O_3 (maghemite).

Magnetic nanoparticle hyperthermia is a minimal invasive technique to treat non-invasive breast cancer and multifocal tumours. The magnetic nanoparticles are coated with dextran to allow free flow in lymph. Dextran also provides as a base for attaching antibodies or other targeting material. These particles are activated by alternating magnetic field applied externally, they absorb energy which is converted into heat required to kill the tumour cells [36-40].

Gold Nanoparticles

Recently gold nanoparticles have been used for diagnosis and treatment of breast cancer. Gold nanoparticles have proved to enhance the proliferation and apoptosis of cancer cells when paclitaxel is used as chemotherapeutic agent. Gold nanocages are used for molecular imaging test for Optical Coherence Tomography, these when conjugated with antibodies can be used for specific targeting [41, 42]. Gold nanoparticles have been used with probes in targeting and detecting BRAC1 gene, this method quantifies the number of genes in a cell. The analysis is done using colorimetric image and spectrometry. On colorimetric imaging the monomers of gold particles appear green and dimers appear red, therefore helps in quantification of the gene. The problem with this technique is that when cells are to be analyzed they are in a group and not a single cell making the detection in each cell difficult [42].

Liposomes

Liposomal form of drugs used in breast cancer helps in increasing the therapeutic index of the drug, apart from reducing the side effects. Clinically it was proven that Doxil (Doxorubicin HCl liposomal formulation) [43] prevents the degradation of the drug by encapsulating but the efficacy of transfer to cancerous cells is doubtful. So further research is going on. In an attempt PEG-glyated liposomes [44] and temperature sensitive liposomes were developed. In temperature sensitive liposomes thermosenstive molecules were added and made to rupture at tumour site by external stimuli. Out of such efforts came

Table 4: Applications of Magnetic Nanoparticles in Breast Cancer

Sl. No.	Application	Drug or coating with SPIO	Target site	Type of cancer or mechanism of action
1.	For imaging	Dextran conjugated with Herceptin	HER-2/neu receptor	Primary malignancies
2.	For imaging	Pyrenyl hyaluronan	CD44	Primary malignancies
3.	For imaging	copolymer of chitosan and PEG + neu antibodies	HER-2/neu receptor	Metastatic breast cancer
4.	For SLNB	Dextran	lymph	Axillary or sentinel lymph nodes
5.	As drug delivery systems	Methotrexate (MTX) grafted	Folate receptors	Peptide bond cleavage by lysozyme, due to overexpression of the receptors on target increased uptake
6.	In hyperthermia	Alternating magnetic field with or without conjugated antibodies on nanoparticles	If antibodies present then HER-2/neu receptor	Primary tumour

liposomes composed of dipalmitoylphosphatidylcholine (DPPC), monostearoylphosphatidylcholine (MSPC), and distearoylphosphatidylethanolamine (DSPE)-PEG 2000 which are currently in Phase II clinical trials for the treatment of recurrent breast cancer [45].

8.4. cMeth DNA Assay

This test detects ten breast cancer specific genes in patients' blood. Hypermethylation is a process which silences the genes which check cancers. This test checks if hypermethylation has occurred in any of the ten genes specific for breast cancer. The test is capable of determining patients' response to treatment 2 weeks prior by a decrease or stabilizing of the DNA methylation [46].

8.5. Thermography

Also called as thermal imaging is based on the idea that cancer cells have higher blood flow and metabolism as a result, their temperature would be higher and this difference is detected for diagnosis. Replacement of mammograms with thermography is not recommended, it is used only as supplementary information. Thermography can't detect deep placed tumours, they can only detect those present closer to the surface [47, 48].

9. CONCLUSION

Breast cancer is having wide coverage in the entire globe as per the statistics, the survival rate is increasing slowly with progress in technology. The disease can be further controlled and treated effectively with early diagnosis and detection. This has been partially achieved due to new techniques. New combinations of drugs are under different stages of clinical trials which in future will provide effective treatment at various stages of the disease.

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