1 Introduction

1.1 Breast cancer

Breast cancer is one of the most overwhelming problems affecting women and is the second leading cause of cancer death in women. Among the most common types of cancers in women (breast, lung and bronchus, and colorectal), which accounts for 51%, breast cancer alone is expected to account for 29% of all new cancer cases. As revealed by the current statistical data on breast cancer in the United States, 232,340 women were diagnosed, and 39,620 women are expected to die of breast cancer in 2013 [1, 2]. Furthermore, the American Cancer Society's (ACS) most recently estimated about 207,090 new cases of invasive breast cancer, about 64,640 new cases of carcinoma in situ (CIS is non-invasive and is the earliest form of breast cancer), and about 40,030 women will die from breast cancer. The incidence of breast cancer is also constantly rising in India, which accounts for 25% to 31% of all cancers [3].

1.1.1 Signs and symptoms of breast cancer

Breast cancer typically produces no symptoms when the tumor is small and most treatable. Larger tumors may become evident as a breast mass, which is often painless. Less common symptoms of breast cancer include persistent changes to the breast, such as thickening, swelling, distortion, tenderness, skin irritation, redness, scaliness, or nipple abnormalities. Breast pain is more likely to be caused by benign conditions and is not a common early symptom of breast cancer [4].

1.1.2 Cause and risk factors

A variety of risk factors have been considered for increasing the incidence of breast cancer. Breast cancer is a complex disease with multiple factors contributing to its development. While the exact cause is often not clear, various risk factors have been identified that may increase the likelihood of developing breast cancer. It's important to note that having one or more risk factors does not guarantee the development of breast

cancer, and individuals without known risk factors can still be diagnosed with the disease [5]. Here are some fundamental causes and risk factors associated with breast cancer

- Gender and age
    - Women are at a higher risk of developing breast cancer than men.
    - The risk increases with age, with most cases diagnosed in women aged 50 and older.
- Genetics and family history
    - Inherited gene mutations, such as BRCA1 and BRCA2, significantly increase the risk of breast cancer.
    - A family history of breast cancer, especially in first-degree relatives (mother, sister, daughter), may elevate risk.
- Personal history of breast cancer or certain non-cancerous breast diseases
    - Individuals with a history of breast cancer in one breast are at an increased risk of developing cancer in the other breast.
    - Certain non-cancerous breast diseases may also raise the risk.
- Hormone replacement therapy (HRT) and hormonal factors
    - Long-term use of hormone replacement therapy (estrogen and progesterone) after menopause may increase the risk.
    - Early onset of menstruation or late menopause can be associated with a higher risk.
- Reproductive and menstrual history
    - Delayed childbirth or never having children may increase the risk.
    - Early onset of menstruation (before age 12) and late menopause (after age 55) may be linked to higher risk.
- Radiation exposure

- Previous radiation therapy to the chest for other medical conditions, especially during adolescence, may increase the risk.

➢ Dense breast tissue

- Women with dense breast tissue, as seen on mammograms, may have a higher risk.

➢ Lifestyle factors

- Lack of physical activity.
- Excessive alcohol consumption.
- Being overweight or obese, especially after menopause.

➢ Environmental and occupational exposures

- Prolonged exposure to certain chemicals or environmental pollutants may be associated with an increased risk.

Additionally, maintaining a healthy lifestyle and discussing personal risk factors with healthcare professionals can help manage and mitigate potential risks.

1.1.3 Breast cancer treatment

Surgery is the primary treatment of breast cancer. Considering tumor size, extent of spread and patient preference, treatment usually involves lumpectomy (surgical removal of discrete lump or surrounding tissue) or mastectomy (surgical removal of the breast). Removal and evaluation of some of the underarm lymph nodes during surgery is usually recommended to determine whether the tumor has spread beyond the breast. Radiation therapy may follow surgery in an effort to eradicate residual disease while reducing reappearance rates. External-beam radiotherapy (EBRT) and partial-breast irradiation (PBI) are two general approaches for delivering radiation therapy [6]. Surgical resection with or without radiation is the standard treatment for ductal carcinoma in situ.

Adjuvant treatment for breast cancer involves radiation therapy and a variety of chemotherapeutic and biologic agents. It is designed to treat micrometastatic disease (or breast cancer cells that have escaped the breast and regional lymph nodes, but which have not yet had an established identifiable metastasis). Treatment is aimed at reducing the risk of future recurrence, thereby reducing breast cancer-related morbidity and mortality [7]. **Table 1.1.** summarizes the various classes of drugs or biological agents recommended for the treatment of breast cancer.

Table 1.1. Various drugs/biological agents for the treatment of breast cancer

Broad class	Representative examples	Mechanism of action
Antimicrotubular	Eribulin (Halaven), Docetaxel (Taxotere, Docefrez), Paclitaxel (Abraxane), Ixabepilone (Ixempra)	Microtubule formation inhibition, G_2/M cell-cycle block
Alkylating agents	Carboplatin (Paraplatin), Cyclophosphamide (Cytoxan)	DNA-cross linking
Anthracyclines	Doxorubicin (Liposomal) (Adriamycin, Doxil), Epirubicin (Ellence)	Intercalation between DNA base pairs, inhibition of type II topoisomerase function, inhibition of DNA helicase
Antimetabolites	Capecitabine (Xeloda), Gemcitabine (Gemzar), Methotrexate (Trexall)	Inhibition of DNA replication and cell growth
Vinca alkaloids	Vinorelbine (Navelbine)	Inhibit tubulin polymerization and inhibit mitosis.
Monoclonal antibodies	ado-trastuzumab emtansine (Kadcyla, TDMI-1), Denosumab (Prolia, Xgeva), Trastuzumab (Herceptin), Pertuzumab (Perjeta)	React with antigens on cancer cells, mainly targeting HER-2 receptors
Aromatase inhibitors	Anastrozole (Arimidex), Letrozole (Femara), Exemestane (Aromasin)	Inhibit the enzyme aromatase
Estrogen receptor antagonist	Tamoxifen (Nolvadex, Soltamox), Raloxifene (Evista), Toremifene (Fareston)	Selective estrogen receptor modulators (SERMs) stimulate or inhibit the estrogen receptors of various target tissues.
Tyrosine kinase inhibitors	Lapatinib (Tykerb)	Inhibits the intracellular tyrosine kinase
Cyclin-dependent kinase inhibitor	Abemaciclib, Palbociclib and Ribociclib	Inhibit cyclin-dependent kinase 4/6

1.1.4 Mechanism of receptor overexpression in breast cancer

Most of breast cancer emerges from epithelial cells in the ducts or lobules as a consequence of genomic and endogenous changes that cause abnormal growth control and intracellular signaling interruption. As a result, breast cancer is regarded as a diverse disease with various sub and cells with different origins and functions. Because estrogen receptors (ER) are expressed in 75% of breast cancer, upregulation of estrogen receptors ER during development has a significant effect. ER transcription factors move to the nucleus after coupling to the estrogen steroid hormone, driving relevant gene expression programs that disrupt growth control pathways [8].

1.1.5 Hormonal receptors over-expressed in breast cancer

Cytotoxic chemotherapeutic agents cannot differentiate tumor cells from normal cells, this may produce unwanted toxic effects. When patients are exposed to higher doses of these agents, which are required to eliminate tumors, this is one of the major challenges faced during cancer therapy with chemotherapeutic agents. However, studies and research show marked differences between the tumor cells and normal cells in terms of their molecular, biochemical and physiological features, including pH levels, leakiness of tumor tissue and vasculature, and overexpression of certain receptors. Therefore, cancer therapy can utilize these differences in order to reduce the damage caused to normal tissues or cells due to chemotherapy with cytotoxic agents [9]. These differences can be exploited with the help of designing targeted nanomedicine delivery i.e, ligand-modified nanocarriers can be utilized for drug delivery; this will target the specific features expressed in cancer cells. Molecular markers that can be utilized for targeting therapeutic agents in cancer mainly include the differential expression of proteins present in the membrane, organelles, and cytosol of tumor cells and tissues. The group of deferentially expressed proteins includes specific receptors that are upregulated in the tumor microenvironment relative to normal cells and tissues; these upregulated receptors in the cancer cells act as specific endogenous

sites for achieving targeted delivery of drugs. Hence protecting normal cells and tissues [15]. In breast cancer, the receptors that are overexpressed and can be targeted with nanomedicine include estrogen receptor (ER), progesterone receptor (PR), and androgen receptors (AR).

1.1.6 Estrogen receptor (ER)

Estrogen is the key female sex hormone which is steroidal in nature, and it helps in the growth, development and differentiation of the reproductive system and also controls multiple physiological processes, which include maintenance and growth of the skeleton and normal working of the cardiovascular and central nervous systems. Estrogens function as a key hormone in maintaining normal physiology, but studies have shown that estrogen is also concerned in several diseases, particularly breast tumor [10]. There are majorly three types of estrogen that have been identified including estrone (E1), estradiol (E2), and estriol (E3). Estradiol (E2) is the major hormone present in non-pregnant females, whereas estrone and estriol are mainly release during pregnancy. ERs play a key role in regulating of the action of estrogen. ER belongs to the nuclear hormone receptor super family which has nearly 150 members. ERs have been classified into two groups, namely ERα located on chromosome 6 and ERβ located on chromosome 14. ERα possesses two isoforms they are ERα 66, which is expressed mostly in the nucleus and regulates the mitogenic effect of estrogens and ERα 46, which mediates the arrest of the cell cycle [11].

The structure of ER contains mainly five functional domains. Both ERα and ERβ isoforms of ER possess these five functional domains. The first functional domain is A/B, which is also called the N-terminus variable region. A/B functional domain of ER is linked with transcription activation through the activation function 1 (AF-1) sequence. Next to N- the terminus region or A/B domain is functional domain C, which is also called the DNA binding domain. Adjacent to domain C is domain D, referred to as the hinge region due to its presence between the ligand binding domain and DNA binding domain. Domain

D, or hinge region, is also involved in the nuclear localization of receptors and their binding to DNA. Domain E, one of the most important regions of ERs, performs multiple functions, including dimerization hormone binding and binding to heat shock proteins, domain E also possesses an activation function 2 (AF2) sequence. The main difference between AF1 and AF2 is noted as AF1 can function independently of hormone attachment, whereas AF2 requires hormone binding. The role of domain F in ER is unclear, but studies show that it modulates estrogen and anti-estrogen responses however, it is not necessary for transcriptional activation or receptor half-life regulation. ERα and ERβ possess five domains, but their functioning is different due to the difference in A/B domain or N-terminus region. AF-1 of ERα is more active than that of ERβ also, β-form lacks many sequences that are present in ERα [12].

Based on the status, ER breast cancers can be ER positive and ER negative. Studies show that around 75% of breast tumors exhibit overexpression of ER receptors and are ER positive; overexpression of ER in breast cancer is found to be dynamic and reversible. ER α acts as an oncogene, while ERβ acts as a tumor suppressant. But some isoforms of ERβ, such as ERβ5, may function as oncogenes in the genesis of breast tumors and tumor proliferation [13]. The mechanisms involved in the abnormal expressions of ER in breast cancers are complicated and involve alternative attachment of ERα and ERβ, transcription factors, and epigenetic and post-transcriptional regulation of ER expression [12]. The growth, development and functions of the mammary gland are controlled by estrogen. Exposure of excessive amounts of estrogens to the breast epithelium can lead to the development of breast cancer due to the activation of oncogene, which may also result in the overexpression of ER receptors, particularly ERα isoforms. ER, which mediates the functions of estrogen in the mammary gland can contribute to the proliferation of breast cancer cells due to their over expression in the breast tissues in tumor microenvironment.

Excessive exposure of estrogens in the mammary gland results in increased cell division that leads to mutation, and the genotoxic waste generated after estrogen metabolism can produce DNA damage this may leads to point mutation consequently this process induces disturbances in DNA repair mechanisms, all these results in tumor generation. Moreover estrogen can also stimulates the secretion of growth factors from breast stroma indirectly which can promote the proliferation of breast epithelial cells validate their role in the development of breast cancer [14]. This changes in the levels of estrogen and estrogen ERcan be utilized for developing nanomedicine to target the breast cancer cells by using suitable nanocarriers modified with targeting ligands.

1.1.7 Progesterone receptor (PR)

Progesterone belongs to the class of steroidal hormones and plays a crucial role in regulating female reproductive tissues, which controls the developmental process as well as proliferation and differentiation during the reproductive cycle and pregnancy. Progesterone exerts its actions and other major physiological functions during and after pregnancy through its association with Progesterone Receptors (PRs) [15]. PRs are members of the steroidal hormone receptor (SHR) family that are composed of ligand-activated transcription factors. The binding of progesterone to the PRs produces several responses that lead to the regulation of gene expression to regulate the development, proliferation, and differentiation of target tissue. It also controls pathophysiological processes in hormonal receptor-expressed cancer [16].

The PRs are generally made up of an amino terminal domain (NTD), a central globular DNA binding domain, and well folded ligand binding domain (LBD) at the C-terminal end. The structure of PR largely comprises intrinsically disordered protein (IDP). Progesterone Receptors (PRs) mainly have two isoforms which include PR-A and PR-B. These isoforms have similar DNA binding domain (DBD) and ligand binding domain (LBD) but differ in amino terminal domain (NTD)[23]. PR-B possesses a length receptor

whereas PR-A is an N terminally shortened version, which means the PR-A isoform deficits 164 amino acids present in the NTD of PR-B. Isoforms of PRs function as homodimers (A:A, B:B) or heterodimers (A:B), and these isoforms are efficient in attaching DNA to transcription factors; also, these isoforms have the ability to affect the behaviors of the same or separate sets of target genes, as well as execute ligand-dependent and independent functions. Progesterone receptors have two transcriptional activation functions (AF), which are AF1 located in amino terminal domain and AF2 located in the ligand binding domain; their function is to offer contact surfaces for co-regulatory proteins [17].

Progesterone receptors are generally evaluated in primary breast cancers using immunohistochemistry; overexpression PRs mainly occur in hormone receptor positive breast cancer, especially luminal A subtype [18]. Biological actions and functions of progesterone vary according to the different conditions of females; for example, progesterone regulates the development of the mammary epithelium in mature female adults in order to promote ductal side branching, whereas, in the early stage of pregnancy, it activates massive expansion of epithelial compartment, during mid to late pregnancy period progesterone promotes differentiation and at the term progesterone stops the differentiation and initiates lactation. The effect of progesterone in breast cancer depends on the cancer subtype and stage of disease progression; the major challenge involved in PR overexpressed breast cancer is to specify how progesterone signaling pathways are altered and to interpret the role of abnormal signaling in breast tumor progression. An important variation identified in the progression of breast tumors is the disruption of balance between the two isoforms of progesterone receptors (PRs) [19]. Compared to PR-A, PR-B is capable of activating transcription strongly, and also, PR isoforms have different biological actions in different target tissues; PR-A mediates actions in the ovary and uterus, whereas PR-B mediates the growth of the mammary gland, which indicates that proliferative effect of

progesterone in the breast cancer cell is due to PR-B [20]. Roughly equal amounts of PR isoforms are expressed in normal breast tissues, while in the tumor microenvironment PR-B to PR-A ratios have been altered, and high ratios are observed most often. Unbalanced PR isoform ratios play a key role in cancer development in the mammary gland [28]. Studies have shown that progesterone receptors can regulate the proliferation and survival of mammary tumor cells and tissues independently without the influence of ER. Therefore, targeting PRs in hormone receptor positive breast cancer became a rational therapeutic approach [21].

1.1.8 Androgen receptors

Androgens are generally known as male hormones but are detected at physiologically relevant amounts in females and perform critical biological functions. The major androgenic hormones circulate at physiologically pertinent levels of females mainly include dehydroepiandrosterone-sulphate (DHEAS), DHEA, androsterone (A4), testosterone, 5α dihydrotestrone etc. [22]. Androgen Receptors (AR) are steroidal hormone receptors that mediate the biological actions of androgens. Several studies show that ARs arise as a new biomarker and a potential target in breast cancer chemotherapy; since AR belongs to the steroid hormone receptors family, it shows high structural, functional, and topographic similarity to ER and PR [23].

The AR is made up of 919 amino acids encoded from a 180kb gene located at the chromosome Xq11-12. The structure of the androgen receptor is mainly composed of three functional domains these are amino-terminal domain (NTD, residues 1-555), DNA binding domain (DBD, residues 555-623), c) and carboxyl-terminal domain (CTD, residues 655-919). There is a hinge region (623-655) which connects DBD and CTD. Amino terminal domain (NTD) is the largest region in the AR structure, and it contains Activation Factor (AF-1), which is composed of two separable transcription units, tau 1 and tau 5; these units are necessary for the full activity of ARs. The DNA binding domain (DBD) is liable for the

binding to DNA, which takes place through the binding with a palindromic consensus sequence 5′ -GGTACAnnnTGTTCT-3′ called Androgen Response Elements (ARE) and is only recognizable by ARs. The nuclear localization signal (NLS) responsible for AR nuclear import is present between DBD and the hinge region. The CTD domain contains a ligand binding domain (LBD) provided with androgen and anti androgen binding sites and activation Factor 2 (AF-2) [24]. Another typical feature of AR is the presence of glycine and glutamine units repeating in the N terminal activation domain, which can be connected to the development of certain cancers and neurological diseases. Isoforms of AR are structurally similar to the isoforms of PR, such as PR-A and PR-B. The isoforms are formed due to the difference in translation initiation sites, which results in the formation of the full-length receptor(110kDa) and N terminal shortened form (87kDa) and are referred as AR-B and AR-A, respectively. Several low molecular weight isoforms are also identified, especially in prostrate cancer cell lines and tumors. Isoforms such as AR-V7 (AR-3) lack the ligand binding domain, and thereby, such isoforms are able to activate transcription even in the absence of androgen i.e, androgen-independent transcription. AR-V7 isoforms are generally found in prostrate cancer cases; however, a substantial number of primary breast cancers and breast cancer cell lines show the presence of this AR isoform. AR is a variant of AR isoform that has been found expressed in some breast tumors and breast cancer cell lines, but they are not present in normal breast tissues and cells [13].

Several research and studies reveal the expression of Androgen Receptor (AR) in breast cancer; the role of ARs depends upon the tumor microenvironment and the relative amounts of estrogens and androgens circulating in the body. It is found that in ER negative breast cancers, ARs are mainly expressed. Studies show that ARs trigger cellular differentiation and proliferation in triple-negative breast cancer (TNBC), and also additionally, ARs interact with ERα and HER2 signaling pathways to regulate breast

cancer [25]. Generally, the actions of androgen and Androgen Receptor (AR) are highly complicated, and it is difficult to explain the mechanism of androgen receptor over expression in breast cancer. The role of AR expression in breast cancer varies depending on the expression of ER and other steroid receptors. As therapies targeting AR have been well established for prostrate cancer, AR can also be utilized as an alternative target in breast cancer therapy as they are well expressed in breast cancers; however, it is necessary to gain better knowledge regarding the action of ARs in different breast cancer subtypes inorder to efficiently exploit this target for clinical benefits [26].