

Empowering Hope: Advances in Breast Cancer Detection and Treatment

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Abstract- Breast cancer consists of different types of tumors, and is widely regarded as a heterogeneous disease with such types and the heterogeneity also implies different occupies a similar position in other respects, too. Various changes associated with the RNA expression, including both individual mRNA and microRNA. Advances in molecular technologies have now made possible the identification of new and more specific biomarkers for diagnosing, forecasting, and forewarning the risk of breast cancer, allowing treatment personalization, therapy optimization and prevention of overtreatment, undertreatment or incorrect treatment. A few biomarkers for breast cancer have been figured out with traditional biomarkers, and together they can be a physician's companion from diagnosis to treatment plan to improve results and increase the number who succeed in therapy. Certain well-studied biomarkers influence more than just therapeutic choices; several have exhibited promising potential for noninvasively screening and detecting breast cancer, such as cell-free tumor DNA circulating in blood, carcinoembryonic antigen, carbohydrate antigen 15-3, extracellular vesicles transporting molecules, circulating microRNAs, hereditary BRCA genes, and an array of chemicals identifiable from urine, nipple fluid extraction, and exhaled breath. Biomarkers employed to gauge the tumor microenvironment can likewise help forecast treatment responses and disease progression over time.

Keywords: Breast cancer, Biomarker, Tumour, RNA, DNA.

INTRODUCTION:

Breast cancer, a malady arising from errant breast cells multiplying and growing into tumors. The tumor now has the potential to develop and spread to every organ in the body. Ignoring these grows fatal. It's the tissues in the breast, possibly the milk ducts and breast's milk-producing lobules, where first breast cancer cells proliferate[1]. The early in situ form has no danger to life. Cancer cells can pass into other breast tissues. As for this, the tumors are focal, unyielding lumps. Invaded by invasive tumors, these next migrate to lymph nodes or other organs through a process known as metastasis. One can die from this. The patient's cancer kind and prognosis determine the course of treatment[2]. Treatment might include crada (medication), radiation because you're only bumping into the scaffold, so we have to build a new one and surgery. The leading cause of women's cancer-related death worldwide, is breast cancer (BC). Among cancers overall, it is second most frequently identified[3]. In these personalized medicine times traditional prognostic markers like metastasis of lymph node tumor size, and histological grading of tumors are no longer sufficient for doctors treating the newly diagnosed BC patient . Recently improved technology has accelerated our understanding of molecular-based progression and treatment responses to breast cancer. The discovery that certain molecular biomarkers might be useful in prognostic or predictive genetics has helped those who prescribe therapeutics to make their decisions , individualizing treatment and thus optimising therapy , rather than inadvertently over- or undertreating--or giving the wrong password markers Prognostic indicators can help clinicians to anticipate how aggressive an invasive tumor is so as to enable better treatment choices[4,5,6] .

Global Impact and Age Dynamics of Breast Cancer:

Globally, there were 685,000 incidences of breast cancer in 2020, accounting for 2.3 million additional deaths from the disease .It's the all-too common occurrence that of an estimated 7.8 million women still living at the end of 2020, because their diagnoses occurred in the previous five years. Globally, breast cancer strikes women from adolescence onwards, yet prevalence is clearly higher with increasing age[7,8].

BIOMARKERS

Blood, tissue, and other body fluids are common sources of biomarkers, which are quantifiable indicators of a biological condition or state. Disease detection, diagnosis, tracking the effectiveness of treatment, and disease progression prediction are all possible with biomarkers. They can refer to a wide range of medical symptoms that can be monitored precisely and consistently[9]. They are useful in clinical research and healthcare because they provide objective measurements of biological processes, disease, or the response to treatment interventions. In medical

research and clinical practice, biomarkers are valuable instruments that can facilitate the creation of novel diagnostic tests, track the advancement of diseases, and assess the efficacy of treatments. They help to further personalized medicine techniques, in which treatment strategies are tailored to individual patients based on biomarker profiles[10].

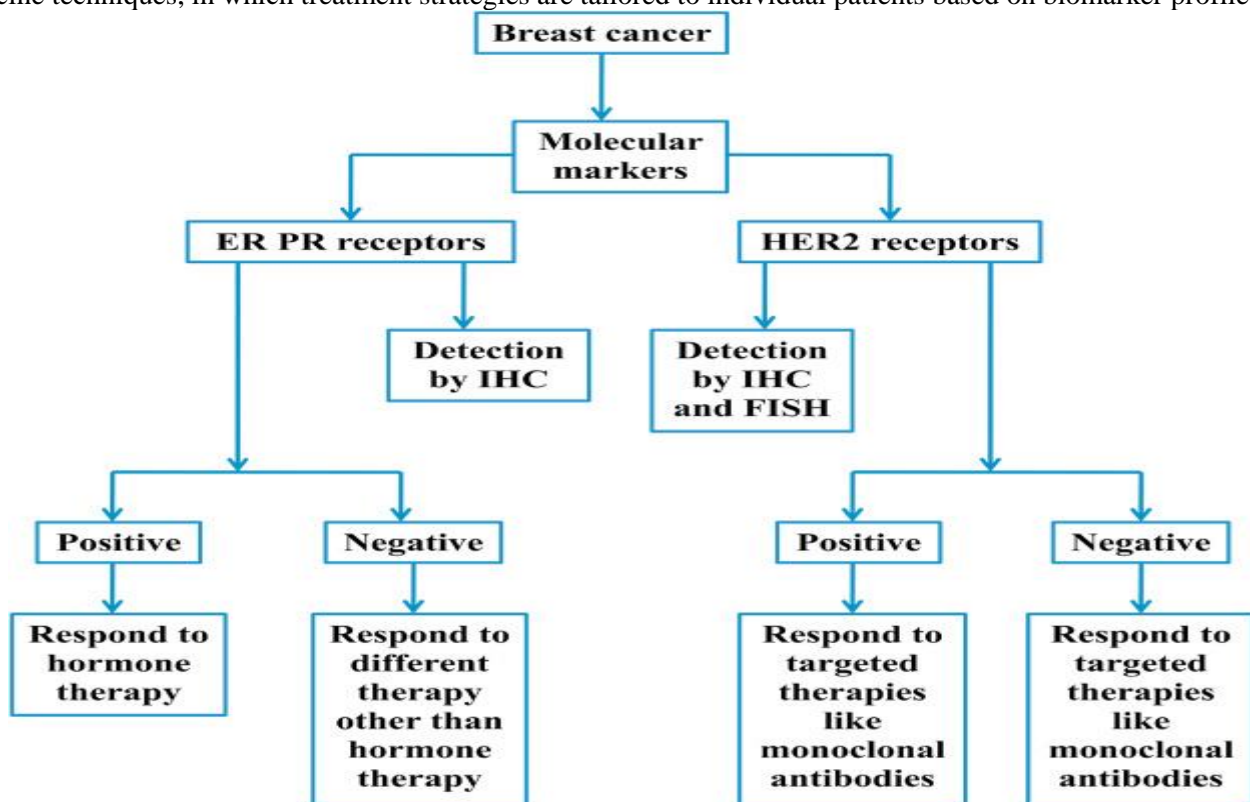


Figure 1: Classification of biomarkers

Importance of biomarkers

The study and therapy of breast cancer depend heavily on biomarkers. The following main ideas emphasize the significance of biomarkers in breast cancer:

Early Detection: By assisting in the early identification of breast cancer, biomarkers can enhance treatment results and enable prompt intervention.

Prognosis: Biomarkers can give important insights into how breast cancer will likely progress, enabling medical experts to adjust therapy regimens based on the disease's expected trajectory.

Treatment Selection: Biomarkers may be used to assist identify the most appropriate courses of action for a given diagnosis. By assisting in the identification of certain molecular targets and directing the use of targeted medicines, they can increase therapy efficacy.

Drug Resistance: By identifying the processes of drug resistance in breast cancer, biomarkers can facilitate the development of countermeasures[11].

LIMITATIONS

Biomarkers have various drawbacks despite their enormous promise in breast cancer research and clinical treatment. The following are a few of the limitations of biomarkers in breast cancer:

Heterogeneity: With several subtypes and variations within each subtype, breast cancer is an extremely diverse disease. The inability of biomarkers to fully represent the intricacy of the illness might make it difficult to predict therapy response or prognosis[12].

Sensitivity and Specificity: Biomarkers' capacity to identify genuine positive cases and weed out false positive instances may not always be strong points of differentiation. False-positive or false-negative findings may arise from this, misdiagnosing a patient or recommending an ineffective course of therapy[13].

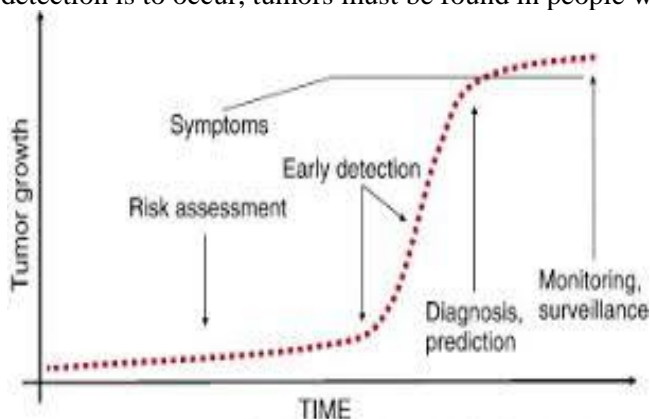
Validation and Standardization: Biomarkers must go through stringent validation and standardization procedures in order to be guaranteed to be dependable and repeatable in various lab and clinical environments. The consistency and quality of biomarker results might be impacted by testing technique variability and a lack of established protocols[14].

Cost and Accessibility: A number of biomarker tests have the potential to be costly and not be easily available to all patients, which may restrict their general application in clinical practice. Disparities in access to therapies guided by biomarkers may result from this.

Dynamic Nature: Over time, biomarker expression levels might fluctuate, particularly in reaction to therapy. Because biomarkers are dynamic, it might be difficult to reliably track the course of a disease or the effectiveness of a treatment with a single biomarker measurement[15].

Biomarkers for early detection:

A disease's detection and diagnosis are not the same thing. Identification of a disease in medical practice necessitates not only symptom recognition but also specific attributes that would without a doubt point to the existence of a specific disease[16]. Additional focused assays are usually required before a diagnosis is made because early symptoms frequently indicate a probable group of diseases with comparable traits. Since signs of cancer typically manifest when tumors are large enough, diagnosing the disease solely on the basis of symptoms is inappropriate. Because of this, if early cancer detection is to occur, tumors must be found in people who are



(<https://www.sciencedirect.com/science/article/abs/pii/S0304416507000402>)

Figure 2. Asymptomatic, elevating the tumor-specific assay to the status of a screening test with correspondingly strict needs. For the assay to identify problems in asymptomatic persons early on, it must first be performed using a small amount of starting material in a non-invasive (or minimally invasive) fashion[17]. In addition, a perfect screening assay must identify the site of tumor formation (it must be site-specific) and be able to identify cancer in many organs. In addition, non-cancerous occurrences in the same organ or tissue must be ruled out for an assay to be effective[18]. Furthermore, the assay must function at the differential diagnosis level, be observer-independent, and provide objective measures of disease biomarkers. Ultimately, depending on the disease's frequency in the community, the screening test must be sufficiently specific to yield an acceptable limit of false-positive results. The table provides a summary of the essential elements of an early detection assay[19,20,21].

Table 01: The early detection assay's requirements[22]

Criteria	Consequences
(1) Prompt identification population screening for apathy	Comprehensive screening
(2) Method with minimal invasiveness bodily liquids	The least possible quantity
(3) Location-specific identification	Particular to an organ or tissue
(4) Diagnostic utility	Specifically related to cancer
(5) independent of the observer	Quantifying biological feature(s) objectively
(6) Particular	Merely seven false-positives
(7) Simple and inexpensive	Universal screening

Molecular markers (or) Biomarkers used in breast cancer detection:-

Among the protein receptors that can bind to hormones are molecular markers. To ascertain how a certain therapy will be received, those produced by malignant cells are utilised. Cancerous tissue contains changed DNA sequences as well as proteins that are utilized as molecular markers[23,24]. To further limit the growth and metastatic spread of neoplastic cell clones without harming healthy cells, certain medications or other preventives, like an antibody, are employed in an appropriate targeted therapy [25].

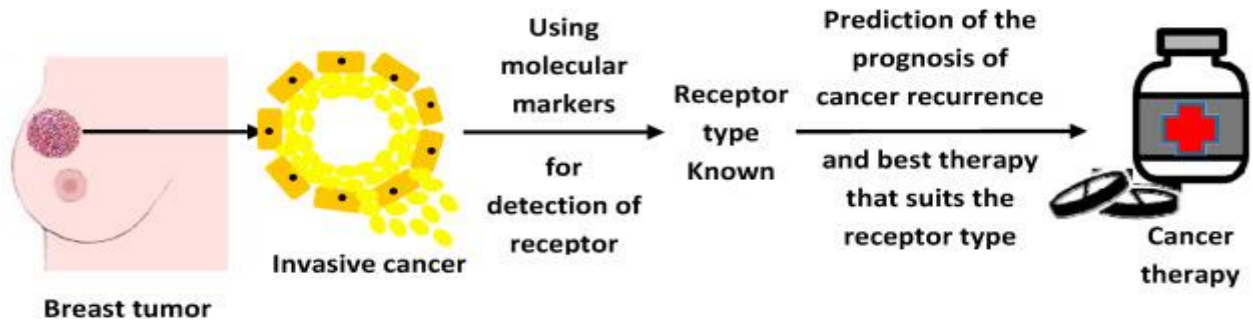


Figure 3. Detection of type of receptor using molecular markers and prediction of prognosis. (<https://www.sciencedirect.com/science/article/pii/S2352304220301628>)

1.Estrogen receptor:-

The estrogen receptor (ER) is a ligand-activated transcription factor belonging to the nuclear transcription receptor superfamily. Oestrogen and other steroid hormones bind to and activate the ER, causing it to translocate to the nucleus. After entering the nucleus, the activated ER can bind to DNA and start the transcription of genes that are involved in invasion, angiogenesis, proliferation, and evasion of apoptosis. ER- α and ER- β are the two isoforms of ER. It is shown that ER- α has a direct role in pathological processes, such as breast cancer [26].

The most widely used and well-established predictive marker for considering endocrine treatment in the management of breast cancer (BC) is the oestrogen receptor (ER), which was the first predictive marker to be developed in the field of cancer [27]. Another important predictor of breast cancer is ER, and research suggests that the clinical, molecular, and epidemiological risk factor profiles of BCs classified as ER positive and -negative vary [28]. Patients with ER-positive BC had a far better prognosis than those with ER-negative tumors. As a result, it is challenging to decide which cut-off should be used the lowest to define ER positivity [29]. That being said, ER has little predictive value in and of itself, and its strongest utility comes from its ability to predict response to endocrine medication. While ER-positive tumors react to endocrine therapy, ER-negative BC is not expected to respond to it, albeit to varying degrees [30]. Endocrine therapy yields a response in at least 70% of patients with significantly ER-positive BC, but less response is revealed by low ER-positive tumors. There is, however, no proof that the relationship between the degree of reaction and the ER expression level in BC is linear. As a result, figuring out the lowest cut-off that should be applied to characterize ER positive is challenging.

Oestrogen receptor and progesterone receptor are detected by **Immunohistochemistry (IHC)**. Immunohistochemistry (IHC) is a method for detecting antigens or haptens in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues. The antibody-antigen binding can be visualized in different manners. Enzymes, such as Horseradish Peroxidase (HRP) or Alkaline Phosphatase (AP), are commonly used to catalyze a color-producing reaction [31]. In clinical practice, a variety of gene expression assays are employed as instruments to forecast the likelihood of recurrence and/or the effectiveness of chemotherapy for BC that is ER positive. Some of these, such as Mrna-based assays, can be used as quality control tools for ER IHC and offer quantitative results for ER Mrna with good levels of concordance with ER assessed by IHC; however, there is no evidence to suggest that these assays can forecast response to endocrine therapy.

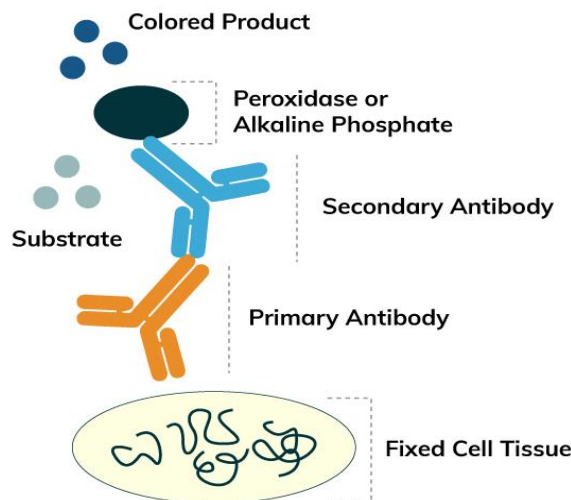


figure 4. Principle of Immunohistochemistry

(<https://www.bosterbio.com/protocol-and-troubleshooting/immunohistochemistry-ihc-principle>)

To identify patients for endocrine therapy, current guidelines do not support using Mrna-based assays[32,33,34]. It is advisable to repeat the IHC test using a different antibody clone or different tumor block if there are inconsistent results between ER status determined by IHC and Mrna-based tests in routine practice. This raises the possibility of a false positive (Table 1).

According to IHC, 80% of BCs express ER [35,36,37]. The published positivity percentage varies depending on the study cohort, the patient’s age, ethnicity, tumor type, and whether the tumor was screen-detected or not. Additionally, data supports the bimodal expression of ER in BC, with >90% of cases exhibiting either very positive (≥70%) or negative (<1%) expression; weak positive cases (≤10%; ER low) and intermediately positive (≥69%) cases are comparatively rare (≤3% and 5-9%, respectively)[36].

2. Progesterone Receptor

The presence of ER is a major need for PgR expression. PgR-expressing tumors that do not express the ER are rare; in some big series, they account for!1% of all occurrences of breast cancer [34]. Tumors expressing PgR but not ER expression should therefore have their ER status retested in order to rule out ER negativity. Although there is a small advantage from tamoxifen in the uncommon cases of patients who only express PgR, endocrine therapy is nevertheless strongly advised[35].

According to research, people with tumors that express both ER and PgR in metastatic breast cancer respond better to anti-estrogen therapy than patients whose tumors only express ER; PgR expression is absent [36]. Although PgR expression has a limited predictive importance, data from adjuvant trials comparing tamoxifen treatment with controls show a strong

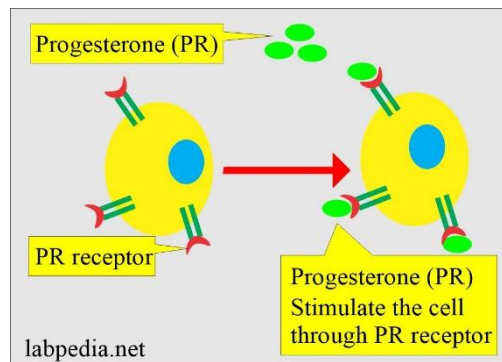


Figure 5. Progesterone stimulates the cells through PR receptors ([Progesterone Receptor \(PR\) For Breast Cancer - Labpedia.net](http://labpedia.net))

Prognostic value for it (Dowsett et al. 2006a). Although the relative benefit from tamoxifen is greater for patients whose breast cancers express high quantities of PgR, lesser expressors do not.

Approximately 80–90% of ER-positive cases and 65–75% of BC cases have PR positivity; the percentage varies based on the positivity cut-off point. There are less studies standardizing and validating IHC assays for PR and concentrating on the cut-off of positivity than there are for ER. Despite the fact that the low 1% cut-off for ER positivity is also advised for PR [26], this seems extremely low given the various clinical indications and purposes of ER and PR status. There isn't any proof that endocrine therapy can treat PR-low positive tumors (which make up 1–10%) if the BC is ER negative, nor is there any proof that this will significantly alter the predicted response of ER-positive tumors [37]. Furthermore, the cut-off points for the prior research on the predictive usefulness of PR were primarily 10%, 20%, or higher[39,40,41,42]. Further rmore, a 10%, 20%, or higher cut-off was typically utilized in earlier research on the predictive usefulness of PR . Here, we suggest that BCs with >10% PR immunostaining are PR positive, tumours with 1%–10% are PR poor, and they should be mixed with cancers that have <1% expression or no PR staining at all (PR negative). Nevertheless, in accordance with the most recent guidelines[38], we also advise disclosing the proportion and strength of stained cells, as these variables reveal the level of PR positive and, in turn, the extent of the prognostic and predictive significance of PR status.

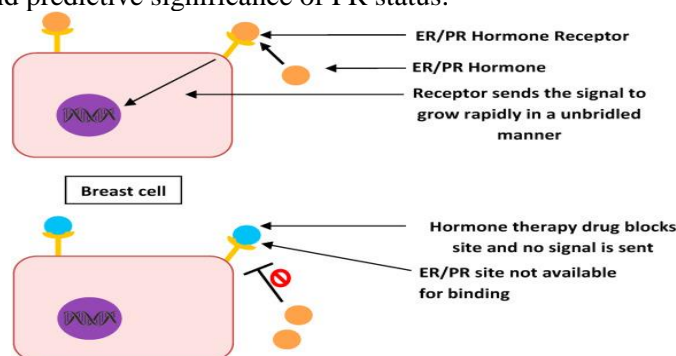


Figure 6. Mechanism of Hormone drug therapy
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3.Human epidermal growth factor receptor type 2 (HER2):

HER2, also called tyrosine kinase, erbB2, CD340 (cluster of differentiation 340), ERBB2 (human), ErbB2 (rodent), NEU, HER2/neu, HER2, NGL, MLN 19, and TKR1, is a growth-promoting oncoprotein generated by the HER2 gene (ERBB2 gene in humans). and mammary cell HER2 protein receptors assist and regulate the mammary cell's ability to divide, repair, and develop in a healthy manner HER2 gene amplification, on the other hand, occurs when the HER2 gene defects and starts to overproduce copies of itself. These additional copies of the HER2 gene subsequently cause the mammary cells to overexpress the HER2 protein, resulting in an excess of HER2 receptors[43,44,45].

A fraction of breast cancers overexpresses HER2, which is mostly as a result of HER2 (ERBB2) gene amplification[29,30]. An elevated HER2 gene or an overexpressed HER2 protein was found in up to 30% of BCs, according to previous study. The percentage is closer to 15% in more recent data, which is likely due to the assessment process's adoption of tight rules and the decrease in false-positive results—which can occur in as many as 19% of cases [31,32] as well as the frequency with which screening mammography can identify early-stage breast cancer in published series. In around 15% of all initial breast tumors, HER2 overexpression and amplification are seen, and anti-HER2 medicines are very beneficial to these patients. Every newly diagnosed instance of breast cancer should have its HER2 status evaluated [33].

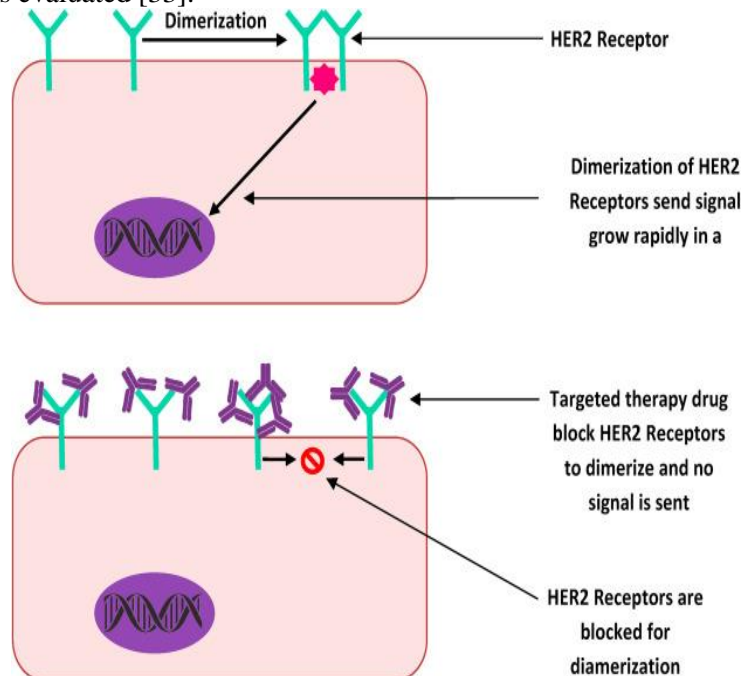


Figure 7. Mechanism of targeted therapy drug.
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4.Triple Negative Breast Cancer (TNBC):

TNBC is classified as a kind of breast cancer in which ER, PR, and HER2 are not expressed[38]. TNBC, a more aggressive and diverse subtype with lower treatment-related survival rates and a worse prognosis, accounts for around 15-20% of individuals diagnosed with breast cancer. The primary treatment for TNBC at this time is still cytotoxic chemotherapy. As a result, more TNBC classification is required for a more specialized and efficient treatment strategy [40,41].

Many biomarkers that can be utilized to further categorize TNBC patients for cellular treatment have been found through current research on TNBC-related biomarkers[41,42]. There are further signs that depict the treatment status and prognosis of people with triple negative breast cancer [42].Angiogenesis signaling through VEGF is required for tumour growth and dissemination [32]. VEGF is present in 30-60% of TNBC patients[41]. Patients with primary operable triple-negative breast cancer had much higher levels of VEGF and lower survival rates. Targeted anti-VEGF therapy enhances treatment results for TNBC patients, according to clinical trials.

The androgen receptor (AR) interacts to androgen in cells, modulating transcription factors and regulating gene expression[43,46]. AR can activate many signaling pathways, causing proliferation, dedifferentiation, apoptosis, and

cell death [46]. AR expression correlates with biological characteristics in triple-negative breast cancer, influencing endocrine therapy and prognosis[44].

EMERGING BIOMARKERS :

Ki-67:

Ki-67 is a proliferation's indicator. Using a mouse monoclonal antibody directed against a nuclear antigen from a Hodgkin's lymphoma cell line, Gerdes et al. (1983) made the initial discovery of Ki67 in the 1980s[47]. Nuclear protein MKi-67 has a molecular mass of 359 kDa and is frequently used to identify and measure cells that are multiplying. Its enhanced expression is linked to the expansion of cells. Since its expression indicates the rate of cellular proliferation, it is frequently employed as a diagnostic marker in a variety of malignancies[48]. Ki67 was further investigated as a proliferation marker because of its property that it was uniformly expressed in proliferating cells and missing in quiescent cells. While the precise role of the protein in cell division is still unclear, Ki67 is expressed throughout the G1, S, and G2 stages of the cell cycle, peaking during mitosis and disappearing during the G0 phase [49]. The Ki-67 gene is located on chromosome 10's long arm (10q26.2). The initial Ki-67 antibody targeted an epitope (FKEL), and this sequence of 22 amino acids is known as the Ki-67 motif. It is extremely conserved across animals. The molecule's carboxy terminal region is home to a putative ATP/GTP site known as P-loop. During interphase, the antigen can only be found in the cell nuclei [50]. It is essential for cell division and is also assumed to be necessary for meiosis, organ regeneration, the metabolic process of DNA, cell sensitivity to heat, and cell proliferation. The lack of similarity with other proteins makes it challenging to determine the functional role of Ki-67. Except for a few Ki-67 counterparts in other animals, no protein until recently showed clear homology with the human Ki-67, despite the existence of conserved domains shared with proteins with defined activities [51].

cyclin D1

It is found on chromosome 11q13 and is made up of 295 amino acids with a molecular weight of around 34 kDa. Cyclin D1 is a member of the cyclin family, a group of proteins that exhibit periodic variation in quantity during the cell cycle [52].

In more than half of instances of breast cancer, including 15% when a gene amplification takes place, cyclin D1 is overexpressed at the mRNA and protein levels (Buckley et al. 1993, Gillett et al. 1994, Ormandy et al. 2003) [53]. D-type cyclins are induced when growth factor stimulation occurs in cells in the G1 phase of the cell cycle (Musgrove et al. 1993) [54]. Following this, cyclin D1 attaches itself to cyclin-dependent kinases, phosphorylating a number of substrates, including the RB protein (Matsushime et al. 1994) [54]. Cyclin D1 is very important in the development of neoplasia. It is an established oncogene for which the amplification and genomic rearrangement leading to overexpression is commonly found in multiple types of human cancer. These particular changes to the cyclin D1 gene demonstrate the significance of cyclin D1 in the neoplastic process and represent the crucial selection advantage granted by its overexpressed gene product. The exact downstream cellular pathways that dysregulate cyclin D1 to cause neoplasia are unknown [55]. Robust data highlights the role that cyclin D1 overexpression and amplification play in human breast cancer. Roughly 13% to 20% of breast tumors have three- to ten-fold amplification of the DNA on 11q13.14 to 11q13.5 [56].

Cyclin E

Similar to cyclin D1, cyclin E is a positive regulator of cell cycle transition that peaks in protein expression during the G1 phase and forms an enzyme complex with cyclin-dependent kinase 2 (Koff et al. 1992) [56]. Many breast cancer cell lines have been shown to have cyclin E gene amplification, and there is compelling evidence that cyclin E contributes to carcinogenesis [57].

A G1 cyclin, cyclin E was initially discovered in 1991. Its 395 amino acid composition and 45 kDa molecular weight are found on chromosome 19q13.1. Periodically, the levels of cyclin E occur during the cell cycle. The G1 phase is when the cyclin E-cdk2 complex has its highest enzymatic activity [58]. It controls how cells move from the G1 to the S phases of the cell cycle. Cyclin E builds up at the G1/S phase boundary in the nucleus and is degraded as the cell moves through the S-phase.

This strong cyclin-mediated control over cell proliferation is lost in cancer [59]. Cyclin E is constitutively expressed in association with cyclin-dependent kinase 2 in a large number of breast tumors. This may result in the removal of crucial checkpoint regulation and incorrect substrate phosphorylation throughout all cell cycle stages. Accelerated S-phase entrance, genomic instability, and cancer are caused by cyclin E dysregulation. Research has demonstrated that chromosomal aneuploidy is caused by overexpression of cyclin E in human mammary epithelial cells[60].

Two investigations conducted in the middle of the 1990s established the crucial function of cyclin E in regulating G1 to S-phase. A reduction in the need for growth stimulants, a shrinkage in the size of the cell, and a shortening of the G1 phase were seen in one research where constitutive overexpression of cyclin E occurred. Cell cycle arrest was seen in the other investigation when anti-cyclin E antibody was microinjected into fibroblasts during the G1 phase[61]. There are cell lines of breast cancer that have an increased cyclin E gene. Cyclin E mRNA can become constitutively

overexpressed as a result of this amplification, up to 64-fold over the course of the cell cycle. The chromosomal instability caused by this overexpression lends credence to cyclin E's involvement in breast cancer^[62].

FUTURE PROSPECTS AND CHALLENGES:-

The creation of biosensors for breast cancer biomarkers has drawn a lot of interest recently. Still in their infancy, biomarker research and diagnostic tool innovation for breast cancer early detection are still relatively new. Even though electrochemical immunoassays were incredibly effective biotransducers, biomarkers identified for breast cancer must be tested for specificity, responsiveness, and efficiency in comparison to the established diagnostic criteria. Early phases of rapid clinical cancer diagnosis will be aided by the development and implementation of these sophisticated cancer screening technologies. However, in order to achieve actual validity of assays and more authentic output, suggested detection methodologies for biomarker detection of cancer inherently require standardization of pre- and post-analytical processes such as sample preparation, storage, and adjustment of experimental conditions. HER2, BRCA1, and p53 are among the biomarkers that have demonstrated therapeutic use; nonetheless, the methods used for assessing them still require improvement. Early clinical decision-making procedures need the assistance of more instruments. Developing biomarkers for clinical use and prospective assessment requires careful consideration, as does the planning and execution of clinical studies. We may identify distinct case-specific patterns of biomarkers to aid in the optimization of customized therapies and the individualized care of breast cancer patients, thanks to the work being put into characterizing the molecular characteristics of individual cancers and the discovery of new biomarkers with promising use in clinics.

CONCLUSION:

Breast cancer biomarkers are exceptionally vital in its conclusion, forecast and treatment; in this way, they play a noteworthy part in the location of breast cancer. On account of personalized treatment approaches that will make strides understanding results, the significance of biomarkers in breast cancer early conclusion and successful administration cannot be overemphasized. The discoveries of the look illustrate the noteworthiness of biomarkers in the determination of breast cancer, especially in circumstances when more routine strategies like mammography might not be as valuable. By considering person hazard components and hereditary cosmetics, biomarkers offer a more personalized screening strategy. These are essential to decide the sort, arrange and reaction of the tumor, all of which help in viable breast cancer treatment. Other than, developments in progression, such as fake bits of information and machine learning, are being utilized to update the region and examination of biomarkers. The alter of novel and more successful biomarkers is essential to move forward the precision and loyal quality of breast cancer screening and conclusion. In order to improve early identification, individualized treatment plans, and overall outcomes for those with breast cancer, biomarkers in the detection of the disease are essential tools with enormous potential. The field of breast cancer diagnosis and management will advance only if they maintain their research and development.

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