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UNRAVELING THE MOLECULAR LANDSCAPE OF BREAST CANCER: PROTEOMIC PERSPECTIVES AND CLINICAL IMPLICATIONS

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ABSTRACT

Breast cancer, a heterogeneous disease with diverse molecular subtypes, poses significant challenges in diagnosis and treatment. In this comprehensive review, we delve into the intricate landscape of breast cancer classification and biomarkers, encompassing various types and their distinct characteristics. From hormone receptor-positive BRCA to triple-negative breast cancer (TNBC) and invasive lobular carcinoma, each subtype presents unique molecular profiles and clinical implications. Through advanced proteomic techniques such as immunohistochemistry (IHC) and reverse phase protein arrays (RPPA), researchers have identified key protein biomarkers associated with prognosis and treatment response. Furthermore, the emergence of innovative tools like the CanAssist-Breast (CAB) algorithm and the integration of multi-omics data offer promising avenues for personalized treatment strategies. Looking ahead, precision medicine approaches driven by collaborative efforts between researchers, clinicians, and industry partners hold the potential to transform the landscape of breast cancer care, ultimately improving patient outcomes and quality of life.

KEYWORDS: Breast cancer, Molecular subtypes, Hormone receptor-positive, Triple-negative breast cancer (TNBC), Invasive lobular carcinoma, Proteomic techniques, Immunohistochemistry (IHC), Reverse phase protein arrays (RPPA), Precision medicine, Personalized treatment, Multi-omics data integration, Prognosis.

1. BACKGROUND

Cancer is that form of disease where some cells of the human body start growing in uncontrolled manner and also spread in different portions of the human body. This uncontrollable growth of cells can start at anyplace in the human body. In normal situations, cells divide and grow with help of cell division by which new cells develops and fulfill body requirements. When these cells become older or damaged, they usually die and with cell division newer cells replace them.[1]

In some situation this process of cell division breaks down or sometimes form damaged or abnormal cells that may convert into tumors (lumps of tissues) that may be carcinogenic or noncarcinogenic (benign). Carcinogenic tumors spread in neighboring tissues and travel to distant part of the body and develops new tumors (metastasis). Carcinogenic tumors also known as malignant tumors. Many of the cancers develops into solid tumors, but in case of leukemias, do not form solid tumors. Benign tumors don't spread in neighboring tissues. After surgical removing benign tumors don't grow again but malignant sometimes do. Benign tumors sometimes becomes very large and cause problem or sometime life threatening as in case of brain tumors.[1]

2. MAIN TEXT

2.1. How Cancerous cells different from Normal cells?

- ✓ Cancer cells are different from normal cells. Like:
- ✓ Cancer cells grow in absence of any signal while, normal cells require signal or messages to grow.
- ✓ Cancerous cells ignore or not respond to those signals which tell the cells to cease division or to die (apoptosis process (programmed cell death)).
- ✓ Cancerous cells spread into neighboring and even distant areas while, normal cells don't invade in neighboring cells and also not moves in other part of bodies.
- ✓ Cancerous cells give signal to blood vessels to develops towards tumors. Blood vessels provide supply of oxygen and nutrients to the tumors.
- ✓ Cancerous cells hide themselves from immunity system which can remove abnormal or damaged cells that may cause cancer.
- ✓ Sometimes cancerous cells also convince immunity system to give the protection in place of attacking the cells.
- ✓ Cancerous cells develop multiple alteration in their chromosomes structure, even some cells have double number of chromosomes.
- ✓ Cancerous cells depend on different types of nutrients as comparison to normal cells, this makes cancerous cells to grow fast.

2.2. Development of cancer

Cancer is a type of genetic disease that cause by alteration in genes that controls functions of a cell i.e., division and growth of cells. Genetics alteration arises due to error in cell division, damage cause to DNA by UV rays, tobacco smoke and harmful substances present in environment, inheritance from parents. Most of the time our immune system eliminates damaged DNA but this immunity becomes weaker along with ages. So, there is always higher chances of cancer in later ages. Each and every cancer has a unique and different combination of genetic alteration.[1]

2.3. Some fundamentals of cancer

- ✓ Cancer is developed when cells multiplication lost its ability to control and starts invading or affecting neighboring tissues.

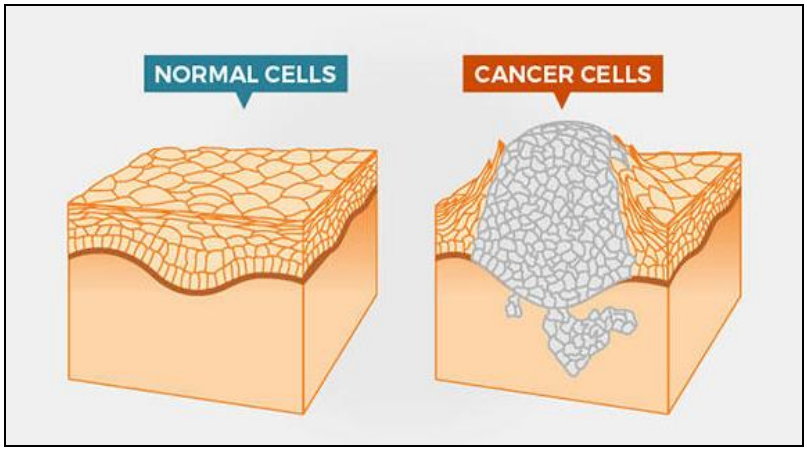

Figure 1: Normal and Cancer Cells.

- ✓ Cancer is generally cause by alteration in structure of DNA. This alteration in DNA structure happens at gene level so, also known as genetic changes.

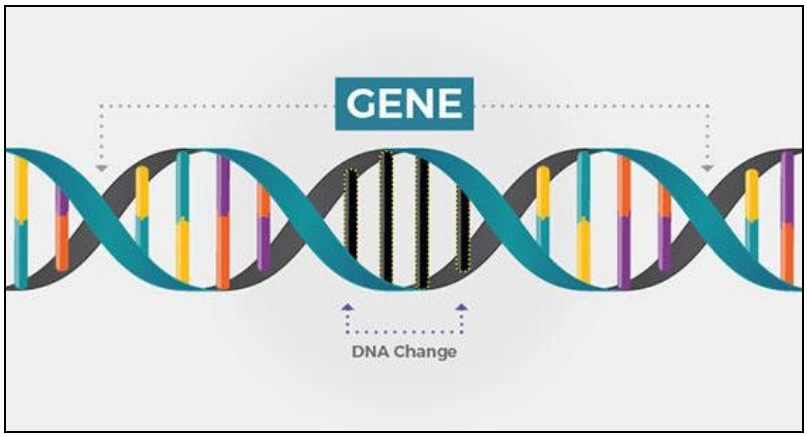

Figure 2: Gene: causing Cancer.

- ✓ After this alteration in DNA structure, normal genes responsible for cell growth converts into oncogenes. Oncogenes are those genes which responsible for uncontrolled growth of cells, these genes can't be stopped.

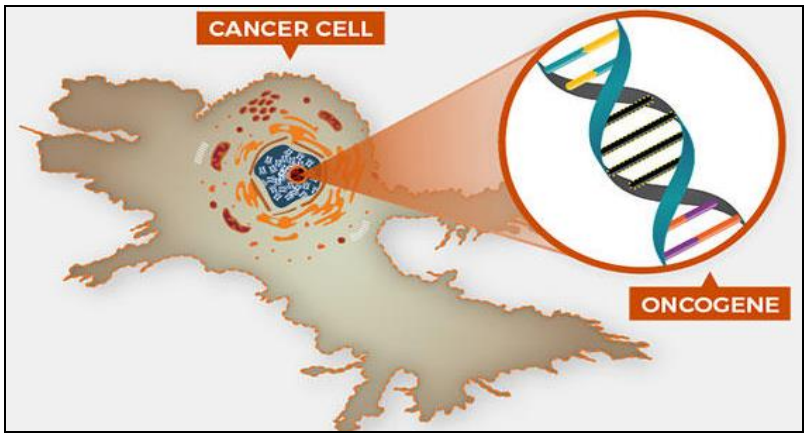

Figure 3: Oncogene.

- ✓ Tumor suppressor genes are presents in a normal cell that has the ability to prevent cancer by stopping or decreasing the growth of cells. But altered DNA deactivate the tumor suppressor genes and cause uncontrolled growth of cells.

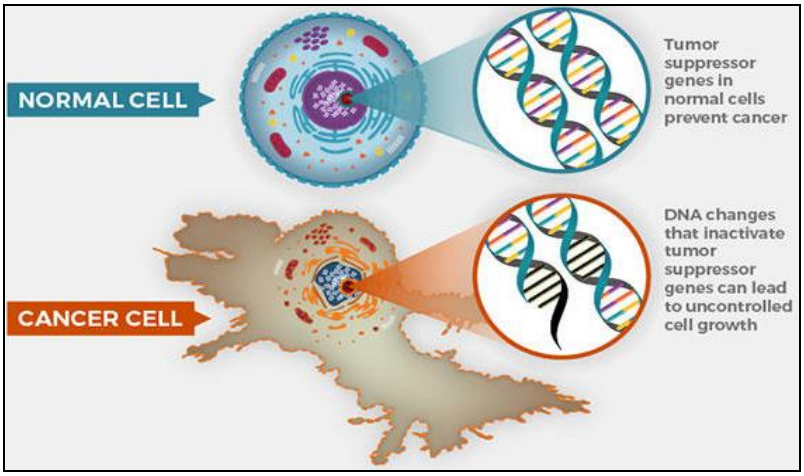

Figure 4: Tumor Suppressor gene.

- ✓ The microenvironment inside a tumor is very complex and shows changes time to time which ultimately affects the growth and spread of cancer cells, a typical cancer cell is totally surrounded by number of blood vessels for nutrient and oxygen along with different molecules, immune cells and fibroblasts.

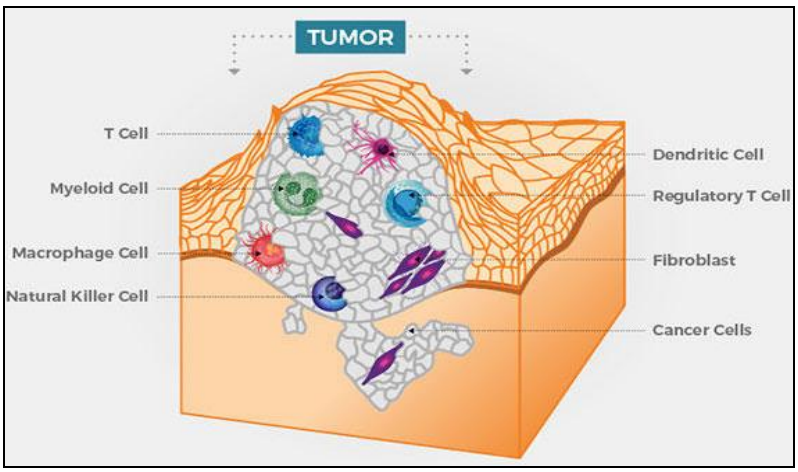

Figure 5: Tumor Microenvironment.

- ✓ Immune cells have the power to detect and then attack the cancer but, some of the cancer cells has the ability to hide or avoid this attack by immune cells. Sometimes treatment or adjuvant treatment of cancer helps the immune cells to kill the cancerous one.

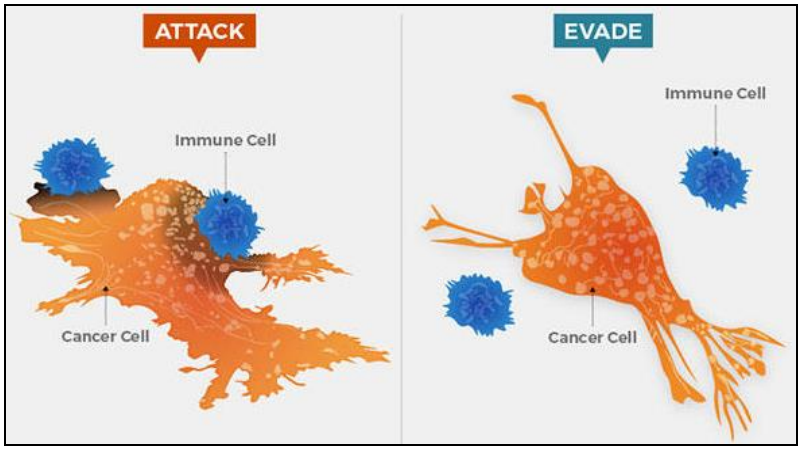

Figure 6: Interaction of immune system with cancer cells.

- ✓ The genetic alteration varies from person to person this cause severe challenges for treatment line so, individual treatment therapy or more molecular inside is necessary for treatment.

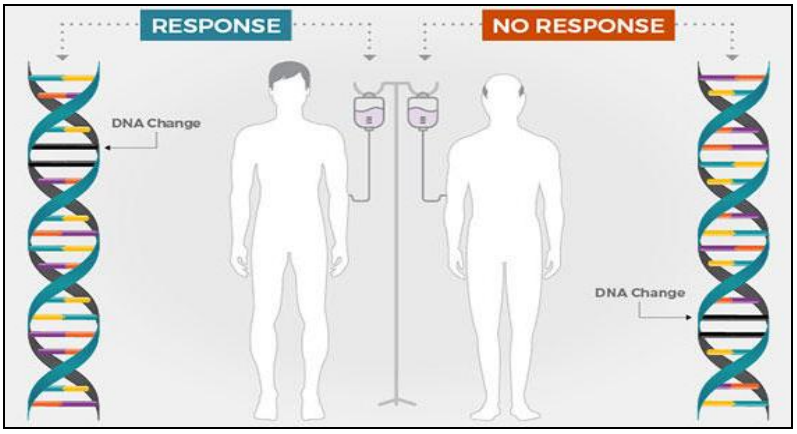

Figure 7: Genetic alterations affecting cancer treatment line.

- ✓ Genetic alteration in human body are generally arises from environmental factors and causes cancer growth, apart from environmental factors sometimes errors in cell multiplication also causes cancer cell growth.

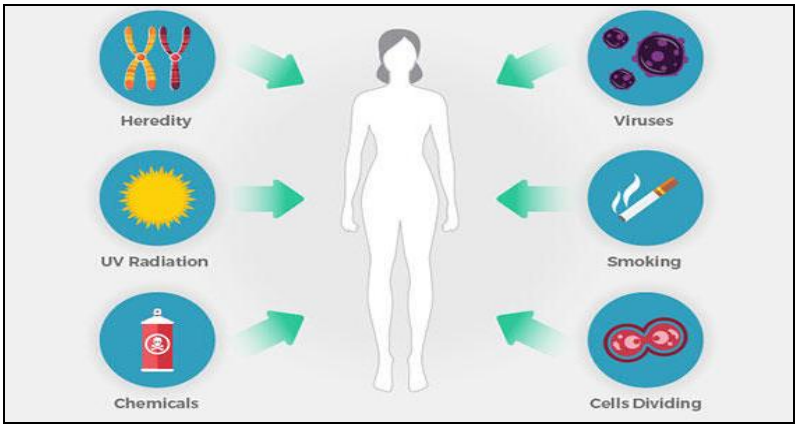

Figure 8: Factors causing genetic changes.

- ✓ Cancer risk increases as the age of person increases, this is due to accumulation of alteration in genes and weakening of immunity that ultimately leads cancer risk as person becomes older.

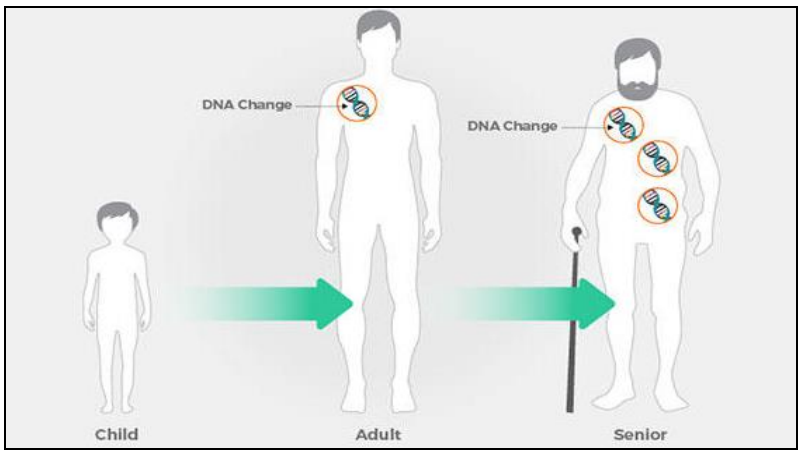

Figure 9: Relation of cancer with Aging.

- ✓ Cancerous cells effect the distant location by spreading through lymphatic system and blood. Cells after reaching distant location comes out from these vessels and starts forming new tumors this process known as metastasis.

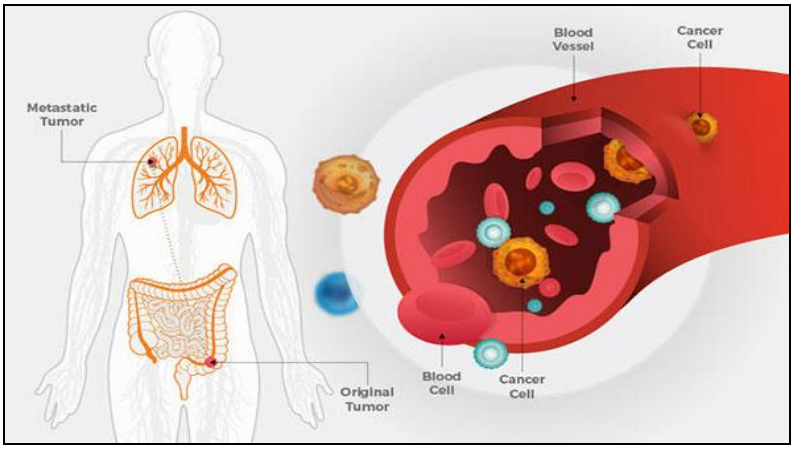

Figure 10: Metastasis.

2.4. Genes responsible for cancer

The alteration take place in genes are usually known as “drivers of cancer”. These alterations majorly target the DNA repair gene, proto-oncogene and tumor suppressor genes. DNA repair genes helps in fixation or repairing of damaged DNA. Those cells having mutation in their genetic parts had also develops tendency to make mutations in additional genes with altering their chromosomes, like deletion and duplication of chromosomal fragments. Ultimately this whole process makes the cells cancerous.

Proto-oncogenes are responsible for multiplication and growth in a normal cell. But, when alteration happens in these genes, they start showing more activity, becomes cancer producing genes known as oncogenes that will allow cells to grow rapidly.

Similarly, tumor suppressor genes are also responsible for controlling growth and multiplication in a normal cell. But, sometimes alteration in these genes cause multiplication of a cell in an uncontrolled way.

So now a days, scientists have a good knowledge about the microenvironment inside a tumor that ultimately helps them to develops such type of novel treatments that also target mutant genes responsible for cancer. This type of treatment line helping the patients having common mutations in their genetic makeup.

2.5. Spreading of Cancer

Cancer has the ability to spread from where it started to the other or distant parts of the human body. Cancer that has such kind of ability is known as metastatic cancer and the method by which cancer invades different parts is known as metastasis.

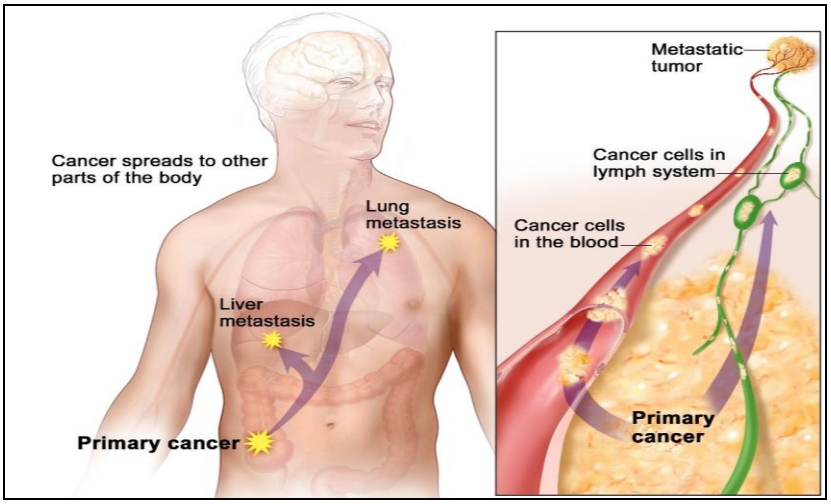

Figure 11: Metastasis.

Cancerous cells present in primary cancer are the similar one that found in distant metastatic cancer. For example, in case of metastasis of breast cancer, the tumor that develops in lungs as metastasis is known as metastatic breast cancer, not as lung cancer. Under microscopic examination one can find that metastatic cancer cells look similar to the primary cancer and even they have common molecular and chromosomal features.

In certain cases, treatment help the patients with metastatic cancer to prolong their lives and in few cases, the main goal of a treatment line is to control the growth or to provide relief from the symptoms causes by metastatic cancer. Most of the patients die is just because of metastasis as it causes severe damage to body parts.

2.6. Non-cancerous tissue changes

Changes at tissue level is not always cancerous, some of them develops into cancer if they remain untreated. Let's discuss some of them that require close monitoring because these tissues sometimes convert into cancer and cause severe problems.

- ✓ **Hyperplasia** is a condition in which tissue multiplication at higher side as compare to normal tissues, this will build additional cells. By seeing under a microscope, the infected tissue still looks normal. This type of condition arises by several conditions like chronic irritation.

- ✓ **Dysplasia** is advance form of hyperplasia, similarly there is built of additional cells but these cells seem very abnormal with unorganized tissues. So, if there is a greater number of abnormal cells and higher abnormality in tissue, there are greater chances of cancer. Sometimes dysplasia requires monitoring and treatment, although not require any attention. For example, dysplastic nevus (abnormal mole) that generally develops on skin. Dysplastic nevus sometimes turns into melanoma.
- ✓ **Carcinoma in situ** is most advance in all these conditions. Sometimes it is also known as stage 0 cancer, this kind of situation arises not because of the invasion to neighboring tissues, but formation of cancer by carcinomas in situ that require treatment.

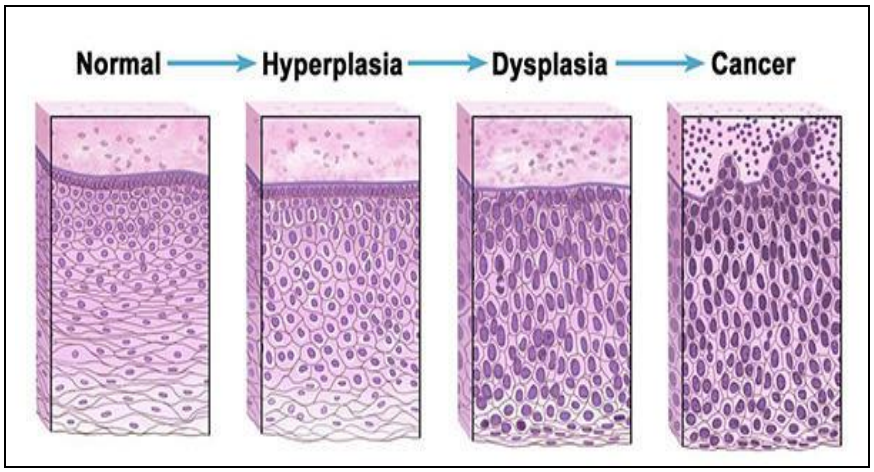

Figure 12: Development of Cancer.

2.7. Types of cancer

Around 100 types of cancer are identified. Cancer types are generally named for the tissues and organs where the cancer developed. For example, Brain cancer develops in brain. Similarly for the cells, like squamous cell or epithelial cell. Classification of cancer also done on basis of age, like cancer in childhood or cancer in adolescents. So, here we discuss basis types of cancer based on cells types.

- ✓ **Carcinomas** are very common type of cancer. This type of cancer develops by epithelial cells, that present as covering inside and outside surfaces of the body. Epithelial cells are of different types which look like column shape under microscopic observation. Carcinomas develops in different epithelial cells have specific names:

- **Adenocarcinoma** develops in epithelial cells that secrets mucus or fluids. These types of epithelial cells are generally present in glandular tissues (secreting tissues). Cancer of colon, prostate and breast are adenocarcinomas.
- **Squamous cell carcinoma** develops in squamous cells, these epithelial cells present just below the outer surface of the skin. Under microscopic observation, squamous cells look like fish scales. Squamous cells line many organs, like lungs, stomach, bladder, intestine and kidneys. Sometimes squamous cell carcinoma called as epidermoid carcinomas.
- **Basal cell carcinoma** develops in basal (base) or lower layer of epidermis, which is outer most layer of skin.
- **Transitional cell carcinoma** is a cancer that develops in epithelial tissue known as transitional epithelium (urothelium). These tissues can become bigger and smaller, that found in inner lining of ureter, bladder and renal pelvis with few other organs. So, cancer of ureter, bladder and renal pelvis are generally transitional cell carcinoma.

- ✓ **Sarcoma** develops in soft tissues and bones, along with muscles, blood vessels, fat, fibrous tissue (ligaments and tendons) and lymph vessels. Osteosarcoma is very common bone cancer. Most common types of soft tissue cancer are liposarcoma, dermatofibrosarcoma protuberans, leiomyosarcoma, malignant fibrous histiocytoma, and Kaposi sarcoma.
- ✓ **Leukemia** develops in blood forming tissue of bone marrow. This type of cancer does not form solid tumors. Although, they build up huge number of abnormal white blood cells (leukemic blast cells and leukemia cells) in bone marrow and blood, gathering out normal blood cells. This severe decrease in normal blood cells results in low oxygen supply to organs and tissues, that leads to uncontrol bleeding and loss of defense against infections. There are four types of leukemia, based on how worse the disease (acute or chronic) and on the type of blood cell where the cancer starts in (myeloid or lymphoblastic). Acute form develops very quickly while chronic develops slowly.
- ✓ **Lymphoma** develops in lymphocytes i.e., T cells or B cells. Lymphocytes are part of immune system that provide defense against diseases, lymphocytes are basically white blood cells. These abnormal lymphocytes store in lymphatic vessels and nodes, as well as in different organs. Lymphomas are generally of two types i.e., Hodgkin (form from B cells) and Non-Hodgkin (B cells or T cells).
- ✓ **Multiple Myeloma** develops in plasma cells, these abnormal plasma cells known as myeloma that store in bone marrow and starts forming tumors inside the bones, plasma cell myeloma also known as multiple myeloma or Kahler disease.
- ✓ **Brain and Spinal Cord Tumors** are named behalf of the cell where the tumor develops first time in central nervous system. For example, when tumor begins in astrocytes cells of brain that keeps nerves healthy. Brain tumors can be non-cancerous (benign) or cancerous (malignant).

If we look cancer scenario in Indian women, Breast cancer (BRCA) is the cancer that comes on the top among all type of cancers.[2] In 2020 this number hit 1.9 million BRCA patients.[3] If we talk about India, here BRCA patients has extremely aggressive tumor biology that's comes with higher number of nodes positivity and higher tumor staging along with younger age. But unfortunately, not every patent gets benefited by chemotherapy even if patient has highly risky factors and here the challenge arises to accurately identify this category of patients.[4]

One can observe persistent development not only in the treatment but also in effective management of BRCA patients by surgical procedures since last 60 years. In 1950s, first combination drug i.e., 5- flurouracil, methotrexate and cyclophosphamide evolved for BRCA[5], now so many new drugs and regimens present in market, apart from that Hormone therapy is also present for Hormone receptor positive BRCA patients. Efficacy of these drugs and regimens are enhanced in few of the cases but so many of these drugs or regimens cause undesirable side effects like reduction in blood cells and majorly cardiotoxicity.[4]

The variation in BRCA treatment arises due to variation at molecular level, Receptor such as progesterone receptor (PR), Estrogen Receptor (ER) along with Human-Epidermal growth factor II (HER2) responds positively or negatively to the cancer treatment.[6] This will eventually give rise to heterogeneity among BRCA patients and cause inter or intra tumor heterogeneity.[7] Now a days, several prognostic or diagnostic tools are available that basically use identification or evaluation of genes associated with the tumor at molecular level but unfortunately most of these tools reports fruitless clinical results, these tools also unable to distinguish clusters of those patients who has very low or minimal risk of distant metastasis or those patients who can avoid any adjuvant chemotherapy.[8-11] This happens due to the

primary focus of these tests on proliferation and leaving behind the microenvironment of tumor or the exchange of various signal between different pathways that help in growth of tumor.[12] Even though, the transcription or Central Dogma do not ever predict that presence of plenty of genes always expressed as proteins[13], even genes expression sometimes unable to capture alteration in expression of proteins. So, here we need to study precise cellular functions along with their responses that can be done by using Quantitative and Qualitative analysis of protein that expressed in tumor biology. This can be done by examining the protein visually at cellular level by using immunohistochemistry (IHC), that might be very challenging to identify new targets of novel drugs but this can ultimately give rise to optimized and personalized cancer therapeutics for BRCA.[14]

2.8. Classification of BRCA or Breast Tumor Biomarkers

The BRCA tumor biology is very complex, to understand it there is requirement of appropriate understanding related to biomarkers or proteomics that helps in differentiation between aggressive and nonaggressive tumors with their treatment line. To understand BRCA tumor biology so many attempts were made which can be classified on behalf of IHC staining techniques that ultimately stains the proliferating proteins or receptors and helps in identification of their aggressiveness. Like CanAssist-Breast (CAB) utilize five biomarkers combination namely, CD44 along with ABCC4 and ABCC11 as membrane biomarkers and N-cadherin along with pan-cadherin as cytoplasmic biomarkers and also included 3 pathological and clinical parameters to predict the possibility of recurrence of BRCA in future with help of IHC, similarly staining of protein that proliferating (Ki-67), Status of hormone receptors (ER, PR along with androgen receptors (AR)), or sometimes absence or presence of some special cytokeratins (CK).[14-21]

CAB shows its robustness in classification of BRCA reoccurrence risk and also not exaggerated by age, luminal subtyping or any geographical area. Performance of CAB is appreciating across any age groups with no impact of menopausal status. CAB ultimately helping patients by avoiding not only overtreatment but also undertreatment of patients those based on tests for prognosis.[4]

Expression by Ki-67 is inversely proportional to the outcomes: High Ki-67 scores relates with poor results. The clinical worth of Ki-67 is highly controversial as the reproducibility of score vary from laboratory to another laboratory also there is differences in method of assay which also produce different thresholds for high and low scores.[22] Apart from this limitation of Ki-67, at high and low thresholds Ki-67 score has demonstrated clinical value for prognosis.

Sensitivity of endocrine therapy is predicted by ER and PR response. There are two forms of estrogen receptor occurs, ERα and ERβ. Both these two forms of estrogen receptor are biologically active and functional. The complexity of this biological environment begins when estrogen receptor starts talking with Human-Epidermal growth factor I (HER1) and HER2.[23] The function of ERα is to promote cell proliferation while ERβ act as antagonist to ERα.[24] In spite both of these forms only ERα has clinical value[25] therefore, here we refer ER as ERα only.

Regulation of Transcription is done by ER and PR that known as Transcription regulators which belongs to a superfamily of nuclear receptor including receptors for vitamin D, thyroid hormone, peroxisome proliferator and steroids.[26] BRCA Tumors that respond ER and PR positively favor the prognosis[22] as compared to tumors that are ER negative.{277] Tumors that are ER positive responds better to endocrine therapy (Aromatase inhibitor, Estrogen receptor modulators or inhibitors), but this type of treatment respond non uniformly and in some cases few patients develops treatment resistance.[16,22] Threshold value of ER positive is ≥ 1%.[28]

Oncogenesis is mainly regulated by Growth factor receptors with the help of their ligands that regulates proliferation of cells.[29] Epidermal Growth factor receptor (EGFR), HER2, HER3, HER4, insulin like growth factor I receptor (IGFIR) and platelet derived growth factor (PDGF) all are types of Growth factor receptors. These receptors regulates proliferation of cells activity thru tyrosine kinase that present in plasma proteins with receptor cross talks and dimerization reaction.[30] Discovery of gene associated with HER2[29-31] and its presence in cell line of BRCA that drive breast tumors[32,33], totally change the biomarker classification of BRCA. Overexpression of HER2 is related to extremely aggressive tumors, that cause poor prognosis and chemotherapy response.[30,34-39]

2.9. Expression of gene as BRCA classifier

IHC based classification of BRCA tumors gives better prognosis and diagnosis[40,41], but BRCA discussion would be unfinished if one cannot consider influence of intrinsic gene expression that act as classifiers of BRCA subtypes. Around 15 years before, when profiling of BRCA tumors was done, it revealed a set of genes those expression significantly varied from one tumor to another and this type of variation not arise due to sampling bias in between tissues.[6,42,43] When analysis of that 500 set of genes done, it revealed around 5 set of gene profiles expression, that were categorized as Luminal A, Luminal B, HER2 +ve, Basal Like, and Usual Breast like.[6,42] The basal, luminal and normal categories were obtained from histomorphology of breast. The duct lumen surrounded by epithelial cells consider as luminal category. The basement membrane or basal layer has lining of myoepithelial cells consider as basal like category. When normal or usual breast tissue are in abundance in a heterogenous breast tumor consider as usual or normal breast like. Most aggressive HER2 +ve category represent increase in amount of HER2neu gene expression and their corresponding receptors.[44] In 2009, Parker JS. et al. proposed Prediction Analysis for Microarrays (PAM50) that helps in prediction of recurrence risk, PAM50 contains 50 sets of genes out of the total intrinsic genes along with use algorithm for categorization.[10] This proves revolutionary and adopted rapidly in regular clinical practices for prediction of recurrence risk generally in ER/PR +ve and HER2 -ve, lymph node either +ve or -ve up to stage II BRCA patients.[45] Generally, now a days may genomic and proteomics studies adopted this classification and nomenclature, also adopting new algorithms and continuing redefining and developing.

In 2015, Milioli HH. et al. use ensemble learning technique and developed a new gene expression score, that score helps in ranking the features between classes by using univariate method. By using this system, they successfully identified 7 different unexplored genes that has the potential to help in prognosis and can generate a predictive clinical value.[46] Currently, so many factors arises after study of intrinsic genes that impact on recurrence risk and prognosis of BRCA.[47] Factor like Race[48-50], age[47-49], interactions between immune cells[51-54] and metastatic sites (48,55–58) also impacting on treatment options, risk of recurrence and metastatic free survival.

2.10. Clonal Heterogeneity and BRCA

Identification of new biomarkers that present at the molecular level is now a days very easy because of the new advance technologies along with new drug discovery and importance of bioinformatics. Classifying different features of breast tumors into their subtypes gives very useful information that help in prognosis, diagnosis, estimation of recurrence risk, and optimized treatment therapy or regimen.[34,38,43,45,59] Categorizing of breast tumors currently relies on proteomics and genomic analysis, including IHC, histomorphology and valuable clinical data but sometimes the consolidative genomic analysis fails to recognize the negative and positive feedback in signal transduction pathways which give rise to clonal heterogeneity that basis of breast tumors.[60] In between tumors clonal heterogeneity arises due

to many of the tumors starts cloning the tumor population.[61,62] Even if anyone of the clone killed or move to static niche by the treatment line, remaining will survive as the assurance provided by clonal heterogeneity.[43,63] More focusing on microenvironment of breast tumors will help to understand tumor clones and their behavior.[64,65] This review article focusing on protein biomarkers and their contribution in diagnosis and prognosis of BRCA. The plethora of article on protein biomarkers limits our potential to summarize and entertain all the current published articles. So, here we selected recent studies that focus on novel protein biomarkers identification that helps in BRCA.

2.11. Male BRCA clas1sifier

Heterogeneity at molecular level also found in male BRCA. BRCA in male patients is around 1% of the total identified BRCA cases.[66] In male BRCA2 mutation is the main reason along with TP53 and PIK3CA as related to the females with equal ER +ve and HER2 -ve phenotype.[66,67] BRCA of male are mostly AR and ER +ve, and it is very rare to found triple negative (ER, PR, HER2 -ve).[66] By IHC and tissue microarrays, BRCA of male can be classify into four groups: low ER +ve, intermediate ER +ve, high ER +ve and hormones receptor negative.[68] Classification of these four groups clearly shows males BRCA is different from female BRCA.[69] Due to very limited literature about proteomics biomarkers for male BRCA, the remaining part of this article mainly focusing on female BRCA.

3. Proteomics and BRCA

Discovery of protein biomarkers is rapidly emerging with the new technologies, we can now quantify even very low strength of protein that help in their identification and characterization, we can also verify identity of protein with help of antibody reaction and pinpoint the treatment therapy. Generally, these protein biomarkers identify at breast microenvironment, sometime within tumor, surrounding blood or lymphatic circulation.[70] There is discordance between Protein and RNA expression, just because of this some of the biological features were not expressed by the intrinsic genes.

Technology regarding this approach evolving but number of proteomics which are clinically validated is very small.[64] This type of failure arises due to biasing in sampling, poor statistical data analysis and faulty experimental design.[64,71]

Today, for pre-clinical biomarker studies so many cell line models are available, these cell lines can categorized on the behalf of protein biomarkers expression.[72] The major drawback behind using cell lines as compared to breast tumor sample are (a) using some genetic mutation like K-RAS in cell line MDA-MD-453 which is not identified in patient sample[73], (b) in cell lines, tumor microenvironment replication not possible, and (c) concordance level between breast proteomics and cell lines protein expression is not satisfactory.[74] To get the valuable information from these available studies, study design and factors affecting experiment should be well evaluated. Variation during analysis need to be minimized by making tissue collection consistent and good processing procedures. Identification of potential biomarkers need to be validated and verified in a good number of peoples that gives an accurate prediction.

These protein biomarkers studies clear all the clinical issues that includes a better prognosis and predict good therapy response with avoiding toxicity and drug resistance.

3.1. Inexpensive and fast IHC score in prognosis of BRCA

IHC4 test is inexpensive, fast and commonly available test that overall decrease healthcare burden on the patient, apart from this IHC4 is reproducible and consistent. ER, PR, HER2 and Ki-67 individually use for prognosis of recurrence risk, by combining all these biomarkers could have additional value added prognosis result.[75]

In 2018, Bakre MM et al., developed a novel proteomic tool for prognosis of those patients having hormone receptor +ve BRCA that help in estimating the risk of distant recurrence by using IHC. This proteomic risk classifier is a combination of 5 biomarkers CD44, ABCC4, ABCC11, and N-cadherin, pan-cadherin along with 3 clinical parameter, tumor size, node status and tumor grades which combined into CAB algorithm.[14] The CAB classify patients into high-risk group and low-risk group for distant risk of recurrence. The threshold value for differentiate between low-risk and high-risk groups was set at 15.5 that resembled to 9% chances for distant risk recurrence.[14]

In 2016, Lakhanpal R et al., assessed IHC4 and Clinical Treatment Score (CTS), that contain tumor size, tumor grade, nodal status, age and kind of drug therapy. This inexpensive tool could predict the recurrence risk in those women who had early stage BRCA and also had conservation surgery for breast (BCS).[76] The IHC4 and CTS scores divide the patients into 3 groups i.e., high-risk, intermediate-risk and low-risk patients that shows promising results in prediction of recurrence risk, along with also deliver cost effective and quality lifespan to those patients who can sidestep adjuvant radiation therapy.[76]

In 2011, Cuzick J. et al. developed a IHC4 tool that has four protein biomarkers i.e., ER, PR, HER2 and Ki-67 for estimation of recurrence risk in those patients who were already treated with hormonal therapy.[75] These biomarkers combined with type of drug regimen, size of tumor, nodal status, grading and age of patient to get IHC4 algorithm. IHC4 score is more reliable than gene based recurrence score like Oncotype Dx in case of prognosis for distant recurrence.[75]

3.2. Triple negative breast cancer analysis by immunocytochemistry

Triple negative breast cancer (TNBC) is a type of BRCA, which is ER/PR/HER2 negative, along with high probability of recurrence risk, high mitotic division and high metastasis. Within all BRCA, TNBC has the poorest prediction. As there is no biomarkers involve for prediction of outcomes and tailoring treatment therapy so, only adjuvant chemotherapy and surgery are the options for treatment of TNBC.

There are 4 categories of TNBC, basal like 1 and 2, luminal androgen receptor and mesenchymal.[77] Even the basal like category, all TNBC are not basal like and vice versa that all basal like tumors not comes under TNBC.

A set of TNBC tumors express both lymphocyte activation gene 3 (LAG3) and programmed cell death 1 (PD1) protein when revealed by IHC.[54] These both the protein expression could be used to recognize those TNBC patient who can get help from immunomodulatory treatment therapy.

CD44 is a protein that helps in adhesion of cells by binding hyaluronic acid, expression of CD44 in breast tumors is very common as associated with tumors or BRCA stem cells. Epithelial Mesenchymal transition (EMT) or dissociation of cancerous cells is under control of CD44, CD44 also act as a coreceptor for Mesenchymal epithelial transition (MET) and HER2. CD44 sometime also modified the shape of cells with help of actin fibers. Around more than 50% TNBC has expression of CD44.[78]

4. Role of Reverse phase protein arrays (RPPA) and BRCA

RPPA is most suitable method for characterization and quantification of protein and their level of expression that helps to discover functional protein signal transduction paths which signify treatments targets.[79,80] RPPA not use for identification of new protein like in case of mass spectrometry, instead it provides better protein analysis by using antibody detection methods.

In 2014, Montero JC et al., revealed that sample derived in TNBC cell lines and tumors had extra cellular signal related protein (ERK I and II) and mammalian target of rapamycin (mTOR) was shown by western blotting and antibody arrays which can controlled by BEZ235 (dual inhibitor of mTOR/PI3K).[81]

In 2013, Craig DW et al., analyzed good number of alterations by transcriptome and genomic sequencing from 14 metastatic TNBC sets. The goal of the study to analyzed potential molecules. They found patient to patient genomic variations, and also found some common variation in genes that controls DNA repairing and those drives signal cascades RAF/MEK/RAS/ERK and mTOR/PI3K/AKT.[82]

These two studies show heterogeneity in between the TNBC patients and there is something to target by the drug therapies within DNA repair pathways and protein translation.

4.1. HER2 and Proteomics heterogeneity

Classification of BRCA is very complicated as receptor cross talk and receptor switching complicate it. HER2 truncation will results in hyperactivation of p95HER2 that cause trastuzumab resistance.[57] Heterodimers form by HER2 and HER3 activates growth pathways and AKT-mTOR protein translation. High level of HER3 or p95 found in HER +ve formalin fixed paraffin embedded (FFPE) samples.[57]

Activation of HER2 signal pathway done by phosphorylated EGFR and HER3, in FFPE blocks that previously said as HER2 -ve by IHC and fluorescent in situ hybridization (FISH).[83] These studies demonstrate that molecular sketching of HER2 requires to include p95HER2 and phosphoHER2 to provide whole horoscope of receptor.

4.2. Heterogeneity and Luminal A BRCA

Luminal A type of BRCA is extremely heterogenous but it has a good prognosis. Despite endocrine therapy sometime patients relapse and some receive endocrine therapy even doesn't require. By using proteomics and genomics data for frozen primary breast tumors, one can identify the correct or potential therapeutic option for these kind of patients.[84]

In 2017, Miriam RA et al., studied data obtain for 148 proteins by using RPPA method from 173 samples, separated the results into 5 categories: HER2 +ve, luminal, basal and reactive I and II, the identified reactive group contains protein which related to the microenvironment and fibroblasts. On behalf of functional protein classification by RPPA, heterogenous 6 protein were found in Luminal A type: Rad51, AMPKa, Caspace 9, 53BP1, p90RSK Thre359/Ser363 and GATA3.[84] Drawback of the study, that is contain widespread range of tumor content that may cause possible overlap among luminal A and reactive groups.

In 2015, Watcher A et al., developed a method that can identify recurrence of BRCA in ER/PR +ve patients group by using gene expression and quantitative analysis of RPPA data.[85] They utilized 3 methods: RPPA, support vector

machines and random forests. In high risk bearing tumors ki-67, NDKA and RPS6 highly expressed.[85] The outcomes had good specificity and sensitivity.

4.3. Invasive lobular BRCA

Around 10% of the total breast cancer are invasive lobular type but in general invasive lobular cancer specimens were not well signified in most of the trials for prognosis and therapeutic regimen.[86] To rectify this, a study was done by using proteomics, transcriptomic and genomic integration to enhance the findings.[86] Genomic expression sub divided into two sub categories, immune and hormone related that identified by pathway analysis. Hormone related category has ER, PR, Cell cycles and GATA3 while, immune related has mRNA upregulation of cytokine receptor transcripts or lymphoid signaling. By doing analysis through RPPA, enhance expression of ER, PR, Ser 118, HER2, GATA3 and fibronectin shown in hormone related group.[86] In transcriptomic and protein level, upregulation of GATA3, fibronectin, PR and down regulation of YAP1 were found.[86]

RPPA contribution is extraordinary and result oriented that helps in quantifying protein expression, gene expression and describing post translation altered protein.

5. EMERGING NOVEL BIOMARKERS

Emerging novel biomarkers have inadequate validation, this is due to assessment of these biomarkers on very limited patient group. Emerging biomarkers are vey useful in the research field for development of new diagnostic tools, for monitoring of disease, checking new drug therapies and drug sensitivity.

6. CONCLUSION

In conclusion, the intricate landscape of breast cancer classification and biomarkers presents a complex yet essential realm for understanding and managing this pervasive disease. With over a hundred identified types of cancer, each named according to the specific tissues and cells they affect, the classification of cancer extends beyond traditional parameters. Among these, breast cancer (BRCA) stands as a prominent concern, especially for women in India where it ranks as a prevalent and aggressive form of cancer.

In this comprehensive review, the discussion delves into the intricate world of protein biomarkers and their roles in BRCA diagnosis, prognosis, and treatment. Through techniques such as immunohistochemistry (IHC), researchers have identified key protein expressions associated with different types of BRCA, aiding in risk assessment and treatment planning. Notably, the development of innovative tools like the CanAssist-Breast (CAB) algorithm has shown promising potential in classifying risk and tailoring treatments for BRCA patients.

The advent of reverse phase protein arrays (RPPA) has amplified the precision of proteomics, enabling a detailed analysis of protein expressions and signaling pathways within breast tumors. This has led to novel insights into the heterogeneity of various BRCA subtypes, such as triple-negative breast cancer (TNBC) and invasive lobular carcinoma. Furthermore, the emergence of predictive markers like LAG3 and PD1 for TNBC has opened doors to immunomodulatory therapies.

However, the path towards effective personalized treatment remains a challenging endeavor. The interplay of factors such as molecular variations, heterogeneity, and patient-specific responses underscores the need for continued research and validation. With the integration of emerging technologies and expanding knowledge, the field of breast cancer

classification and biomarkers holds the promise of refining diagnostics and therapies, ultimately leading to improved patient outcomes. As science advances, the hope is that the insights gained from these endeavors will transform the landscape of breast cancer care, providing patients with optimized and tailored treatment strategies.

7. FUTURE ASPECTS

Looking ahead, the future of breast cancer research and treatment holds great promise as novel approaches and technologies continue to emerge. The complexities discussed in this review pave the way for several exciting future aspects:

Precision Medicine: The increasing understanding of heterogeneity within breast cancer subtypes is likely to propel the era of precision medicine forward. As more data is gathered from proteomics, genomics, and clinical parameters, advanced algorithms and AI-driven approaches will allow for more accurate prediction of disease progression and personalized treatment plans. Biomarker-guided therapies can minimize unnecessary interventions and side effects, leading to improved patient quality of life.

Advanced Proteomics Techniques: Rapid advancements in proteomics techniques will provide deeper insights into the molecular mechanisms underlying breast cancer. Techniques like single-cell proteomics and multiplex immunofluorescence are expected to unravel complex cellular interactions, enabling a more comprehensive understanding of tumor microenvironments and treatment resistance mechanisms.

Liquid Biopsies: Non-invasive liquid biopsies that analyze circulating tumor cells, cell-free DNA, and extracellular vesicles hold the potential to revolutionize cancer diagnosis and monitoring. These assays could provide real-time information about tumor dynamics, treatment response, and the emergence of resistance, enabling timely adjustments to therapy.

Integration of Multi-Omics Data: Integrating proteomics, genomics, transcriptomics, and other -omics data will provide a holistic view of breast cancer biology. This comprehensive approach can uncover novel biomarkers, therapeutic targets, and therapeutic vulnerabilities, leading to the development of more effective treatment strategies.

Immunotherapy Advancements: As the role of the immune system in cancer becomes clearer, immunotherapies are likely to play an increasingly vital role in breast cancer treatment. Developing combination therapies that target both cancer cells and the tumor microenvironment will enhance the effectiveness of immunotherapy approaches.

Validation and Clinical Translation: The ongoing validation of emerging biomarkers and technologies in large, diverse patient cohorts will be crucial for their successful translation into clinical practice. Rigorous validation will ensure that biomarkers are reliable, reproducible, and provide clinically meaningful insights.

Global Collaborations: International collaborations and data sharing initiatives will accelerate progress by pooling resources and expertise. This will be particularly beneficial for rare subtypes of breast cancer, where large datasets are essential for meaningful analyses.

In this dynamic landscape, interdisciplinary collaborations between clinicians, researchers, data scientists, and industry partners will be essential for driving innovation. By harnessing the power of emerging technologies and integrating

diverse data sources, the field of breast cancer research and treatment is poised to make significant strides towards improved patient outcomes and ultimately a world with more effective cancer management strategies.

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