Biomarkers of Breast Cancer

Endah Indriastuti[1], Fatimah Nur Fitriani[1*]

[1]Faculty of Medicine and Health, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia

*Corresponding Author. Email: typeyouremail@here.com

Telp: +62-12345678

Abstract

Background: Breast cancer is the primary cause of cancer-associated mortality among women worldwide. There have been many studies related to biomarkers in breast cancer.

Content: A Biomarker is a molecule in the body that is present in blood, other bodily fluids, or tissue and serves as an indicator of an aberrant or normal process, a condition, or an illness like cancer. Biomarkers in breast cancer that have been widely used in clinical settings include estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER2), Ki67, CEA, and Ca15-3. Circulating tumor cell (CTC) and genome sequencing are currently being researched and expected to be useful in the management of breast cancer patients.

Summary: Biomarkers are crucial for diagnosing, classifying, and determining the most effective treatment strategies such as personalized and targeted therapies for individuals with breast cancer. It also can be used as a prognostic tool to predict response toward therapeutic intervention and to detect disease recurrence. Technologies of breast cancer biomarkers are evolved and expected will produce a better patient outcome.

Keywords: breast cancer, biomarker, early detection, diagnosis, monitoring

Introduction

Cancer is a worldwide public health concern. Globally, breast cancer is the most prevalent cancer to be diagnosed.[1] Many studies on breast cancer have been conducted in the Asian region. The results demonstrated that breast cancer was the most prevalent cancer among Chinese and Japanese women. Cases were also found to increase over time in China, India, and Thailand, although the possible cause was an increase in early identification capabilities.[2] The most common cancer-related death in women is still breast cancer. However, despite this, the mortality rate for breast cancer is now starting to decline due to technological advances in diagnosis and therapy.[3]

With a mortality rate of 21.5 per 100.000 and an incidence of 40.3 per 100.000, breast cancer is Indonesia's leading cause of death. Government programs related to screening involve general practitioners and midwives in conducting clinical breast examinations. In women, breast cancer is the most prevalent cancer as well as the leading cause of death, followed by lung cancer.[2] Currently, there are many laboratory modalities for early breast cancer detection, diagnosis, and monitoring. Updates of breast cancer biomarkers in clinical practice are needed

Breast Cancer Classification

Breast cancer is a diverse disease that results in varying characteristics, aggressiveness, response to therapy, and prognosis in each type.[3] Therefore, breast cancer is commonly classified based on histologic type, immunophenotypic, and molecular genotypic classification.[4]

Based on histology type, breast cancer can be categorized into infiltrating duct carcinoma no special type (IDC-NST) which is the most prevalent histology type among invasive breast cancer, invasive lobular carcinoma (ILC), erbiform, mucinous, micropapillary, papillary, tubular, apocrine, and medullary metaplastic types.[4], [5]

Classification based on immunophenotyping is generally based on the presence or absence of ER, PR, and HER2 expression in breast cancer. Classification based on immunophenotyping expression is a simple classification that is widely used as a guide in determining therapy and prognosis. Based on this classification, breast cancer can be distinguished into breast cancer that expresses ER, PR, and or HER2, as well as triple-negative breast cancer (TNBC).[4], [6]

Improved technology has made it possible to investigate breast cancer from a molecular perspective. Classification based on existing gene-expressing profiles differentiates breast cancer into luminal type A, luminal B, HER2 overexpressing, basal-like breast cancer (BLBC), and normal-like tumor.[4], [6] However, normal-like tumor is currently excluded from the classification because it is considered as contamination of normal breast tissue.[6] Molecular-based classification requires cost and more advanced technology that may hinder the scope of its use. In clinical practice, molecular classification could be substituted by IHC analysis based on ER, PR, HER2, and Ki67.[4], [6]

Cancer biomarker

Any material, structure, or mechanism that can be measured in the body or its byproducts and affects or forecasts the occurrence of an event or disease is referred to as a biomarker. A biomarker is a molecule in the body that is present in blood, other bodily fluids, or tissue and serves as an indicator of an aberrant or normal process, a condition, or an illness like cancer, according to the National Cancer Institute.[7] Biomarkers are often protein

markers, nucleic acids, peptides, or antibodies. Biomarkers can also represent changes in a gene, such as gene expression or signatures of metabolomics and proteomics.[7], [8] Biomarkers should be objective, quantifiable laboratory measures that characterize biological processes. [9]

The evaluation of biomarkers in patients with breast cancer is essential not only for early identification but also for diagnosis, therapy consideration, and monitoring and detection of recurrences. Biomarkers in breast cancer that have been widely used in clinical settings include estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER2), Ki67, CEA, and Ca15-3.[10]–[16] Circulating tumor cell (CTC) and genome sequencing are currently being researched and expected to be useful in the management of breast cancer patients.[17]–[19]

Estrogen Receptor

Estrogen receptor (ER) activity has a major impact on the development of breast cancer. Estrogen hormone-induced ER activation is the primary factor influencing the majority of breast cancer cases.[20] The estrogen receptor is the receptor predominantly in nuclear locations, a nuclear steroid receptor that acts as a transcriptional regulator. Initially, two different estrogen receptor forms, α and β, are coded by two different genes, ESR 1 and ESR 2, respectively. ERα is a homodimer coded in human chromosome 6, while ERβ is a heterodimer coded in chromosome 14. The G-protein coupled estrogen receptor (GPER)-1 is a novel class of estrogen-binding protein that has recently been identified in target cells. Unlike Erα and Erβ which are nuclear estrogen receptors, the GPER1 is a membrane estrogen receptor.[21] Estrogen stimulates the nuclear receptors ERα and ERβ, which in turn encourages the growth and survival of both healthy and malignant cells in breast tissue. This activation will induce the transcription of genes favorable to survival as well as the triggering of cellular signaling.[21], [22]

The ERα mechanism as a transcription factor is already understood. ERα activation is involved in cell cycles and proliferation. The dysregulation of ERα expression, action, or deregulation of the target cell plays an important role in the development of breast cancer. Activation of ERα to estrogen has oncogenic potential promoting the growth of cancer cells and the likelihood of DNA damage. In contrast to ERα, activation of Erβ generally inhibits proliferation as well as trigger apoptosis, even though these effects are also dependent on the coactivator, the tissue, the cell, and the coexpression of ERα.[22] The effects of Erβ in breast cancer have not been fully established since some studies showed Erβ expression tends to correlate with favorable prognosis whereas other studies don't.[23]

The immunohistochemical stain is currently used to assess the presence of ER in breast cancer tissue. These are considered as ER+ if the staining is located in the cell nucleus while cytoplasmic staining is ignored.[24] Breast cancer tissue is classified as ER-negative (ER-) by the American Society of Clinical Oncology/College of American Pathologists if less than 1% or 0% of the tumor cell nuclei are immunoreactive. The percentage of 1-10% of immunoreactive cell nuclei is considered as ER low positive.[25] ER status examination is important in considering the administration of therapy and monitoring of metastatic conditions.[10], [26] In almost all patients with ER-positive breast cancer, adjuvant endocrine therapy is suggested to prevent metastasis, local or regional recurrence as well as contralateral tumors.[10] The presence of ER conversion in patients who experience metastasis, from ER- to ER+ corresponds with an increased overall survival rate compared to patients whose ER status remains negative.[26]

Progesterone Receptor

Hormone progesterone, a 21-carbon steroid, binds to progesterone receptors (PR) to promote the development of humans and other species, the female menstrual cycle, and

pregnancy. PR belongs to the family of ligand-dependent transcription factors known as steroid nuclear receptors.[27] Approximately 15–30% of luminal epithelial cells were positive for PR and ER. Progesterone binds to progesterone receptors and induces cell proliferation and differentiation through the activation of the receptor of the nuclear factor κB (NF-κB) ligand (RANKL) path.[28] On the other hand, PR plays a role in modulating ER activity. PR acts as a molecular rheostat that affects the chromatin binding and transcriptional activity of the ER. Progesterone that interacts with the ER can change the location of ER binding on chromatin. The presence of progesterone can inhibit estrogen-mediated growth in tumors in addition to having anti-proliferative effects.[29]

PR expression is determined by IHC examination of tumor tissue. PR+ samples are defined as having immunoreactivity between 1% and 100% of their tumor cell nuclei. Samples are classified as PR- if less than 1% or none at all of the tumor cell nuclei exhibit immunoreactivity.[25]Studies conducted on male breast cancer patients show that patients with ER+/PR- tend to be found at a younger age, have a higher histology grade, larger tumor size, and have a greater tendency to invade the lymph node and metastasis. ER+/PR- patients have a lower overall survival rate than ER+/PR+ breast cancer patients.[11] Evaluation of PR especially in ER+ breast cancer patients is important to determine the prognosis of therapy. Patients with PR+ have a good response to tamoxifen when compared to patients with no PR expression.[12], [13] PR evaluation is important to follow up as is the ER receptor. The presence of PR conversion is associated with a shortened overall survival rate compared to patients who do not experience PR status conversion.[26]

HER-2

Human epidermal growth factor receptor-2 (HER2) belongs to the human epithelial receptor group. HER family consists of 4 subtypes namely HER1. HER2, HER3 and HER4.

Activation of the receptor by its ligand can trigger homodimerization and heterodimerization which further activates the tyrosine kinase signal cascade. The activated cascade will then trigger cell proliferation, migration, invasion as well as cell survival.[30]

HER2 is a type of HER that has no ligand but is a preferred partner for other HERs's dimerization. ERBB2 gene, formerly known as neu, encodes HER2.[30] HER overexpression can be found in 20-30% of breast cancers.[31], [32] Breast cancers that are HER2 positive typically exhibit higher levels of aggressiveness and metastasis.[33] HER2 expression can be evaluated through immunohistochemical (IHC) examination of tumor tissue. HER2 gene amplification can be performed on tumor cells using in situ hybridization techniques with fluorescent, chromogenic, or silver markers.[34] HER2 is considered positive (+3) by IHC when more than 10% of the cells exhibit complete membrane staining. It is also considered positive by in situ hybridization (ISH) if there are six or more copies of the HER2 gene, HER2/chromosome 17 (CEP17) ratio of two or and HER2 copies of four, or HER2/CEP17 <2 and HER2 copies of six.[34], [35]

HER2 screening is not only important in considering targeted therapy for invasive breast cancer patients but also provides sufficient information on the prognosis among breast cancer patients.[36]–[38] Giving trastuzumab to breast cancer patients with an IHC score of +1 or +2 or patients with a FISH ratio <2, or HER2 gene copy <4 does not provide any benefit.[38] In contrast, patients with advanced breast cancer HER2 positive will be recommended therapy using HER2-targeted therapy such as trastuzumab and pertuzumab.[36]

Historically, an unfavorable prognosis has been linked to HER2 overexpression.[37] The prognosis of patients can be better estimated by looking at HER2 status along with other factors. Breast cancer patients with low HER2 expression are associated with better recurrence-free intervals in patients with HR+ and age less than 65 years.[39] Among HER2(+)

breast cancer patients, prognosis was related to histologic subtype. Patients with invasive lobular carcinoma subtype are associated with bone metastasis while patients with infiltration duct and lobular carcinoma (IDC & ILC) histology subtypes are associated with lung metastasis.[14] ER+/HER2+ patients tend to metastasize in the bone with better overall survival compared to ER-/HER2+ patients who tend to metastasize in the liver brain and lung.[40]

The extracellular domain of HER2 can be examined considering that part of HER2 can be released into the blood circulation through the extracellular domain shedding mechanism. Shedding-HER2 (sHER2) may increase especially in metastatic conditions. The examination of sHER2 can be done using serum samples so that it is non-invasive. However, the results of studies related to the correlation between HER2 in tissues and sHER2 have not been consistent. However, sHER2 can be an alternative examination where sHER2 can increase above 15ng/ml in 16.7-23.5 cases of early-stage breast cancer.[41]

Ki67

Johanes Gerder made the initial discovery of Ki67 and discovered that it was linked to an increasing histological grade of breast cancer. Nuclear antigen Ki67 is linked to cell proliferation. Each phase of the cell cycle, except G0 and G1, expresses the Ki67.[42] Further studies found that Ki67 expression can be found in G0 and G1, but is not detected in standard IHC.[43]

Ki67 expression was discovered and could be considered as a predictive biomarker in breast cancer.[44] The application of a cut-off value for Ki67 as a prognostic and predictive marker in patients with stage I or stage II breast cancer is the Ki67 expression of less than 5% or more than 30%.[45] Another study on Ki67 in the general population with breast cancer

discovered that low disease-free survival and overall survival were linked to an expression cut-off of 5–30%.[46], [47]

Ki67 has been assessed as a potential predictive biomarker for chemotherapy response and has shown prognostic significance in early ER-positive disease.[48] A prior study demonstrated that higher Ki67 expression following two weeks of endocrine therapy was significantly correlated with decreased recurrence-free survival, as opposed to higher Ki67 expression at baseline.[49] However, the use of Ki67 as a treatment decision is still not recommended due to analytical validity constraints that have not been standardized so it is still not fully reliable.[45]

CEA dan Ca 15-3

CEA stands for carcinoembryonic antigen. CEA was first discovered in colorectal carcinoma in 1965.[50] The human CEA gene family now has about 29 different genes. CEA can be expressed in cancer tissues but can also be expressed in normal adult tissues.[51] CEA levels from nipple aspirate fluid (NAF) samples in patients with breast cancer were found to be higher when compared to benign breast tumor patients and normal controls.[52]

Ca 15-3 is the MUC-1 protein in soluble form. This marker has been widely used especially in breast cancer. Under normal circumstances, the transmembrane protein MUC-1 is expressed at the apical portion of secretory epithelial cells' plasma membrane. In malignant changes, MUC-1 can be overexpressed both on the surface of the membrane and the cytoplasm, and MUC-1 will shed into circulation or soluble form. The increase of Ca 15-3 can be used as a tumor marker.[53]

CEA and Ca 15-3 are widely used as biomarkers in tumors because of their non-invasiveness, wide availability, and ease of performance.[54], [55] High levels of CEA and Ca

15-3 were found to have an association with the molecular type of breast tumor. High CEA is associated with HER2-positive and luminal B2 status. High Ca 15-3 was found in luminal B as well as TNBC.[56] Another study showed high levels of CEA and Ca 15-3 were found in patients with hormone receptor-positive. HER2-negative patients also had elevated CEA and Ca 15-3 levels.[15] Ca 15-3 levels were also found to be independently associated with molecular subtypes. Patients with HER2-positive luminal B had the highest Ca 15-3, followed by patients with HER2 overexpression ad luminal B with HER2 negative.[16]

CEA and Ca15-3 can be regarded as tumor markers for the prompt detection of metastasis from breast cancer. High CEA levels in breast cancer is correlated with the size of the tumor and metastatic status. High CEA levels were also associated with large tumor size and nodal metastasis.[56] Another study that indicated higher levels of CEA and CA15-3 in patients with metastatic breast cancer compared to non-metastatic patients corresponded to this finding.[15] The European Tumor Marker in Breast Cancer in their recommendations has recommended CA15-3 more than 50 U/mL and or CEA more than 20 ng/ml as tumor markers that can be used to screen for metastasis so that special attention can be given to patients of breast cancer with high CA15-3 and or CEA.[18]

Preoperative CEA as well as Ca15-3 measurements can also be used to estimate the prognosis of breast cancer patients. A cohort research conducted on more than 10,000 cases of breast cancer in women showed that high levels of Ca15-3 and CEA were correlated with low survival rates and disease-free survival. When compared with various molecular subtype groups, patients with luminal subtype A with high Ca15-3 and CEA showed the most reduction in survival rate and disease-free survival.[57]

A meta-analysis conducted by Shifu Tang found that CEA levels in breast cancer secretion can be considered as a method for diagnosing breast cancer. Based on this study, the

CEA test has a specificity of 87% with an area under curve result of 0.8570.[58] CEA and Ca15-3 are biomarkers that can be considered in terms of monitoring therapy. Patients who respond well to therapy will have decreased levels of CEA and Ca15-3. Conversely, patients who experience worsening clinical conditions will show increased levels of CEA as well as Ca15-3.[18]

CTC

Liquid biopsy has garnered significant attention as a potent tool in personalized medicine since it allows for real-time cancer patient follow-up and monitoring of the cancer progress. Circulating tumor cells (CTC) as part of liquid Biopsies already have a significant influence on cancer patients' prognosis, minimal residual disease (MRD) detection, treatment choices, and follow-up, and new research indicates that they may also be useful for early cancer detection. CTC is part of liquid biopsy. These cancer cells are separated from the main tumor and subsequently enter the bloodstream which may enable them to disperse to different body areas and form new tumors. Detection of circulating tumor cells (CTCs) from clinical samples can serve as a useful instrument for diagnosing cancer, treatment monitoring, and prognosis through liquid biopsy.[59]

CTCs present in peripheral blood retain a wide range of variations despite their minimal number. Effective techniques for identifying and catching CTCs are highly challenging and complex. The gathered CTCs can serve as the principle and basis for the subsequent evaluation and description. In order to accomplish this, several commercialized technologies for CTC capture have been developed in over a decade, each has a unique feature for sensitive CTC identification.[60] In this section, we typically categorize these methods as label-dependent or independent based on the cell surface markers they use. Label-dependent technologies apply the immunoaffinity principle for identifying and gathering CTC based on their surface marker.

The most commonly used, label-dependent technologies are based on the principle of using magnetic microbeads with antibodies attached. CTC is detectable by immunoreactive recognition of markers associated with cancer that are non-expression in leukocytes, i.e. EpCAM and CK, while CD45 is the opposite. The first and only system authorized by the US Food and Drug Administration (FDA) for CTC assessment in metastatic malignancies is CellSearch®. This method is widely regarded as the gold standard.[17], [61]

Label-independent technologies come from a consideration that CTC expression of epithelial markers such as EpCAM and CKs to variable degrees in different types of cancer as well as different stages, some isolation must be based on the other approach. To overcome this possibility, CTCs were identified based on their size, density, and electrical characteristics.[60] Based on their size, CTCs can be selected using membrane filters, microfluidic chips, and hydrodynamics approaches. Bigger isolated cells from other cells will be gathered without considering the surface marker.[61]

The potential use of CTC in breast cancer includes various aspects, i.e. in early detection, diagnosis, treatment monitoring as well as helping diagnosis of breast cancer. The CTC can be detected in blood samples before the tumor is visible on imaging tests. In the field of monitoring treatment, CTC levels can indicate the effectiveness of the treatment as well as detect disease recurrences. The CTC can also predict the prognosis of breast cancer patients as high CTC levels are associated with poor outcomes. The CTC can provide information on molecular characteristics of the tumor that can be considered to be used in treatment selection and predict treatment response. The CTC can also be used in identify new treatment targets and develop personalized treatment strategies.[17]

Genome sequencing

The genome is a whole of genetic material in an organism. It is composed of DNA or RNA in some viruses that contain genes and accompanying elements that regulate gene activity. The human genome is the same in each individual, although some variations can cause variations in the appearance and health tendencies of each person.[62] Genome sequencing is a procedure to determine the sequence of bases in the genome of the cells in a single process. Genome sequencing examination technology has developed rapidly.[63], [64]

Currently, genome sequencing technology has developed from the first generation to the third generation.[63], [64] Radioactive labels were utilized by Sanger to identify nucleotide sequences in the initial generation of genome sequencing.[65] The first generation has further updated the label with non-radioactive, fluorescent labels.[66] The second generation of genome sequencing is based on the principle of pyrosequencing.[63] Pyrosequencing technology is a sequencing technique based on the release of pyrophosphate during DNA synthesis. Through several enzymatic cascades, visible light will be generated in proportion to the number of nucleotides.[67] Next-genome sequencing (NGS), which is distinguished by high throughput and single-molecule DNA sequencing, started in second-generation genome sequencing. Second-generation genome sequencing is a short-read sequencing technology that has the principle of sequencing fragmented DNA that occurs individually simultaneously and massively. Third-generation genome sequencing is distinguished from second-generation based on its ability to perform the sequencing process on unfragmented DNA in real time, minimizing genome-wide repeats and structural variant detection.[64]

The important role of genome sequencing in breast cancer management has been studied in previous studies. Generally, genome sequencing could find the drivers of mutations in breast cancer that can affect the development of the disease, the possibility of genomic variations that cause variations in the results of therapy, and the choice of therapy that might be given to patients with certain mutations.[19], [68] Among the crucial functions of genome

sequencing in breast cancer is to advance the understanding of somatic mutations in breast cancer patients. Types of mutation commonly found in driver mutations are substitution mutations, copy number aberrant, insertion-deletion, and rearrangement. The mutation of BRCA1 and BRCA2 is a signature mutation. The majority of BRCA1 or BRCA2 mutations are germline although some are somatic mutations. Several genes are thought to be recessive which were not previously found including MED23, FOXP1, MLLT4, XBP1 and ZFP36L1.[19]

A previous study also found that the common mutation in patients with metastatic breast cancer is ESR1 mutation. This mutation is responsible for resistance to the main therapy of aromatase inhibitors.[68] Another study conducted in a great enough population of advanced breast cancer patients who had experienced metastasis also tried to determine the genomic alteration in those patients. Patients with a history of resistance to hormonal therapy for breast cancer showed the results of ESR1 mutations, disruption of the MAPK pathway, and disruption of estrogen receptor transcriptional regulation, so that in clinical applications, breast cancer patients with this picture will provide the possibility of unsatisfactory results against aromatase inhibitor therapy.[69] To address this condition, other therapies like selective degraders and modulators of the estrogen receptor are still being developed to overcome therapy in ESR1 mutation patients.[68]

A study of BRCA1/2 in breast cancer patients found that BRCA1/2 mutation patients had a good response to Olaparib. Therapy tailored to genomic alteration based on the European Society of Medical Oncology (ESCO) scale for clinical actionability of molecular targets (ESCAT) can improve the progression-free survival rate.[70] Previous research also showed five percent of patients with metastatic breast cancer had hypermutation in their genome sequencing results. Patients with hypermutation had a good response to pembrolizumab therapy, a programmed death-1 (PD-1) inhibitor.[71]

Genomic study in metastatic breast cancer tried to identify driver mutations based on hormone receptor expression. Cases with hormone receptor expression (estrogen and/or progesterone receptor) (HR+) but low HER2 (HER2-) levels were more likely to have nine driver mutations, namely NCOR1, AKT1, NF1, RIC8A, RB1, TP53, ESR1, GATA3, KMT2C, when compared to the early-stage breast cancer. An unfavorable prognosis is associated with HR+/HER2-metastasized breast tumors that had several hallmark mutations coupled with TP53, RB1, and NF1 alterations.[72]

Conclusion

Biomarkers are crucial for diagnosing breast cancer, classifying breast cancer types, and determining the most effective treatment strategies such as personalized and targeted therapies for individuals with breast cancer. It also can be used as a prognostic tool to predict response toward therapeutic intervention and to detect disease recurrence. The examination of ER, PR, HER2, and Ki-67 are prognostic and predictive factors as well as become considerations in determining patient therapies. HER2 screening is important in considering targeted therapy for invasive breast cancer patients. Shedding-HER2 (sHER2) may increase especially in metastatic conditions, despite previous studies that have not shown a consistent correlation between HER2 in tissues and sHER2 in circulation. Both CEA and Ca15-3 can be taken into consideration when monitoring therapy and are used for the early detection of metastases from breast cancer. CTC has an important role in early cancer diagnosis and detection of recurrences as it can be detected before the tumor is visible on imaging tests. Genome sequencing could find the drivers of mutations in breast cancer that can affect the development of the disease. The genomic variations may affect consideration in the therapeutic regiment selection and the results of therapy. Technologies of breast cancer biomarkers are evolved, however, comprehensive genomic researches are expected to be conducted so that personalized therapy will produce a better patient outcome.

Authors' Contributions

EI drafted, wrote, edited the manuscript. FF wrote the manuscript. All authors have agreed with the final manuscript.

References

[1] M. Arnold *et al.*, "Current and future burden of breast cancer: Global statistics for 2020 and 2040," *Breast*, vol. 66, pp. 15–23, Dec. 2022, doi: 10.1016/j.breast.2022.08.010.

[2] S. V. S. Deo, J. Sharma, and S. Kumar, "GLOBOCAN 2020 Report on Global Cancer Burden: Challenges and Opportunities for Surgical Oncologists," *Ann Surg Oncol*, 2022, doi: 10.1245/s10434-022-12151-6.

[3] E. A. Rakha and A. R. Green, "Molecular classification of breast cancer: what the pathologist needs to know," *Pathology*, vol. 49, no. 2, pp. 111–119, Feb. 2017, doi: 10.1016/j.pathol.2016.10.012.

[4] J. Y. S. Tsang and G. M. Tse, "Molecular Classification of Breast Cancer," 2019. [Online]. Available: www.anatomicpathology.com

[5] G. Cserni, "Histological type and typing of breast carcinomas and the WHO classification changes over time," *Pathologica*, vol. 112, no. 1. Pacini Editore S.p.A., pp. 25–41, Mar. 01, 2020. doi: 10.32074/1591-951X-1-20.

[6] E. Provenzano, G. A. Ulaner, and S. F. Chin, "Molecular Classification of Breast Cancer," *PET Clinics*, vol. 13, no. 3. W.B. Saunders, pp. 325–338, Jul. 01, 2018. doi: 10.1016/j.cpet.2018.02.004.

[7] N. L. Henry and D. F. Hayes, "Cancer biomarkers," *Molecular Oncology*, vol. 6, no. 2. John Wiley and Sons Ltd, pp. 140–146, 2012. doi: 10.1016/j.molonc.2012.01.010.

[8] J. L. Wilson and R. B. Altman, "Biomarkers: Delivering on the expectation of molecularly driven, quantitative health," *Exp Biol Med*, vol. 243, no. 3, pp. 313–322, Feb. 2018, doi: 10.1177/1535370217744775.

[9] K. Strimbu and J. A. Tavel, "What are biomarkers?," *Current Opinion in HIV and AIDS*, vol. 5, no. 6. pp. 463–466, Nov. 2010. doi: 10.1097/COH.0b013e32833ed177.

[10] H. J. Burstein, "Systemic Therapy for Estrogen Receptor–Positive, HER2-Negative Breast Cancer," *New England Journal of Medicine*, vol. 383, no. 26, pp. 2557–2570, Dec. 2020, doi: 10.1056/nejmra1307118.

[11] J. L. Wei, J. X. Zhang, and D. Y. Fu, "Characterization and prognosis of estrogen receptor-positive/progesterone receptor-negative male breast cancer: A population-

based study," *World J Surg Oncol*, vol. 16, no. 1, Dec. 2018, doi: 10.1186/s12957-018-1539-7.

[12] A. Nordenskjöld, H. Fohlin, T. Fornander, B. Löfdahl, L. Skoog, and O. Stål, "Progesterone receptor positivity is a predictor of long-term benefit from adjuvant tamoxifen treatment of estrogen receptor positive breast cancer," *Breast Cancer Res Treat*, vol. 160, no. 2, pp. 313–322, Nov. 2016, doi: 10.1007/s10549-016-4007-5.

[13] M. Stendahl, L. Rydén, B. Nordenskjöld, P. E. Jönsson, G. Landberg, and K. Jirström, "High progesterone receptor expression correlates to the effect of adjuvant tamoxifen in premenopausal breast cancer patients," *Clinical Cancer Research*, vol. 12, no. 15, pp. 4614–4618, Aug. 2006, doi: 10.1158/1078-0432.CCR-06-0248.

[14] Y. Wang *et al.*, "Histologic heterogeneity predicts patient prognosis of HER2-positive metastatic breast cancer: A retrospective study based on SEER database," *Cancer Med*, vol. 12, no. 18, pp. 18597–18610, Sep. 2023, doi: 10.1002/cam4.6469.

[15] M. M. Uygur and M. Gümüş, "The utility of serum tumor markers CEA and CA 15–3 for breast cancer prognosis and their association with clinicopathological parameters," *Cancer Treat Res Commun*, vol. 28, Jan. 2021, doi: 10.1016/j.ctarc.2021.100402.

[16] D. Ruswendro, S. A. Syamsu, R. Thabry, A. Seweng, and A. N. Usman, "Association between molecular subtype of local advanced breast cancer with Ca 15-3 level," *Breast Dis*, vol. 40, no. S1, pp. S119–S122, 2021, doi: 10.3233/BD-219018.

[17] H. Zhang *et al.*, "Detection Methods and Clinical Applications of Circulating Tumor Cells in Breast Cancer," *Frontiers in Oncology*, vol. 11. Frontiers Media S.A., Jun. 02, 2021. doi: 10.3389/fonc.2021.652253.

[18] R. Molina *et al.*, "Tumor markers in breast cancer - European group on tumor markers recommendations," *Tumor Biology*, vol. 26, no. 6. IOS Press BV, pp. 281–293, 2005. doi: 10.1159/000089260.

[19] S. Nik-Zainal *et al.*, "Landscape of somatic mutations in 560 breast cancer whole-genome sequences," *Nature*, vol. 534, no. 7605, pp. 47–54, May 2016, doi: 10.1038/nature17676.

[20] A. B. Hanker, D. R. Sudhan, and C. L. Arteaga, "Overcoming Endocrine Resistance in Breast Cancer," *Cancer Cell*, vol. 37, no. 4. Cell Press, pp. 496–513, Apr. 13, 2020. doi: 10.1016/j.ccell.2020.03.009.

[21] N. Fuentes and P. Silveyra, "Estrogen receptor signaling mechanisms," in *Advances in Protein Chemistry and Structural Biology*, vol. 116, Academic Press Inc., 2019, pp. 135–170. doi: 10.1016/bs.apcsb.2019.01.001.

[22] L. Clusan, F. Ferrière, G. Flouriot, and F. Pakdel, "A Basic Review on Estrogen Receptor Signaling Pathways in Breast Cancer," *International Journal of Molecular Sciences*, vol. 24, no. 7. MDPI, Apr. 01, 2023. doi: 10.3390/ijms24076834.

[23] Y. Choi, “Estrogen Receptor β Expression and Its Clinical Implication in Breast Cancers: Favorable or Unfavorable?,” *Journal of Breast Cancer*, vol. 25, no. 2. Korean Breast Cancer Society, pp. 75–93, Apr. 01, 2022. doi: 10.4048/jbc.2022.25.e9.

[24] C. A. Lamb, S. I. Vanzulli, and C. Lanari, “HORMONE RECEPTORS IN BREAST CANCER: MORE THAN ESTROGEN RECEPTORS”.

[25] K. H. Allison *et al.*, “Estrogen and Progesterone Receptor Testing in Breast Cancer: ASCO/CAP Guideline Update,” *J Clin Oncol*, vol. 38, pp. 1346–1366, 2020, doi: 10.1200/JCO.19.

[26] Z. B. Yi *et al.*, “Profile and outcome of receptor conversion in breast cancer metastases: a nation-wide multicenter epidemiological study,” *Int J Cancer*, vol. 148, no. 3, pp. 692–701, Feb. 2021, doi: 10.1002/ijc.33227.

[27] A. R. Daniel, C. R. Hagan, and C. A. Lange, “Progesterone receptor action: Defining a role in breast cancer,” *Expert Review of Endocrinology and Metabolism*, vol. 6, no. 3. pp. 359–369, May 2011. doi: 10.1586/eem.11.25.

[28] Z. Li, H. Wei, S. Li, P. Wu, and X. Mao, “The Role of Progesterone Receptors in Breast Cancer,” *Drug Design, Development and Therapy*, vol. 16. Dove Medical Press Ltd, pp. 305–314, 2022. doi: 10.2147/DDDT.S336643.

[29] H. Mohammed *et al.*, “Progesterone receptor modulates ERα action in breast cancer,” *Nature*, vol. 523, no. 7560, pp. 313–317, Jul. 2015, doi: 10.1038/nature14583.

[30] D. F. Hayes, “HER2 and Breast Cancer — A Phenomenal Success Story,” *New England Journal of Medicine*, vol. 381, no. 13, pp. 1284–1286, Sep. 2019, doi: 10.1056/nejmcibr1909386.

[31] C. R. King, M. H. Kraus, and S. A. Aaronson, “Amplification of a Novel v-erbB-Related Gene in a Human Mammary Carcinoma.” [Online]. Available: www.sciencemag.org

[32] D. J. Slamon, G. M. Clark, S. G. Wong, W. J. Levin, A. Ullrich, and W. L. Mcguire, “Human Breast Cancer: Correlation of Relapse and Survival with Amplification of the HER-2lneu Oncogene.” [Online]. Available: http://science.sciencemag.org/

[33] H. M. Asif, S. Sultana, S. Ahmed, N. Akhtar, and M. Tariq, “HER-2 positive breast cancer - A mini-review,” *Asian Pacific Journal of Cancer Prevention*, vol. 17, no. 4. Asian Pacific Organization for Cancer Prevention, pp. 1609–1615, 2016. doi: 10.7314/APJCP.2016.17.4.1609.

[34] A. C. Wolff *et al.*, “JOURNAL OF CLINICAL ONCOLOGY,” *J Clin Oncol*, vol. 36, pp. 2105–2122, 2018, doi: 10.1200/JCO.

[35] F. Cardoso *et al.*, "Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up," *Annals of Oncology*, vol. 30, no. 8, pp. 1194–1220, Aug. 2019, doi: 10.1093/annonc/mdz173.

[36] S. H. Giordano *et al.*, "Systemic Therapy for Advanced Human Epidermal Growth Factor Receptor 2-Positive Breast Cancer: ASCO Guideline Update," 2022. [Online]. Available: www.asco.org/breast-cancer-guidelines.

[37] C. Corti, F. Giugliano, E. Nicolò, P. Tarantino, C. Criscitiello, and G. Curigliano, "HER2-Low Breast Cancer: a New Subtype?," *Current Treatment Options in Oncology*, vol. 24, no. 5. Springer, pp. 468–478, May 01, 2023. doi: 10.1007/s11864-023-01068-1.

[38] L. Fehrenbacher *et al.*, "NSABP B-47/NRG Oncology Phase III Randomized Trial Comparing Adjuvant Chemotherapy With or Without Trastuzumab in High-Risk Invasive Breast Cancer Negative for HER2 by FISH and With IHC 1+ or 2+," *J Clin Oncol*, vol. 38, pp. 444–453, 2019, doi: 10.1200/JCO.19.

[39] X. Yang, Y. Li, X. lu, X. Ren, and B. Hua, "Clinicopathological features and prognosis of patients with HER2-low breast cancer," *BMC Cancer*, vol. 23, no. 1, Dec. 2023, doi: 10.1186/s12885-023-11421-0.

[40] C. A. Arciero *et al.*, "ER+/HER2+ Breast Cancer Has Different Metastatic Patterns and Better Survival Than ER−/HER2+ Breast Cancer," *Clin Breast Cancer*, vol. 19, no. 4, pp. 236–245, Aug. 2019, doi: 10.1016/j.clbc.2019.02.001.

[41] A. Perrier, J. Gligorov, G. Lefèvre, and M. Boissan, "The extracellular domain of Her2 in serum as a biomarker of breast cancer," *Laboratory Investigation*, vol. 98, no. 6. Nature Publishing Group, pp. 696–707, Jun. 01, 2018. doi: 10.1038/s41374-018-0033-8.

[42] Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U, and Stein H, "Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. ," *J Immunol*, vol. 133, no. 4, pp. 1710–1715, Oct. 1984.

[43] J. Bullwinkel, B. Baron-Lühr, A. Lüdemann, C. Wohlenberg, J. Gerdes, and T. Scholzen, "Ki-67 protein is associated with ribosomal RNA transcription in quiescent and proliferating cells," *J Cell Physiol*, vol. 206, no. 3, pp. 624–635, Mar. 2006, doi: 10.1002/jcp.20494.

[44] B. S. Finkelman, H. Zhang, D. G. Hicks, and B. M. Turner, "The Evolution of Ki-67 and Breast Carcinoma: Past Observations, Present Directions, and Future Considerations," *Cancers*, vol. 15, no. 3. MDPI, Feb. 01, 2023. doi: 10.3390/cancers15030808.

[45] T. O. Nielsen *et al.*, "Assessment of Ki67 in Breast Cancer: Updated Recommendations From the International Ki67 in Breast Cancer Working Group," *J Natl Cancer Inst*, vol. 113, no. 7, pp. 808–819, Jul. 2021, doi: 10.1093/jnci/djaa201.

[46] R. Yerushalmi, R. Woods, P. M. Ravdin, M. M. Hayes, and K. A. Gelmon, "Ki67 in breast cancer: prognostic and predictive potential," *The Lancet Oncology*, vol. 11, no. 2. pp. 174–183, Feb. 2010. doi: 10.1016/S1470-2045(09)70262-1.

[47] J. S. Reis-Filho and N. E. Davidson, "Ki67 Assessment in Breast Cancer: Are We There Yet?," *Journal of the National Cancer Institute*, vol. 113, no. 7. Oxford University Press, pp. 797–798, Jul. 01, 2021. doi: 10.1093/jnci/djaa202.

[48] G. Viale *et al.*, "Predictive value of tumor Ki-67 expression in two randomized trials of adjuvant chemoendocrine therapy for node-negative breast cancer," *J Natl Cancer Inst*, vol. 100, no. 3, pp. 207–212, Feb. 2008, doi: 10.1093/jnci/djm289.

[49] M. Dowsett *et al.*, "Prognostic value of Ki67 expression after short-term presurgical endocrine therapy for primary breast cancer," *J Natl Cancer Inst*, vol. 99, no. 2, pp. 167–170, Jan. 2007, doi: 10.1093/jnci/djk020.

[50] "phil gold".

[51] S. Hammarstrom, "((((( ))))) The carcinoembryonic antigen CEA family: structures, suggested functions and expression in normal and malignant tissues," 1999.

[52] Y. S. Zhao *et al.*, "Nipple aspirate fluid collection, related factors and relationship between carcinoembryonic antigen in nipple aspirate fluid and breast diseases in women in Harbin, PRC," *Cancer Epidemiology Biomarkers and Prevention*, vol. 18, no. 3, pp. 732–738, Mar. 2009, doi: 10.1158/1055-9965.EPI-08-0715.

[53] M. J. Duffy, D. Evoy, and E. W. McDermott, "CA 15-3: Uses and limitation as a biomarker for breast cancer," *Clinica Chimica Acta*, vol. 411, no. 23–24. pp. 1869–1874, Dec. 14, 2010. doi: 10.1016/j.cca.2010.08.039.

[54] F. Fazli Khalaf, M. Asadi Gharabaghi, M. Balibegloo, H. Davari, S. Afshar, and B. Jahanbin, "Pleural CEA, CA-15-3, CYFRA 21-1, CA-19-9, CA-125 discriminating malignant from benign pleural effusions: Diagnostic cancer biomarkers," *International Journal of Biological Markers*, vol. 38, no. 2, pp. 81–88, Jun. 2023, doi: 10.1177/03936155231158661.

[55] P. Hogendorf *et al.*, "Elevated preoperative levels of CA19-9 and CA125 predicts overall survival time in the pancreatic adenocarcinoma. Single institution series."

[56] W. Zhao *et al.*, "Association of Preoperative Serum Levels of CEA and CA15-3 with Molecular Subtypes of Breast Cancer," *Dis Markers*, vol. 2021, 2021, doi: 10.1155/2021/5529106.

[57] J. Li *et al.*, "Tumor markers CA15-3, CA125, CEA and breast cancer survival by molecular subtype: a cohort study," *Breast Cancer*, vol. 27, no. 4, pp. 621–630, Jul. 2020, doi: 10.1007/s12282-020-01058-3.

[58] S. Tang *et al.*, "CEA in breast ductal secretions as a promising biomarker for the diagnosis of breast cancer: a systematic review and meta-analysis," *Breast Cancer*, vol. 23, no. 6, pp. 813–819, Nov. 2016, doi: 10.1007/s12282-016-0680-9.

[59] Z. Deng, S. Wu, Y. Wang, and D. Shi, "Circulating tumor cell isolation for cancer diagnosis and prognosis-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)," *EBioMedicine*, vol. 83, p. 104237, 2022, doi: 10.1016/j.

[60] Z. Feng *et al.*, "Circulating tumor cells in the early detection of human cancers," *Int J Biol Sci*, vol. 18, no. 8, pp. 3251–3265, 2022, doi: 10.7150/ijbs.71768.

[61] H. Zhang *et al.*, "Detection Methods and Clinical Applications of Circulating Tumor Cells in Breast Cancer," *Frontiers in Oncology*, vol. 11. Frontiers Media S.A., Jun. 02, 2021. doi: 10.3389/fonc.2021.652253.

[62] A. David Goldman and L. F. Landweber, "What Is a Genome?," 2016, doi: 10.1371/journal.pgen.1006181.g001.

[63] E. E. Hilt and P. Ferrieri, "Next Generation and Other Sequencing Technologies in Diagnostic Microbiology and Infectious Diseases," *Genes*, vol. 13, no. 9. MDPI, Sep. 01, 2022. doi: 10.3390/genes13091566.

[64] T. Hu, N. Chitnis, D. Monos, and A. Dinh, "Next-generation sequencing technologies: An overview," *Hum Immunol*, vol. 82, no. 11, pp. 801–811, Nov. 2021, doi: 10.1016/j.humimm.2021.02.012.

[65] F. Sander and A. R. Goulson, "A Rapid Method for Determining Sequences in DNA by Primed Synthesis with DNA Polymerase," 1976.

[66] W. Ansorge, B. S. Sproat, J. Stegemann, and C. Schwager, "A non-radioactive automated method for DNA sequence determination," 1986.

[67] M. Ronaghi, "Pyrosequencing Sheds Light on DNA Sequencing," *Genome Res*, vol. 11, no. 1, pp. 3–11, Jan. 2001, doi: 10.1101/gr.150601.

[68] J. O. Brett, L. M. Spring, A. Bardia, and S. A. Wander, "ESR1 mutation as an emerging clinical biomarker in metastatic hormone receptor-positive breast cancer," *Breast Cancer Research*, vol. 23, no. 1. BioMed Central Ltd, Dec. 01, 2021. doi: 10.1186/s13058-021-01462-3.

[69] P. Razavi *et al.*, "The Genomic Landscape of Endocrine-Resistant Advanced Breast Cancers," *Cancer Cell*, vol. 34, no. 3, pp. 427-438.e6, Sep. 2018, doi: 10.1016/j.ccell.2018.08.008.

[70] F. Andre *et al.*, "Genomics to select treatment for patients with metastatic breast cancer," *Nature*, vol. 610, no. 7931, pp. 343–348, Oct. 2022, doi: 10.1038/s41586-022-05068-3.

[71] R. Barroso-Sousa *et al.*, "Prevalence and mutational determinants of high tumor mutation burden in breast cancer," *Annals of Oncology*, vol. 31, no. 3, pp. 387–394, Mar. 2020, doi: 10.1016/j.annonc.2019.11.010.

[72] F. Bertucci *et al.*, "Genomic characterization of metastatic breast cancers," *Nature*, vol. 569, no. 7757, pp. 560–564, May 2019, doi: 10.1038/s41586-019-1056-z.