Breast cancer, screening and diagnostic tools: All you need to know

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Review

Breast Cancer, Screening and Diagnostic Tools: All You Need to Know

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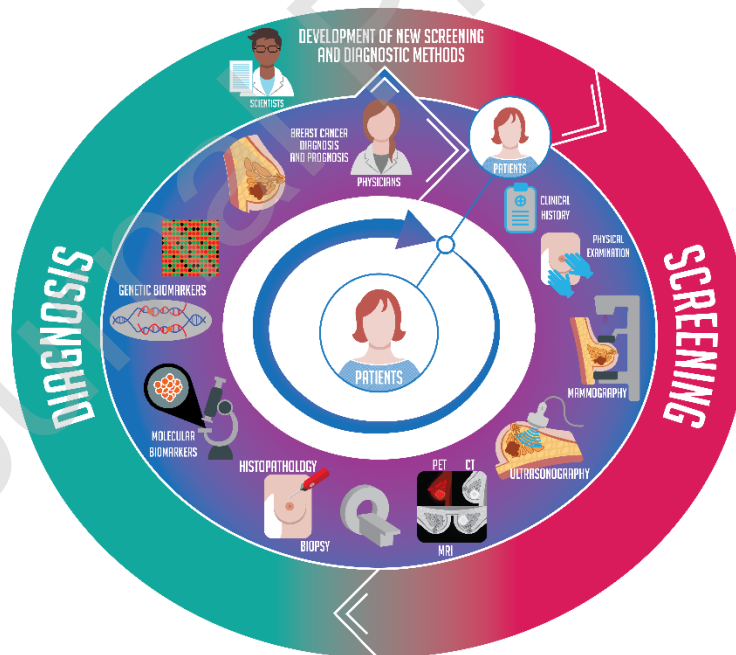
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Graphical Abstract

Highlights

- Nearly two million cases of breast cancer are diagnosed each year worldwide.

- Multidisciplinary approaches are used to develop screening and diagnostic tools.
- Key information is provided on breast cancer for medical and research professionals.
- Breast cancer biology, standard and innovative detection tools are presented.
- This information is needed to develop better diagnostic and screening tools.

Abstract

Breast cancer is one of the most frequent malignancies among women worldwide. Methods for screening and diagnosis allow health care professionals to provide personalized treatments that improve the outcome and survival. Scientists and physicians are working side-by-side to develop evidence-based guidelines and equipment to detect cancer earlier. However, the lack of comprehensive interdisciplinary information and understanding between biomedical, medical, and technology professionals makes innovation of new screening and diagnosis tools difficult. This critical review gathers, for the first time, information concerning normal breast and cancer biology, established and emerging methods for screening and diagnosis, staging and grading, molecular and genetic biomarkers. Our purpose is to address key interdisciplinary information about these methods for physicians and scientists. Only the multidisciplinary interaction and communication between scientists, health care professionals, technical experts and patients will lead to the development of better detection tools and methods for an improved screening and early diagnosis.

Abbreviations

ACCE	Analytical validity, Clinical validity, Clinical utility, Ethical, legal, and social implications
ACS	American Cancer Society
ADC	Apparent Diffusion Coefficient
AI	Artificial Intelligence
AIB1	Coactivator Amplified in Breast Cancer 1
AJCC	American Joint Committee on Cancer
ACR	American College of Radiology
anti-CTLA-4	Anti-Cytotoxic T-Lymphocyte-Associated Protein 4
anti-PD1	Anti-Programmed Cell Death Protein 1
anti-PD-L1	Anti-Programmed Cell Death-1 Ligand 1
AR	Androgen Receptor
ARFI	Acoustic Radiation Force Impulse Imaging
ASCO	American Society of Clinical Oncology
BCSC	Breast Cancer Surveillance Consortium
BI-RADS	Breast Imaging-Reporting and Data System
BMI	Body Mass Index
BPx	Breast Physical Examination
BCS	Breast Cancer Screening
CAD	Computer-Aided Detection
CAFs	Cancer-Associated Fibroblasts

CBE	Clinical Breast Examination
CDC	Centers for Disease Control and Prevention
CEM	Contrast-Enhanced Mammography
CE-MRI	Contrast Enhanced-MRI
Chi3L1	Chitinase 3-like 1
CSCs	Cancer Stem Cells
ctDNA	Circulating Tumor DNA
CTS	Clinical Treatment Score
CTX	Chemotherapy
CXCL4	Chemokine (C-X-C motif) Ligand 4
DBT	Digital Breast Tomosynthesis
DCIS	Ductal Carcinoma *In Situ*
DNA	Deoxyribonucleic Acid
DITI	Digital Infrared Thermography Imaging
DOT	Diffuse Optical Tomography
DW-MRI	Diffusion Weighted-MRI
ECIBC	European Breast Guidelines of the European Commission Initiative on Breast Cancer
EGFR	Epidermal Growth Factor Receptor
EMT	Epithelial to Mesenchymal Transition
EORTC	European Organization for Research and Treatment of Cancer
EPclin®	EndoPredict Clinical®
ER+/-	Estrogen/Estradiol Receptor Positive/Negative
EREs	Estrogen Response Elements
ERRs α/β/γ	Estrogen-Related Receptors α/β/γ
ESMO	European Society for Medical Oncology
EXET	Extended Endocrine Trial
EXOs	Exosomes
FES	16α-18F-fluoro-17β-estradiol
FDA	Food and Drug Administration
FDG	Fluorodeoxyglucose
FDS	Fiberoptic Ductoscopy
FFA	Free Fatty Acids
FFDM	Full-Field Digital Mammography
GEP/S	Gene Expression Profiling/Signatures
GLOBOCAN	Global Cancer Observatory
HbT	Hydrogen Breast Test
HDA1	Histone Deacetylase 1
HER1/2/3 +/-	Human Epidermal Growth Factor Receptor 1/2/3 Positive/Negative
HR+/-	Hormone Receptor Positive/Negative

HT	Hormonal Therapy
IDC	Invasive Ductal Carcinoma
IDO	Indoleamine 2,3-Dioxygenase
IHC	Immunohistochemistry
IHC4	Immunohistochemical 4
IL	Interleukin
IoT	Internet of Things
IR	InfraRed
LCIS	Lobular Carcinoma *In Situ*
LN+/-	Lymph-Nodes Positive/Negative
mAbs	Monoclonal Antibodies
MHC-I	Major Histocompatibility Complex I
MINDACT	Microarray in Node-Negative Disease May Avoid Chemotherapy Trial
miRNAs	MicroRNAs
ML	Machine Learning
MRE	Magnetic Resonance Elastography
MRI	Magnetic Resonance Imaging
MSCs	Mesenchymal Stem Cells
mTOR	Mammalian Target of Rapamycin
NADH	Reduced Nicotinamide Adenine Dinucleotide
NCCN	National Comprehensive Cancer Network
N-CoR	Nuclear Receptor Co-Repressor
NmU	Neuromedin U
OCT	Optical Coherence Tomography
PET/CT	Positron Emission Tomography/Computed Tomography
PGC1α/1β	Peroxisome Proliferator-Activated Receptor Gamma Co-Activator 1α/1β
PPARγ	Peroxisome Proliferator-Activated Receptor-γ
PR+/-	Progesterone Receptor Positive/Negative
RASTER	microarRAy-prognoSTics-in-breast-cancER
ROI	Region of Interest
ROS	Reactive Oxygen Species
RTK	Receptor Tyrosine Kinase
SBI	Society of Breast Imaging
SEER	Surveillance, Epidemiology and End Results
SG	St. Gallen International Breast Cancer Conference
SND	Spontaneous Nipple Discharge
SoC	System-on-Chip
STAT3	Signal Transducer and Activator of Transcription 3
StO$_2$	Oxygen Saturation
TA-MUC1	Tumor-Associated Transmembrane Glycoprotein Mucin 1

^{99m}Tc-MIBI	Technetium-99m Sestamibi
TFF1	Trefoil Factor 1
TMA	Tissue Microarray
TME	Tumor Microenvironment
TNM	Tumor, Node and Metastasis
TransATAC trial	Trans Arimidex, Tamoxifen, Alone or in Combination trial
UCPs	Uncoupling Proteins
US	United States
USPSTF	U.S. Preventive Services Task Force
VEGF	Vascular Endothelial Growth Factor
YAP	Yes-Associated Protein

Key words: Breast cancer, screening, diagnosis, tools, mammography, biopsy, genetic profiling, artificial intelligence.

1. Introduction

Each year, 2.1 million women are diagnosed with breast cancer [1]. In the United States (US), the five-year relative survival after diagnosis for localized breast cancer is 98.8%, for regional cancer is 85.5%, for distant cancer is 27.4% and for unknown disease stage is 54.5% [2]. Early diagnosis strategies aim to increase the number of accurately identified early stage breast cancers by increasing the access to diagnostic services and by providing opportune cancer treatment [1]. Early diagnosis is key for improving patients' survival, as it gives insight regarding the most appropriate therapeutic strategy for each case [3]. Breast cancer screening (BCS) is defined as testing women before any evident symptoms appear, generally by mammography and clinical breast examination (CBE) to detect and treat cancers or pre-cancers [1]. Public and professional knowledge with patient awareness of the disease is necessary to take actions such as determining what cases need to use screening and diagnostic tools **(Fig. 1)**. It is our opinion that multidisciplinary communication between technology experts, biomedical researchers and physicians should be improved with the goal to ameliorate the survival rate of patients by providing reliable screening and diagnostic tools.
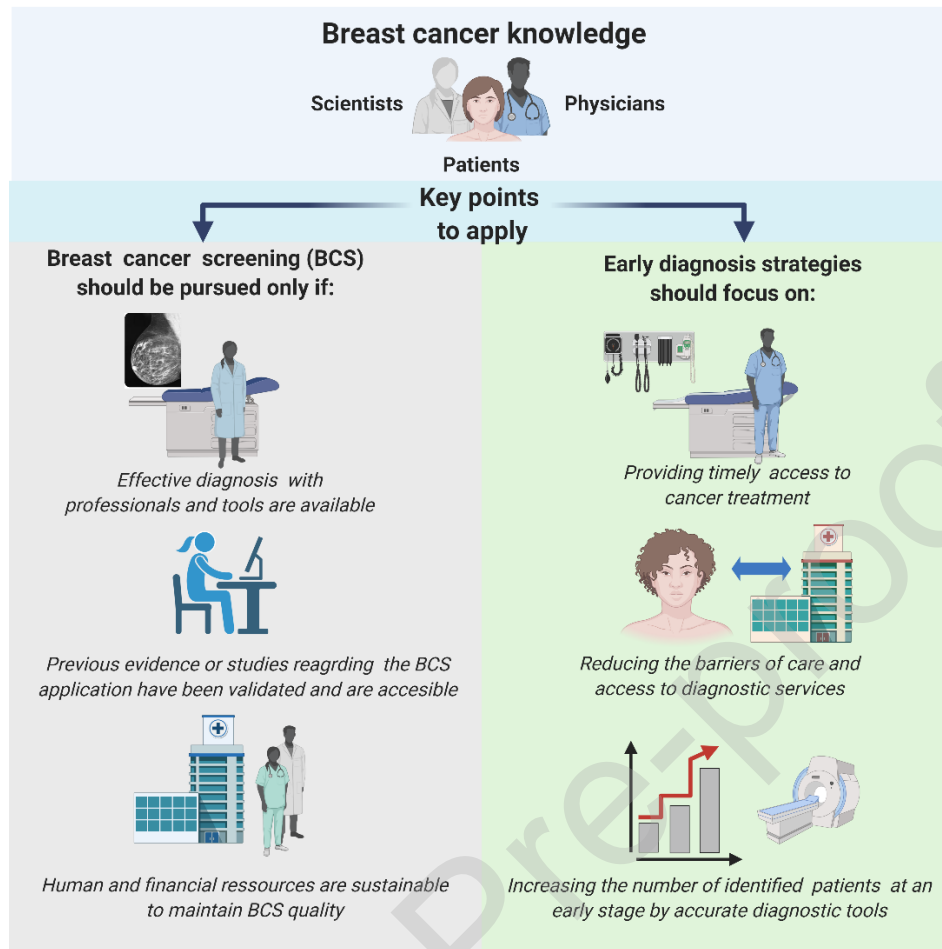
Figure 1. Breast cancer knowledge from scientists, physicians and patients lead to the implementation of adequate screening and breast cancer early diagnosis. The implementation of breast cancer screening must be done only if professionals and tools are available to provide effective diagnosis. Previous evidence reports the success of a screening campaign/initiative and assure that the BCS campaign or initiative is sustainable with allocated human and financial resources to ensure its quality. Even if these recommendations are concise, there is no evidence still that the interventions for raising breast cancer awareness in women work, as there is a lack of high-quality studies with larger samples and follow up for a longer time. Early diagnosis is the best strategy so far that increase the survival rate of patients and allows an effective therapeutic decision. The best strategies for an early diagnosis aim to provide a timely access to cancer treatment, reduce the barriers between patients and health care services and finally being able to increase the number of correctly identified patients with breast cancer with accurate diagnostic tools. Created with BioRender.com.

The US National Comprehensive Cancer Network (NCCN) 2019 guidelines recommended performing an annual CBE and mammography for women over 40 years old, as there are no randomized studies that suggest a benefit in screening women under this age [4–7]. This

recommendation is the preferred screening approach, making it the gold standard in the US [4–6]. It has been observed that BCS for women of all ages, with an average risk of having the disease, is associated with a mortality reduction in the US [8]. However, more studies regarding which specific populations in terms of age and ethnicity are recommended for early BCS as there is still uncertainty [4–6,8]. The practice of BCS lies in the clinical judgement of health care professionals based on scientific evidence. Nevertheless, the interaction between physicians, researchers and technology experts is necessary to refine the application of BCS.

The results obtained from BCS and diagnostic tools could be misinterpreted leading to false positives, false negatives, re-tests, and overdiagnosis [9]. Hence, artificial intelligence (AI) based algorithms are under constant development and evaluation by technology experts, biomedical researchers and physicians to decrease the possibility of misinterpretations. Another issue is the discomfort and stress generated during screening and diagnostic procedures which reduce the willingness of patients to perform the tests [10–14]. It is common that diagnostic equipment and tests are mostly accessible in specialized hospitals and cannot be easily reached by the population living in rural areas or that have transportation impairments [15]. Due to this possible obstacle, BCS campaigns have been done, emphasizing rural areas, which have helped women at risk to increase their survival rate [16]. In definitive terms, improving accessibility and comfort of BCS and diagnostic tools could improve the detection of patients living in rural areas allowing early therapy if required.

Molecular analysis such as the identification of mutations and gene expression profiling/signatures (GEP/S) are commonly used to determine breast cancer predisposition, subtype and aggressiveness, respectively. It has been observed that 5% to 10% of breast cancer patients have a genetic predisposition to develop the disease [17]. After initial screening, the analysis of GEP/S in breast biopsies is a complex task as each cancer develops differently in many aspects from one person to another, impairing the accuracy of the analysis [18]. The comprehension of gene-set expression abnormalities and their correlation with the aggressiveness involves an exhaustive and accurate task using bioinformatics and machine learning (ML) tools [19]. Recent studies support the use of GEP/S to improve treatment decisions which could lead to better patient outcomes [6,20,21].

A great amount of patient's data has been collected worldwide during different phases of breast cancer with the use of multiple screening and diagnostic tools **(Graphical abstract)**. These data correspond to a variety of tests, including physical examination, imaging, immunohistochemical analysis, GEP/S, and large-scale genomics. The analysis of these data would help in the understanding of what is needed to develop devices or diagnostic algorithms updating clinical guidelines. Advances in systems biology could integrate the available information, to generate new biomarker panels and devices [22]. However, many low-to-middle income countries lack the capacity to condense information and integrate an international medical

and scientific network such as the Global Cancer Observatory (GLOBOCAN) [23–25]. Combining different low-cost analysis procedures would lead to better access for more accurate recommendations and the treatment of cancer at an early stage in developing countries [26,27].

It is important to provide the necessary information concerning the most common diagnostic procedures and the use of new combinatorial approaches. This will support the decision of health professionals regarding the application of one tool over another. In addition, understanding the physiology of the breast and how it changes during cancer is key to develop new screening and diagnostic tools **(Graphical abstract)**. In this comprehensive review, we introduce and summarize key information about the normal breast tissue, cancer biology, current methods of screening and detection, emerging methods of detection, staging, grading and classification of breast cancer, as well as molecular and genetic biomarkers **(Fig. 2)**. Nowadays, the ability to comprehend the main aspects of breast cancer biology and the most used methods of detection will allow the development of innovations to satisfy patient needs in three aspects: accuracy, comfort and accessibility.
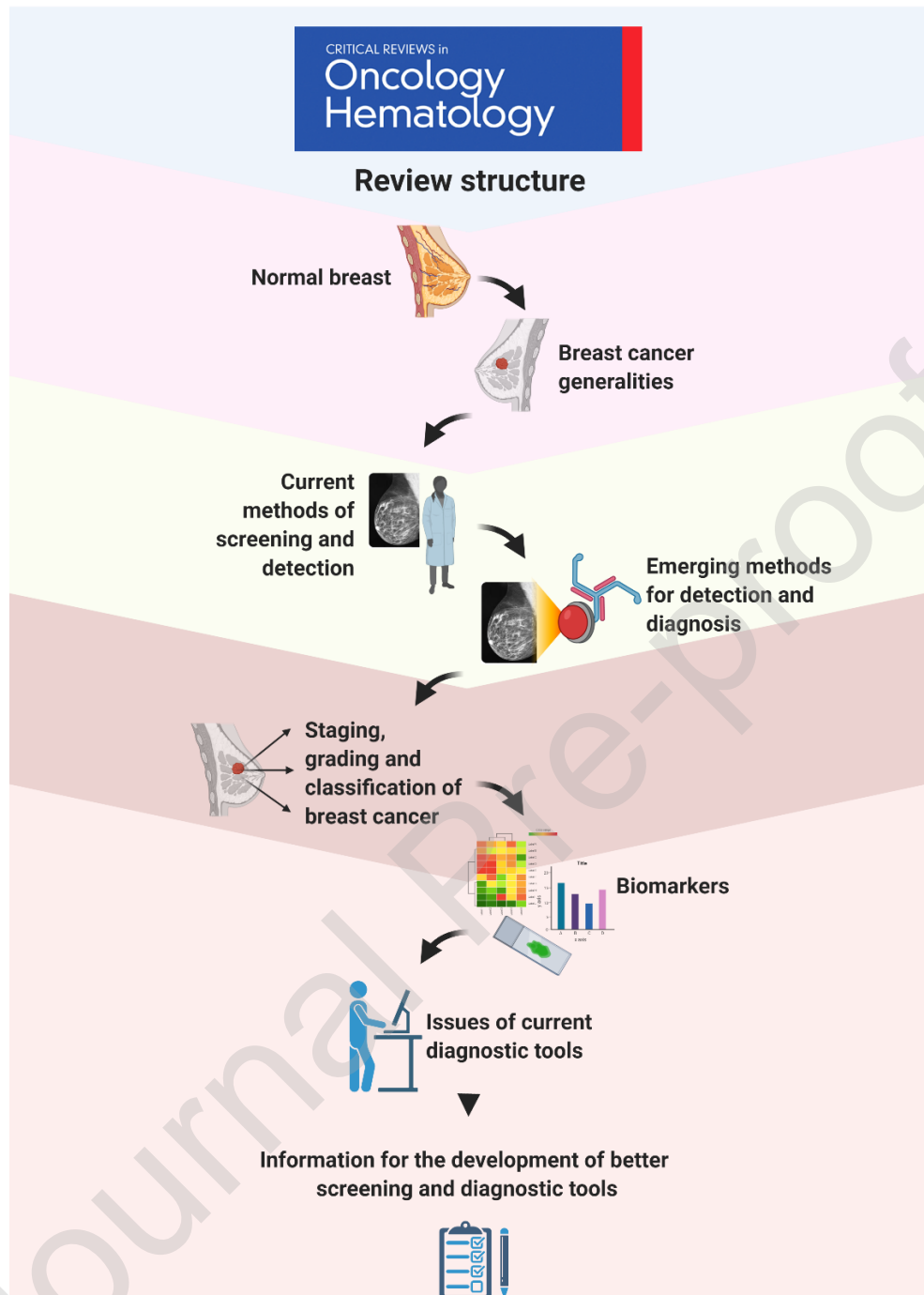
Figure 2. Review structure. The aim of this review is to provide patients, technology experts, physicians and scientists with key information to understand breast cancer screening and diagnosis. We will provide you with the information concerning the normal breast and cancer biology, the established and emerging methods for screening and detection, staging and grading, molecular and genetic biomarkers. Finally, we discuss current key issues in the journey of screening and diagnosis, and how these information could help to generate better tools. Created with BioRender.com.

2. Normal Breast Tissue

Breast cancer awareness entails the understanding of the differences between the normal physiology of the breast and a lesion that could result benign, precancerous or cancerous [28–30]. The NCCN recommends that women should be familiar with their breast and be able to communicate with the health care provider to transmit any sign of alarm [7]. Strengthen the knowledge of the normal physiology would prevent or decrease the negligence of women regarding self-inspection and search for help from a physician for CBE timely [31]. It is our belief that having basic knowledge of breast anatomy would help technology experts to communicate better with physicians and develop better screening and diagnostic procedures (**Fig. 3**).

The micro and macro-anatomy of mammary glands change according to age and hormonal secretions throughout a female's lifetime. Breast development stages are: fetal, neonatal/prepubertal and post pubertal [32]. It continues with pubertal expansion (only women who experiment pregnancy and childbirth experiment all breast developmental stages which includes pregnancy/lactation cycle and post-lactation involution) and postmenopausal involution [32]. Mammary glands start forming at the sixth week of gestational growth. By the end of the fetal formation, only the main lactiferous ducts are completely structured and the mammary glands do not develop further until puberty [32,33]. In females, breasts start growing around 8-12 years of age in response to hormonal changes related to puberty. This includes an increase in plasma concentration of estrogen, prolactin, luteinizing hormone, follicle-stimulating hormone and growth hormone. Later, the breast duct development continues until menstruation [32,34]. A mature breast is made up of multiple tissue layers such as fatty, glandular and dense tissue [35].

Anatomically, the breast is located over the pectoral muscles and sustained by the suspensory ligaments of the breast (Cooper's) [36]. It is composed of a complex network of arteries, veins and lymph vessels that carry blood, nutrients and chemical messengers as well as drain waste material. Blood supply of the breast is provided by the internal mammary and the lateral thoracic arteries in addition to small blood vessels. The venous drainage of the breast consists of veins localized in the thoracic, axillary and cephalic regions. The lymphatic drainage from the breast comprehends the axillary nodes and the internal mammary nodes. The lymph is mainly drained from the medial and lateral nodes to the axillary node [32]. The fat layer surrounds the glandular tissue which is organized into 15 to 20 sections called lobes of adipose tissue, and within each lobe there are smaller structures called lobules that contain between 10 and 100 alveoli (0.12mm diameter) [32,36]. These glands become fully mature during pregnancy and lactation cycle, where the organ is prepared to synthesize and secrete milk, a process that requires about 25% of daily maternal energy [32,33].

Normal interindividual differences in breast tissue distribution are influenced by genetic background and lifestyle [37]. The heterogeneous distribution of breast tissues does not permit to establish standard measures. However, the breast density ratio between fibro glandular and adipose tissue is estimated to be 1:1 by mammography screening [32]. According to the Centers for Disease Control and Prevention (CDC), 10% of women possess fatty breasts, 40% breasts with few areas of dense tissue, 40% heterogenous breasts and 10% have extremely dense breasts. Studies have shown that around 66% of premenopausal women and 33% of elderly women (75-79 years) have breasts with a 50% of density or more [38,39]. The Breast Imaging-Reporting and Data System (BI-RADS) classifies breast density into: BI-RADS A: mostly composed by fatty tissue (low density), BI-RADS B: scattered fibro glandular tissue (medium density), BI-RADS C: heterogeneous tissue (high density) and BI-RADS D: extremely dense tissue (very high density) [38,39]. Dense breast tissue is considered an independent biological risk factor for the development of breast cancer. Additionally, it restrains the evaluation of breast cancer by health professionals and limits the detection of early stage tumors due to its masking effect, decreasing the sensitivity of mammograms [38–40].

Breasts are in constant change due to several factors such as hormonal changes, genetics, breast density and lifestyle. Understanding the anatomy and physiology of normal breast tissue is critical to conceive breast cancer development and could support early detection of suspicious lesions. Breast cancer awareness provides valuable information to reduce negligence in women about their health. It could also encourage presentation to a healthcare professional and promotes communication among patients and physicians (**Fig. 3**).
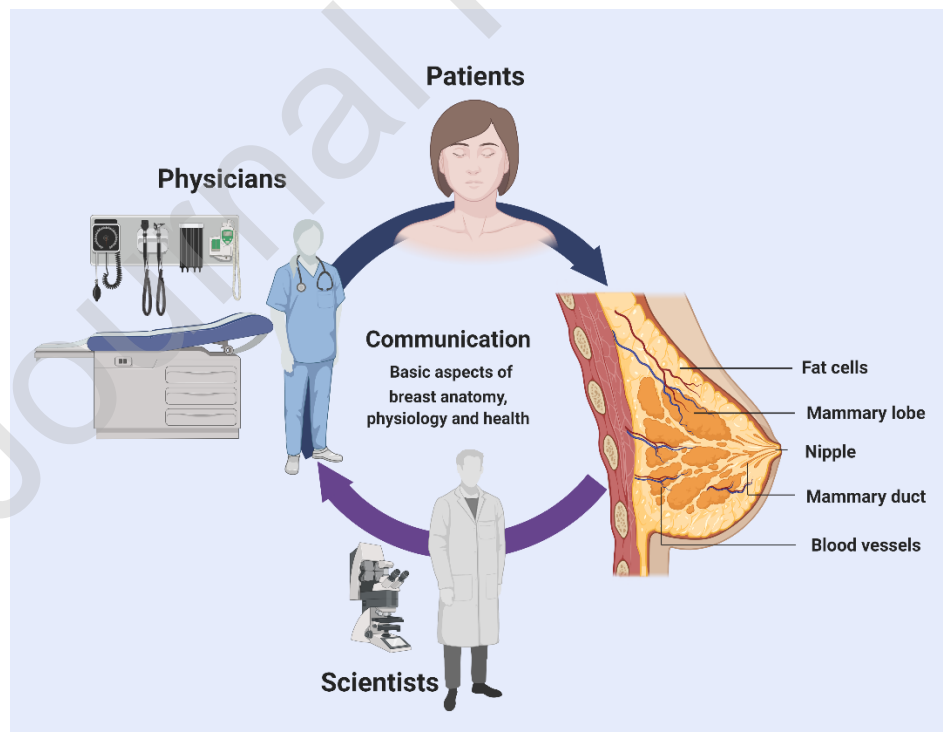
Figure 3. Communication among patients, physicians, and scientists is essential to understand the basic aspects of breast anatomy and health. Transmitting information about normal breast tissue, its physiology, lesion symptoms, and clinical management is key for an early diagnosis and development of better diagnostic approaches. Created with BioRender.com.

3. Breast Cancer Generalities

Cancer can be caused by a mix of genetic predisposition, environmental agents and lifestyle (**Fig. 4**) [2]. Age and breast density are naturally occurring factors that could increase the risk of developing the disease [41]. In addition, it is well known that changes in the circadian rhythm, as well as alcohol and tobacco consumption are agents that augment the probability of having breast cancer [42–44]. These factors can cause cellular stress, increase the production of reactive oxygen species (ROS) and induce changes in hormone concentrations of estrogen and progesterone, amplifying the aggressiveness of the tumor [45,46]. It has been observed that poor eating habits augment the risk of breast cancer [47–49]. Women with a body mass index (BMI) over 25 are at higher risk of developing cancer and recurrence in comparison to those with a normal BMI. Estrogen overproduction within adipose breast tissue, in combination with chronic inflammation due to obesity, stimulates the growth of hormone receptor positive (HR+) tumors [50,51]. An effective immune-surveillance as a result of a healthy lifestyle could reinforce the detection and elimination of abnormal cancer cells in an early stage, thereby decreasing the possibilities of cancer progression [48,49,52]. However, when cancer cells are already installed, they modify their microenvironment generating immune-scape and facilitating the growth and invasion of other tissue compartments [53,54].
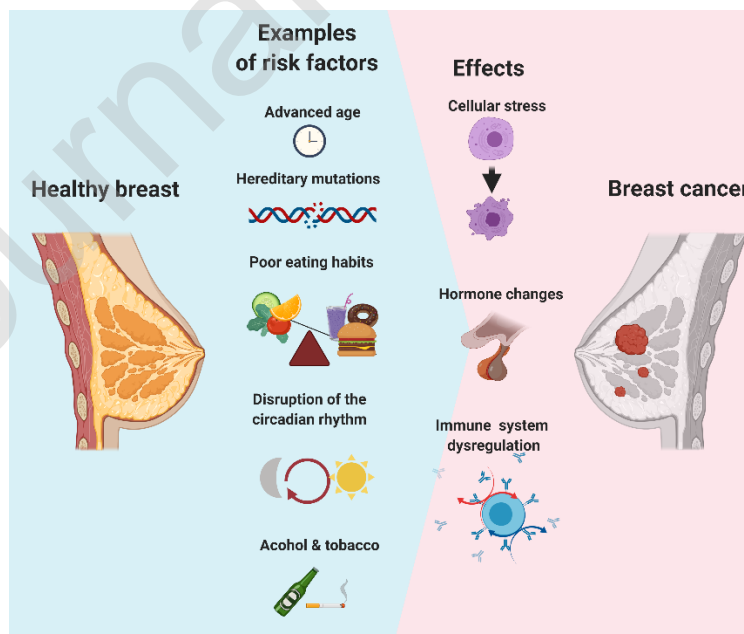
Figure 4. Breast cancer risks and effects. Factors such as advanced age, hereditary mutations, poor eating habits, disruption of the circadian rhythm, alcohol and tobacco consumption can cause cellular stress, hormone changes and perturbations in the immune cells leading to cancer. Created with BioRender.com.

Cancer could start from a variety of cell types within breast tissue **(Refer to Breast Cancer Classification)**. Both differentiated and adult stem cells could carry inherited or acquired mutations [55,56]. These mutations grant cells independence from their self-control mechanisms, such as division checkpoints, leading to excessive proliferation, resistance to cell death and survival, treatment resistance and even replicative immortality [57]. These hallmarks result in an advantage to intratumor selective pressure and natural selection leading to adaptation and tumor progression [56–58]. Cancer stem cells (CSCs) and differentiated cells at the origin of cancer will grow with increasing complexity and generate multiple interactions with the surrounding stroma [55,59]. Cancer cells interact with host cells such as mesenchymal stem cells (MSCs), immune cells, vascular endothelial cells, and adipocytes [55,59–62]. These interactions shape the tumor microenvironment (TME) and play an essential role in the development of the disease. TME interactions which involve the secretion of micro vesicles, cytokines and other molecules can be measured and correlated with the rate of tumor development or aggressiveness [57,63–65]. During tumor progression, cells lose their epithelial markers, causing them to remodel their interaction with the TME's extracellular matrix, detach themselves from each other and from the underlying basement membrane and become mobile [66]. This process is known as the epithelial to mesenchymal transition (EMT), which is associated with an increased tumor-initiating capacity, metastatic potential and resistance to chemotherapy (CTX) agents [57,66].

Multiple interactions occur inside the breast's TME promoting or interrupting its development [67]. The presence and number of immune cells in the TME vary among individuals, as various factors can influence their homing to the tumor and their activity [68]. Depending on individual immunity, innate and adaptive immune cells could promote or stop tumorigenesis. Inflammation in the TME can trigger molecules such as indoleamine 2,3-dioxygenase (IDO) in a first instance, however, this molecule can promote immune tolerance to cancer in a later stage [69]. Cancer cells can also generate multiple adaptations to be unrecognized by immune cells. These cells can express specific surface antigens to diminish the response of the immune system, can lose expression of major histocompatibility complex I (MHC-I), and can lose cell death inducers, among other mechanisms [70–72]. Cancer cells can also change the phenotype of stromal cells such as fibroblasts and MSCs, transforming them in cancer-associated fibroblasts (CAFs) which provide the cancer with nutrients, survival factors and intracellular material, such as mitochondria [61,73,74]. CAFs can also impair the activation of immune cells by secreting interleukin (IL)-4, IL-6 and IL-8, inducing myeloid cell differentiation [75]. Expression of chemokine (C-X-C motif) ligand 4 (CXCL4) by CAFs affect the recruitment of macrophages to the tumor, and the secretion

of factors such as Chitinase 3-like 1 (Chi3L1), which promote the transformation of macrophages in M2 (immune-regulatory function) [75,76]. Chronic inflammation in the tumor can also promote its progression and metastasis as it suppresses the immune responses [77]. Yes-associated protein (YAP), Signal transducer and activator of transcription 3 (STAT3) and Notch are stem cell activation factors that respond to inflammatory signals promoting the emergence of CSCs in the tumor [77,78]. Chronic inflammation has been observed to change the physical and temperature dynamics in the breast, which can be measured together with the aberrant levels of inflammatory cytokines during cancer progression [79–81].

The great cell heterogeneity of breast cancer is due to the increasing number of mutations supported by a high proliferation rate and individual environmental risk factors that influence and promote carcinogenesis [82,83]. During the process of cancer development, multiple interactions between mutations and signaling pathways inside cells drive the tumor and its malignancy. It has been shown that tumor cells can pass through metabolic changes and overexpress proteins related to thermogenesis such as the uncoupling proteins (UCPs), which could increase and modify normal temperature dynamics in the breast [84]. Breast cancer cells can switch to a glycolytic metabolism and increase lactate production in a process called the "Warburg effect" [85]. The most aggressive breast cancers tend to have an increased glucose consumption, using glucose carbon sources to support tumorigenesis. This process is activated by several receptors including the receptor tyrosine kinase (RTK), epidermal growth factor receptor (EGFR), among others which are highly expressed in cancers with the worst prognosis [86,87]. Glycolysis has been correlated with the overexpression of the neuropeptide Neuromedin U (NmU), inflammatory factors such as IL-6 and the presence of CSCs [88]. The presence of free fatty acids (FFA) in blood, a factor associated with obesity, has shown to be related to higher proliferation and aggressiveness in breast cancer cells. These FFA activates the mammalian Target of Rapamycin (mTOR) pathway increasing the metabolic rate and growth of cancer. These adaptations rewire the metabolism of tumor cells, allowing their adaptation to poor perfusion, lack of nutrients and accumulation of metabolic byproducts such as lactate in the TME [57,89]. The characteristics of cellular energetics deregulation are being used as targets for diagnosis and development of treatments in breast cancer [57,90,91].

Breast cancer acquires specific biological characteristics during its development that can be used to generate better diagnostic tools. Tumor metabolic changes, temperature variations and texture dynamics could offer the possibility to increase the number of measurable factors for sensors. Devices and software could use these factors for the development of an integrative analysis of cancer aggressiveness leading to better prognosis. Liquid biopsies that could contain micro vesicles, tumor circulating deoxyribonucleic acid (DNA), cytokines and other factors located in the TME are becoming more studied to develop minimally invasive procedures. Before the arrival of all these potential devices, software and new biopsy procedures in clinical practices,

they must be validated in a process that could take time and investment as they move along each step of clinical trials generating evidence of safety and efficacy [92,93].

4. Breast Cancer Screening Recommendations

Recommendations for BCS depend on the risk factors of each woman. Nonetheless, patients can be classified into two major groups: average population risk and high associated risk (e.g. BRCA1/2 mutations).

4.1 Women with Average Risk

The NCCN recommends breast awareness and CBE as part of routine check-ups, every one to three years from the age of 25 to 39, and annually from the age of 40 [7]. On the contrary, the American Cancer Society (ACS) does not recommend BSE or CBE, however it highlights the need for women to become familiar with their breasts and to promptly inform their doctor if they notice any changes [94].

The most recommended screening method for average-risk women is mammography. Even though the test has its limitations, it has been shown to decrease breast cancer mortality and detect smaller cancers leading to less invasive treatments. Beyond this agreement, there is controversy regarding the range age and frequency for mammography screening. Several organizations publish recommendations based on scientific evidence that assess benefits, risks, and side effects of screening methods for its inclusion in clinical practice [7,94,95]. Current guidelines consider the variables noted above for screening recommendations. According to the NCCN [7] and American College of Radiology (ACR) [95], mammography is suggested from the age of 40. The European Breast Guidelines of the European Commission Initiative on Breast Cancer (ECIBC) [96] and the ACS [94] propose to begin mammography at 45 years of age. Nonetheless, the ACS raises the possibility to start the screening at 40 years of age in patients who agree with their physician. Regarding the frequency of screening, the NCCN and the ACR recommend annual mammography [7,94,95]. The ACS suggests annual screening in women aged 45 to 54, and biennial screening from 55 onwards or annual depending on the patient preference [94]. The ECIBC proposes biennial or triennial mammography in women aged 45 to 49, biennial between 50 and 69 (higher level of evidence on mortality reduction), and triennial for patients aged 70 to 74. The ECIBC establishes an upper age limit of 75 for breast screening [96]. However, the NCCN, ACS and ACR guidelines do not determine an age limit, but highlight that such decision should be assessed considering the comorbidities and life expectancy of each patient [7,94,96].

Clinical guidelines also recommend digital breast tomosynthesis (DBT) for BCS considering its risks and benefits. The NCCN suggests its use since it can decrease the rate of clinical recall and increase cancer detection. However, the guidelines warn of higher radiation

exposure and lack of evidence on mortality reduction. The ACS agrees with the benefits of DBT in accordance with current and general recommendations pointing out a possible higher cost of examination [94]. The ACR highlights its usefulness in women under 50 years of age with dense breasts, spiculated masses and asymmetries in mammograms [95]. Regardless, previous indications, the ECIBC recommends mammography over DBT alone or combined to mammography. The guidelines justify this decision, based on the increased human, time and economic resources involved in DBT as well as its limited evidence on mortality improvement [96].

4.2 Women with High Risk

The NCCN, ACS and ACR agree to incorporate magnetic resonance imaging (MRI) in addition to annual mammography at younger age, for the population with a high risk of developing the disease: 1) lifetime risk greater than 20% measured with risk assessment tools based on family history; 2) carriers of genetic mutation (BRCA1/2) or their unproven first-degree relatives; 3) non-BRCA mutations associated with hereditary breast cancer syndromes (Li-Fraumeni, Cowden, Bannayan-Riley-Ruvalcaba syndrome or first degree relatives with the syndrome); 4) history of mantle radiation for Hodgkin lymphoma between the ages of 10 and 30. There is absence of strong evidence in favor or against MRI screening in women with history of breast cancer, *in situ* lobular neoplasia, atypical ductal and lobular hyperplasia, or life risk of 15-20% [7,94–96].

Since the sensitivity of mammography is limited by high breast density, the NCCN, ACR and ECIBC suggest that manual/automated ultrasound or DBT increases breast cancer detection; however, ultrasound could result in a high false positive rate and unnecessary biopsies. Finally, MRI is not recommended for mammographically high dense breasts. [7,94–96]. Recommendations on breast screening will continue to change as new techniques are developed and current methods evolve. The goal is to provide an informed and personalized BCS with a balance between risks and benefits.

5. Established Methods of Screening and Diagnosis

Current methods of screening and detection in clinical practice include Breast Physical Examination (BPx), Mammography (including full-field digital mammography (FFDM) and DBT), Ultrasonography and MRI (**Fig. 5**). Positron Emission Tomography/Computed Tomography (PET/CT) is commonly used for diagnosis and staging. All these methods vary between one another regarding their utility, sensitivity and specificity.
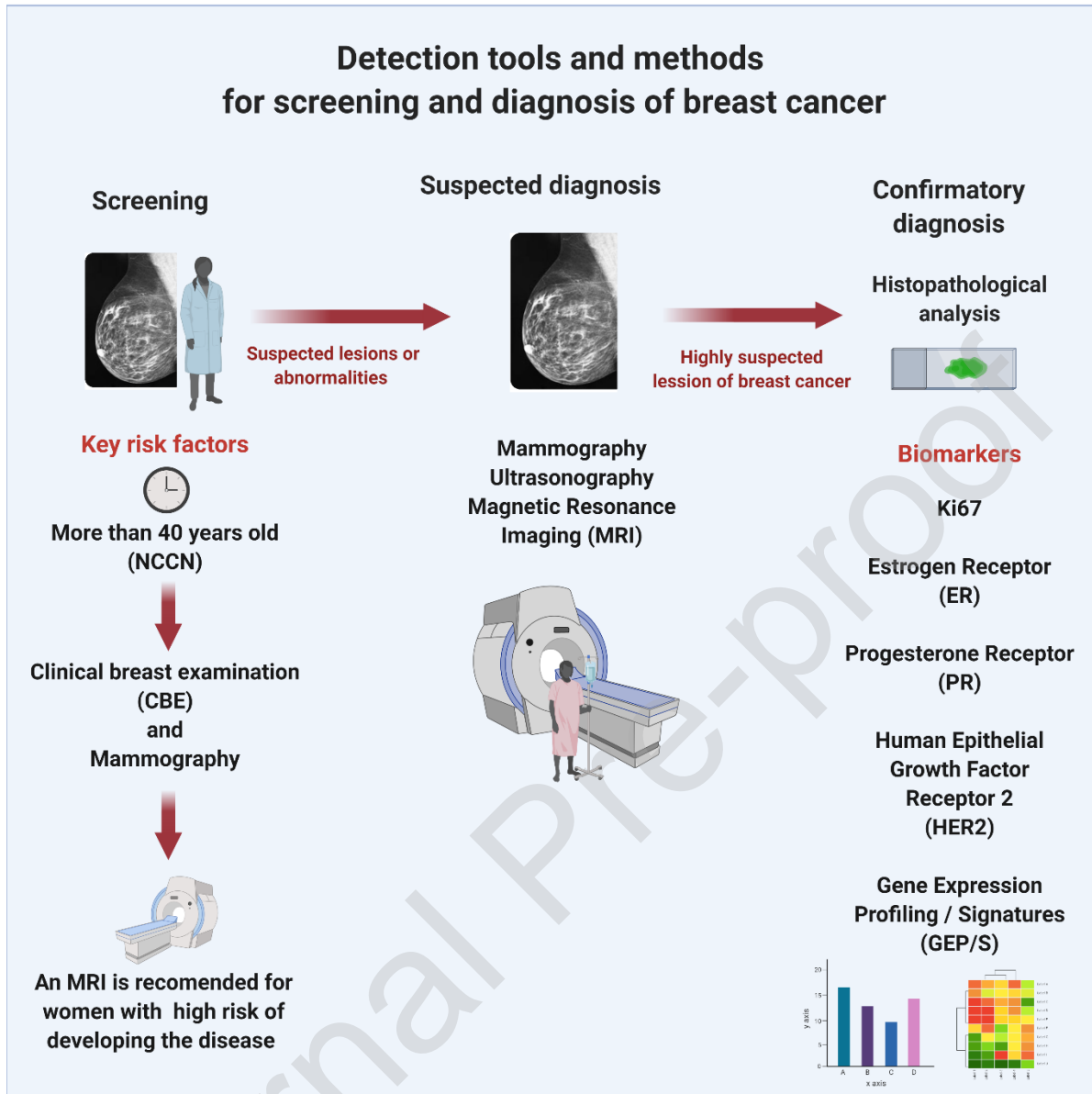
Figure 5. Detection tools and methods for screening and diagnosis of breast cancer. Screening should be performed with a tool that is appropriate according to each patient's breast cancer risk (e.g. The NCCN recommends annual mammography for average risk women of >40 of age). If a lesion is detected during screening, further examinations are required to evaluate such breast abnormality. Finally, if all studies suggest a high probability of cancer, it is suitable to confirm the diagnosis and assess the prognosis with biopsy analysis and biomarkers. Created with BioRender.com.

As mentioned before, mammography is recommended for BCS by the ACR, Society of Breast Imaging (SBI), ACS, U.S. Preventive Services Task Force (USPSTF), NCCN, European Society for Medical Oncology (ESMO), and others [6,7,94,97–100]. However, this imaging

technique is limited by dense breast tissue for which other methods such as Ultrasonography could be used in combination to provide a more accurate analysis [101]. In this section we explore the established methods of detection, representative studies with recommendations to be taken in consideration for their use.

5.1 Breast Physical Examination

BPx refers to the inspection and palpation of the breast, nipple and lymph-draining areas surrounding the armpit. This can be performed by a physician (CBE) or by the patient itself (BSE). BPx has low cost and no special equipment is required [102,103]. Abnormal physical features detected by CBE include breast and axillary lumps, thickening, redness, swelling, asymmetry of the breasts and nipple abnormalities such as ulceration and discharge [102,103]. CBE has a sensitivity of 40% to 69% and a specificity of 88% to 99%. For BSE, sensitivity ranges from 12% to 41% [104]. However, BSE can detect tumors of similar size to those found by CBE (SD: 22.1mm versus CBE: 21.9mm of diameter; p=0.991). Moreover, BSE usually detects tumors in younger women than CBE, since the median age of detection is 60 years and 67 years respectively (p<0.001). This is due to older women tend to omit their palpable lesions or do not attend screening consultation. Such situations could be prevented with more frequent CBE as an integral part of general care [105]. Nonetheless, it has been evidenced that BSE does not decrease breast cancer mortality [7].

5.2 Mammography

Mammography is the current gold standard method for BCS. This method aims to identify malignant tumors before they are noticed. A mammogram consists of an X-ray examination of the breast in the standard craniocaudal and mediolateral oblique views. The test can detect abnormal areas but cannot prove that it is cancer. If there is any suspicion of cancer, a biopsy of the breast tissue is recommended for histopathology analysis and molecular evaluation [9]. A major pitfall of conventional mammography is overdiagnosis, where benign tissue abnormalities or non-aggressive tumors can be misinterpreted, leading to unnecessary procedures and treatment [106]. In addition, the sensitivity of mammograms is influenced by age and the density of breast tissue [107,108]. Dense tissue layers limit detection of cancer tumors in mammograms which could lead to additional screening to confirm detection [109].

According to the Breast Cancer Surveillance Consortium (BCSC), conventional mammography has a sensitivity of 86.9% and a specificity of 88.9% [110]. A study conducted by Luczynska et al. showed a sensitivity of 90% and an accuracy of 62% [111]. Moreover, Helal et al. reported a sensitivity of 52.4%, a specificity of 50% and an accuracy of 51.4% [112]. However, alternative mammogram modalities such as FFDM and DBT could help to overcome conventional mammography issues, especially dense breast tissue.

5.2.1 Full-Field Digital Mammography

FFDM uses digital detectors to transform X-ray photons into electrical signals with the aid of analog-to-digital converters to create the digital image. FFDM allows easier access to images and post-processing manipulation to improve resolution and contrast through computer-aided detection (CAD) [113,114]. Physical performance and discomfort are similar to conventional mammography, however, digital mammography has a slighter lower radiation exposure [115]. A study conducted by Li et. al showed a sensitivity of 88.8%, a specificity of 75.2%, a positive predictive value of 62.1% and a negative predictive value of 93.6% corresponding to FFDM [116]. A multicenter trial that included 49,528 asymptomatic women, reported overall FFDM sensitivity of 70% and specificity of 92%. In addition, the study found a sensitivity of 78% for FFDM vs 51% for conventional mammography, in women younger than 50 years. Similarly, the sensitivity of FFDM in women with dense breast tissue was higher compared to conventional mammography (70% vs 55%) The specificity value did not vary between both mammography methods and among populations [117]. FFDM appears to benefit young women and women with heterogeneously or extremely dense breasts [7,117]. Since its approval in 2000, FFDM has largely replaced conventional film-screen mammography [113].

5.2.2 Digital Breast Tomosynthesis

DBT is an advanced mammography technique that uses a low dose X-ray system to make a 3D high-resolution reconstruction of the breast tissue using multiple images taken from thin sections (1mm). Fiona and colleagues reported that DBT has potential to increase detection rates and decrease recall rates [118,119]. However, the major pitfalls of this technique are the high cost of the test, uncomfortable procedure and use of radiation. The combination of 2D and 3D mammography results in a better screening for more invasive cancers [120–123]. A study conducted by Gennaro et al., which enrolled 200 women with a breast lesion previously identified by mammography and/or ultrasonography. The study showed no significant difference regarding overall performance of DBT compared to FFDM [124]. In 2012, Svahn and colleagues tested the ability of radiologists to identify anomalies in 185 patients using DBT. The study showed that the sensitivity of DBT compared to digital mammography were approximately 90% vs 79%, and no significant difference was found in the specificity between the two imaging techniques [125]. This technique is commonly used as an adjunct to mammography for breast cancer diagnosis since its approval by the US Food and Drug Administration (FDA) in 2011 [113] However, using both techniques could surpass the radiation limit in normal mammography allowed by the FDA. This situation could be avoided by using newer DBT techniques that produce synthetic 2D images using less radiation [7].

5.3 Ultrasonography

Ultrasonography is a diagnostic imaging technique that uses high-frequency sound waves to produce images of internal body structures, allowing the detection of anomalous tissues such as breast cancer [113]. An ultrasound can also be used to discern fluid-filled cysts and solid tumors. [107,126] Moreover this is the primary imaging modality for guiding interventional procedures such as core biopsy, cyst aspiration, preoperative needle localization and drainage [127–129]. Advantages of ultrasonography also include the absence of ionizing radiation and intravenous contrast. Nevertheless, ultrasonography has a low detection rate for calcifications, has a lower specificity compared to mammography and a highly trained technician is required to carry out the test [107].

Zanello and colleagues analyzed the diagnostic performance of ultrasonography in 241 patients with a previous mammography result of BI-RADS category 0. Ultrasonography was considered diagnostic when the BI-RADS changed to a category 2, 4 or 5 and indeterminate when results required a mammographic follow-up. The imaging technique was diagnostic in 60.6% patients and indeterminate in 39.4% patients. Additionally, ultrasonography had a sensitivity and specificity of 100% and 89.1% respectively, and an overall accuracy of 89.6% [130]. Studies have shown that ultrasonography can be useful as an adjunct to mammography to increase detection rates in women with dense breasts [131–133]. Currently, this technique is usually used in conjunction with mammography for a better diagnosis [107,134].

5.4 Magnetic Resonance Imaging

MRI uses strong magnetic fields and low-energy electromagnetic waves to produce detailed images or information about physiological processes of the body such as blood flow and nerve activity. Contrast Enhanced-MRI (CE-MRI) for breast cancer diagnosis relies on the neovascularity generated by tumors during growth and development. Intravenous administration of a gadolinium-containing contrast material is required in order to visualize lesions and malignant angiogenesis as it is carried by the bloodstream into the affected breast [135,136].

Breast MRI is a non-invasive tumor detection tool with a reliable sensitivity that does not use radiation. MRI has improved BCS in high-risk women [107,136]. If used as an adjunct to conventional imaging, MRI can detect breast cancers with an incremental sensitivity of 58% in high risk women compared to mammography alone. MRI in addition to conventional mammography has reported a sensitivity of 93 to 100% [137]. A prospective study conducted by Schelfout et al. described a greater capacity of CE-MRI at detecting ipsilateral and contralateral breast tumors in comparison to other imaging techniques. The study reported that CE-MRI was able to identify 96% of multifocal and 95% of multicentric breast tumors, while mammography detected 37% and 18%, and ultrasound found 41% and 9% accordingly [138]. The major

drawbacks are the high cost of MRI examinations and high rates of false positives. It is important to note that patients with specific psychological conditions (e.g. claustrophobia) cannot perform the test without being previously prepared for examination (e.g. sedation). Moreover, patients with implantable electronic devices (e.g. peacemakers) or tissue expanders (e.g. for breast reconstructions) are usually contraindicated for MRI testing [136,139].

5.5 Positron Emission Tomography/Computed Tomography

PET/CT refers to the technique which combines PET and X-ray CT scanners to obtain images that identify the anatomic location of anomalous metabolic activity within the body. PET provides information of cancer physiology at the molecular level using radiotracers (such as F-18 fluorodeoxyglucose or FDG, and other lesion-specific radiotracers), while CT produces pictures with the anatomic information [140–142]. FDG-PET uses glucose upregulated transport in cancer cells to visualize tumors, therefore the patients should fast for several hours before PET/CT to optimize the study [143].

The main benefit of the PET/CT procedure is its combination of functional and anatomical imaging, permitting clearer visualization of the lesions than conventional screening [142]. The NCCN 2020 guidelines do not suggest PET/CT scanning in patients with clinical stage I or II and operable stage III breast cancer. FDG-PET/CT is most helpful in situations where standard staging tests are equivocal or doubtful, especially in the setting of inflammatory breast cancer, regional nodal disease and/or whole body distant metastasis [6,141,144].

Wahl et al. enrolled 360 women with positive primary invasive breast cancer to determine the accuracy of FDG-PET in the detection of axillary nodal metastases. The sensitivity and specificity of the method were 61% and 80% respectively [145]. In another study, 47 FDG-PET/CT screens of women with a history of breast cancer were used to evaluate the sensitivity, specificity and accuracy of PET/CT versus CT. The results were 85% vs 70%; 76% vs 47% and 81% vs 59% respectively [146]. The main drawback of this technique is the radiation exposure to the patient and the operator, which cannot be mitigated [142].

As presented earlier, BCS is based on physical examination and imaging-based methods. Among the last ones, mammography is the primary screening tool, and has become the gold standard technique for BCS. Improvements in conventional mammography that led to the development of FFDM have provided a more accurate tumor detection in terms of sensitivity and specificity, especially in women with high dense breast tissue. Moreover, clinical practices could include an adjuvant imaging technique, such as DBT and ultrasonography to improve cancer detection. The remaining imaging methods covered previously target specific subpopulations. For example, MRI screening is suggested only for women with high risk of breast cancer, while PET/CT is commonly used for cancer monitoring, specially to determine nodal and distant

metastasis. Even though these are well established techniques, ongoing studies aim to enhance current screening practices. The process of BCS culminates when radiologists categorize imaging findings based on the BI-RADS lexicon scale to recommend further analysis for diagnosis (e.g. biopsy), if required.

6. Emerging Methods of Screening and Diagnosis

Besides current methods of BCS such as BPx, mammography, ultrasonography and MRI, new technologies have emerged fusing biophysics characteristics with clinical data [147]. For instance, tissue rigidity and other mechanical characteristics of the tumor during breast cancer progression may lead to novel diagnostic tools [147,148]. It has been suggested that changes in the rigidity and stiffness of the extracellular matrix persist for longer periods of time in cancer and may have less heterogeneity in the results in comparison to other methods such as GEP/S [147]. In addition, detecting breast temperature variations using thermograms could reveal abnormalities since temperature can be influenced by the increased metabolic activity of cancer cells in comparison to healthy breasts [79,149,150]. In this section we show how beyond improving hardware for the biophysical analysis of breast cancer and the use of new software tools for better analysis and interpretation of data is key for the future applicability of these tools. As new methods of diagnosis continue to emerge, it is important to evaluate their clinical validity before being recommended by clinical guidelines [7]. This section reviews relevant information regarding the application of these innovations including: Optical Imaging, Digital Infrared Thermography Imaging (DITI), Contrast-Enhanced Mammography (CEM), Scintimammography, emerging PET radiotracers, Magnetic Resonance Elastography (MRE), Acoustic Radiation Force Impulse Imaging (ARFI), Diffusion Weighted-MRI (DW-MRI) or DWI, Ductoscopy and Ductal Lavage, Radiomics and Internet of Things (IoT).

6.1 Optical Imaging

Optical Imaging is a detection method that uses light to identify anomalies inside body tissues. This technology uses the electromagnetic spectrum from ultraviolet to infrared waves of light to provide information regarding tissue structure, physiology, and biochemical processes [147,151]. This imaging modality can detect femto or even picomolar concentrations of an optical reporter or contrast element [152]. Optical images are contrasted with elements such as exogenous agents (e.g. dyes or probes), endogenous molecules (e.g. reduced nicotinamide adenine dinucleotide (NADH), hemoglobin or collagen), or reporter genes (e.g. Green Fluorescent Protein) [152,153]. This technology includes modalities such as Fluorescence Imaging, Bioluminescent Imaging, Optical Coherence Tomography (OCT), Photoacoustic Imaging, Diffuse Optical Tomography (DOT), Super-resolution Microscopy and Terahertz Tomography [152,154,155]. In addition, according to Grosenick et al., optical breast imagers can be classified in time-domain, frequency-domain, and continuous-wave systems taking into account the measurement geometry

and the temporal profile of the laser radiation employed [156]. Optical imaging has the potential to be noninvasive, nontoxic and cost-effective to quantify vascularization, permeability, hemoglobin, oxygen saturation (StO_2) and concentration in breast tumors. Studies have shown that vascularization and StO_2 of breast tumors are related to hypoxia, which is highly associated with malignant tumors due to their size [151,153].

Several clinical trials have been conducted to evaluate optical imaging modalities mentioned above in the detection and characterization of breast cancer, dynamic response to external impact, assessment of breast density and cancer risk estimation [156]. Nowadays, large clinical results comparing functional properties (Hydrogen Breast Test (HbT), Tissue blood StO2, water, lipids and scatter power) from breast cancer lesions and healthy tissue are available. Grosenick et al. found that the average HbT and StO2 of healthy tissue are 17.3 ± 6.2 and 74 ± 7 while in malignant lesions the average is 53 ± 32 and 72 ± 14 respectively [157]. In another study, Cerussi et al. determined that the average of HbT and StO2 in healthy tissue are 17.5 ± 7.5 and 67.7 ± 9.3 while in malignant lesions they are 24.7 ± 9.8 and 67.5 ± 8.4 respectively [156,158,159].

Various optical imaging clinical studies have demonstrated that functional properties can improve breast cancer diagnosis [156]. In addition, other trials have been performed to evaluate dynamic changes of functional properties when external factors such as pressure are applied for the distinction among malignant lesions, benign lesions and healthy tissue [156]. Local pressure in tumors can cause an accelerated reduction of StO2 compared to healthy tissue and benign lesions. Fournier et al. using dynamic optical mammography reported a statistical difference in the characterization of benign and malignant lesions. The study revealed a 74% and 92% of sensitivity and specificity respectively [160]. However, Xu et al. in a trial using a handheld probe with continuous waveform light sources reported that pressure appliances in 36 cases had no significant difference in the decrease of StO2 [156,161]. The actual knowledge on functional and scattering properties is improving the future perspectives of optical imaging in breast cancer diagnosis [156].

Optical imaging research has a promising role in cancer detection. It represents an interesting tool to provide functional and molecular characteristics assessment in breast cancer [152,154]. However, methodological preparation and optical data can be time-consuming and difficult to interpret. The ACR establishes that further evidence is required to support the use of optical technology in BCS [162]. Currently, these modalities are in early phases of clinical trials [153]. Further studies regarding how it captures and interprets images to provide better detection of cancer are still needed [151,154].

6.2 Digital Infrared Thermography Imaging

DITI is a technique that detects the heat patterns of blood flow in body tissues. This technique is based on the principle that temperature increases in areas of the tumor due to

neovascularization, increased vasodilation and inflammation influenced by cancer cells [163]. The human body and its different constituents radiate infrared heat energy from the skin. These heat patterns can be detected by specialized cameras and interpreted by a software that generates temperature values. As a result, a thermograph is created with the temperature distribution of the breast. This technique is promising since it could enable a total exploration of the breast with the great advantage of being non-invasive and without radiation exposure [164].

Temperature distribution patterns are measured, recorded, and processed to pinpoint areas related to cancer development during preventive and diagnostic evaluations of the breast. However, since DITI provides a thermal image, it is unable to reveal specific anatomical borders in tissue abnormalities for biopsy analysis. Additionally, the measurement is carried out only on the surface, so deep tumor diagnosis is excluded for such detection[164]. Moreover, the image processing at some points is an extrapolation of the real measurements. In other words, one could easily assume errors and mistakes possibly leading to erroneous diagnosis. To overcome these limitations, an image processing technique has been introduced that enables the creation of a 3D picture of thermography. This technique is the implementation of the inverse problem[165], which is well documented by mathematics. In this technique, the collected values of the skin temperature are computed to deduce the temperature and the location of the source. This is done using heat equations[165] and the physical characteristics of the biological tissues such as the thermal conductivity[166].

A study carried by Kontos et al. determined the sensitivity and specificity of DITI in 63 symptomatic patients who had undergone surgical excision or core biopsy of breast lesions (benign and malignant). They found sensitivity and specificity values of 25% and 85% respectively [167]. A prospective clinical trial conducted by Arora et al. included 92 patients with biopsy referrals based on mammograms or ultrasound results to evaluate DITI performance. Results reported a sensitivity of 97% and specificity of 44%. Authors conclude that DITI could be valuable as an additional method to mammography and ultrasonography for breast cancer detection [168]. Several studies have shown that thermography could be used as a method for early diagnosis of the disease. Still, its effectiveness is questioned due to the large variation of results in terms of sensitivity and specificity. Currently, thermography has not been approved as a standalone method for breast cancer diagnosis [163,169,170].

6.3 Contrast-Enhanced Mammography

CEM is an imaging modality that uses X-rays, a dual-energy technique and a standard iodinated intravenous contrast agent to highlight breast abnormalities on the basis of neovascularization and morphologic assessment of breast lesions. After the intravenous administration of the contrast agent, paired low-energy and high-energy images of the breast are obtained. The low-energy image resembles an unenhanced conventional 2D mammogram,

whereas the high-energy image reveals areas of iodinated contrast uptake. Post-processing results in a recombined image showing areas of contrast enhancement with subtracted background breast tissue [171–173].

The main benefit of CEM is that both breasts can be imaged using a contrast agent in a noninvasive manner [172]. CEM was approved by the FDA in 2011 and originally conceived as an alternative method to CE-MRI when it was not available or limited [113]. Nonetheless, CE-MRI is still the technique of choice over CEM for BCS in high-risk patients [174]. CEM has been reported to have better sensitivity and specificity than conventional 2D imaging, and similar performance, lower cost, and shorter examination time than CE-MRI [172]. Creation of CEM-specific BI-RADS lexicon would improve sensitivity of the technique and provide a more accurate biopsy referral [174]. Mokhtar and Mahmoud assessed the diagnostic accuracy of CEM in 60 women with suspected anomalies detected by mammography and/or ultrasound. They found a sensitivity of 97.7% and a specificity of 50% for CEM and concluded that is more accurate than mammography alone and mammography plus ultrasound, when diagnosing anomalies in women with dense breasts [175]. Lobbes et al., found CEM sensitivity of 100% and specificity of 87.7% in a study that compared its diagnostic performance to conventional mammography. Additionally, they found that tumor localization and diameter measurements described by CEM are in accordance with MRI and histopathology analysis [176]. Since no new equipment is needed apart from an upgrade to conventional mammography units, there is not a significant extra cost for CEM. Something that needs to be considered before screening is that CEM increases radiation exposure by about 20-80% when compared to standard mammography. However, this higher radiation exposure still lies within the range of accepted radiation levels in patients. [172,177].

6.4 Scintimammography

Scintimammography is a nuclear medicine imaging test that uses radioactive isotopes such as Technetium-99m sestamibi (^{99m}Tc-MIBI) to investigate a breast anomaly previously detected by mammograms. The radioactive isotope accumulates in the breast and gives off a small amount of gamma rays which are detected by a specialized camera. The image resulting from this test, provides details in the structure and function of the breast allowing a better cancer detection and estimation of its aggressiveness. This technique could be used to assess cancer treatment response or when other imaging methods (e.g. mammography) are limited to interpretation or when MRI is not feasible [140,178,179]. In addition, scintimammography could be valuable for screening women with dense breast tissue and breast implants [178,180]. However, it exposes the patient to a considerably higher radiation dose than mammography. This high radiation risk contraindicates its use in pregnancy. A trained technologist is required to perform the test and in some cases the image resolution is not as clear as in other techniques such as mammography and ultrasonography. [7,178–180]. Since false positives and negatives results can occur, a negative scintimammography

should not stop diagnostic confirmation by biopsy after a suspicious mammography and/or ultrasonography [179].

A systematic review that included twenty-five studies with a total of 4,094 patients, evaluated the diagnostic performance of scintimammography vs mammography. The study established a sensitivity of 86.64% and a specificity of 83.42% for the nuclear medicine method compared to 75.82% and 59.58% respectively for standard mammography [181]. A systematic review and meta-analysis conducted by Guo and colleagues investigated the efficiency of MIBI scintimammography as a predictor of neoadjuvant CTX response in 503 patients with proven breast cancer. They found a pooled sensitivity of 86% and a pooled specificity of 69% [182]. More trials are required to validate the efficacy of this technique, since it is still considered as an emerging screening method for breast cancer in mammographically dense breasts [7,183].

6.5 Future Role of Positron Emission Tomography and New Tracers

Due to the PET's high sensitivity and spatial resolution compared to other imaging techniques, several new radiopharmaceuticals are in ongoing trials [184,185]. The tracers in late clinical phases are commonly small molecules or peptides labeled with short-live isotopes (^{18}F and ^{68}Ga), longer-lived isotopes (^{64}Cu, ^{89}Zr, ^{124}I, ^{52}Mn), monoclonal antibodies (mAbs) or nanoparticles. FDG PET/CT has limitations in the detection of mixed and low FDG uptake cancers, therefore, new agents that can easily detect specific receptor status or antigens in the disease are mostly developed as FDG replacement. Also, agents are being developed for disease characterization and to evaluate target treatment for patients ([185].

Instead of FDG, other alternatives such as the analog of estradiol, the 16α-18F-fluoro-17β-estradiol (FES) is used in combination with PET. This technique is non-invasive, reproducible and provides valuable information of the tumor stage. In addition, [^{18}F]-FES uptake is correlated with ER overexpression, such correlation can predict the patient's response to hormonal therapy (HT) [186]. [^{18}F]-FES PET as a diagnostic tool can differ from early stage tumors and metastases, this modality is usually used instead of biopsy or when it has undetermined results [186–188]). A study carried by van Kruchten et al. in 33 patients who underwent [^{18}F]-FES PET reported [^{18}F]-FES positive lesions in 22 patients, higher bone metastases lesions were detected by [^{18}F]-FES PET compared with other conventional imaging methods (341 vs 246 bone metastases lesions respectively), additionally, an 88% improvement in diagnosis and 48% of the patients reported a change in therapy [186,188]. Several studies performed with [^{18}F]-FES PET have shown a 84% and 98% sensitivity and specificity respectively [186].

Another modality is immuno-PET, which uses radiolabeled mAbs as markers. The mAbs have shown high specificity and selectivity in tumor targeting, antibodies such as anti-cytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4), anti-programmed cell death-1 ligand 1 (anti-

PD-L1), anti-programmed cell death protein 1 (anti-PD1) and GGSK-1/30 are labeled with long-lived isotopes such as ^{89}Zr. Immuno- PET has shown fewer or no side effects, however further studies are needed [184,189]. An example of immuno-PET is the mAb GGSK-1/30, which exclusively targets the human tumor-associated transmembrane glycoprotein Mucin 1 (TA-MUC1), a protein expressed in over 90% of all breast cancer cases. In a study conducted by Stergiou et al., 10 sections of healthy human breast tissue and 144 sections of HT+ breast cancer tissue were stained by mAb GGSK-1/30. Results reported that 96.5% of breast cancer tissue sections stained by the radiolabeled mAb were positive and 3.5% were negative, while 100% of the stained healthy tissue was negative. The authors described this tracer as a promising agent with potential use as a diagnostic tool, prognostic biomarker, and a companion diagnostic test [189].

In oncology, PET and PET/CT have shown promising results to provide an accurate assessment in breast cancer patients. FDG PET/CT technique is widely used in clinical imaging and remains as tracer by excellence due to its wide applicability [185]. Nevertheless, new tracers and PET imaging tools are emerging in order to achieve a better diagnosis, earlier detection and personalized assessment in therapy selection [187].

6.6 Magnetic Resonance Elastography

MRE is a dynamic elasticity imaging technique useful to obtain a visual map of the body. MRE uses a combination of MRI and mechanical shear waves to obtain information about the stiffness of tissues. The process involves three steps: first, shear waves are induced in the tissue, then, the propagating waves are imaged with MRI and finally, waved data is processed to generate a stiffness map (elastogram) [190]. None of the conventional methods of detection (e.g. mammography, ultrasound, MRI, CT, PET) can measure mechanical properties of tissues except for physical palpation, which is commonly used by physicians to detect masses near to the breast surface. However, not all tumors are superficial, as breast cancer lesions can be hidden in dense breast tissue where palpation or established imaging methods cannot detect. Several studies have shown that malignant tumors are stiffer than benign, thus, MRE can provide a truthful shear modulus or modulus of rigidity of tumors to provide a better diagnosis [190–194].

MRE has been tested as a complement to contrast-enhanced MRI, which has very high sensitivity but low specificity for breast tumor detection [192]. Sinkus et al. investigated viscoelastic tissue properties in 68 patients using CE-MRI and MRE. They found that CE-MRI alone showed a sensitivity and specificity of 100% and 40% respectively, whereas combining CE-MRI and MRE techniques reported an improved specificity from 40% to 60%, retaining a sensitivity of 100% [195]. Another study conducted by Siegmann and colleagues combined CE-MRI and MRE to evaluate breast tumor identification in 57 patients. They found an improved specificity from 75% to 90% when α0 (expression of the hardness of the tissue) from MRE was combined to CE-MRI, maintaining a sensitivity of 90% [196]. Thus, studies have shown that MRE

may improve diagnosis of breast lesions, specially by enhancing the performance of CE-MRI specificity which may lead to the reduction of false positives and unnecessary biopsies. However, further research is required to validate previous results and decrease patient discomfort during examination [197].

6.7 Acoustic Radiation Force Impulse Imaging

ARFI is a new acoustic imaging modality based on ultrasound elastography. This technique uses a short-duration, focused, high intensity ultrasound to provide information about the mechanical properties of tissue by generating radiation force (the interaction of an acoustic wave with an object) and using ultrasonic correlation-based methods [121,198]. This new ultrasound-based elastography is a promising tool which provides quantitative and semiquantitative measurements of the strain of hardness of a tissue lesion [199]. Several studies have shown that malignant tumors are usually stiffer than benign masses and the transmission of shear waves differ in both cases [121,199]. Jayaraman et al., revealed that velocity of shear waves of 34 benign lesions (2.08 m/s) were lower than that of 16 cancer specimens (6.28 m/s) [199]. A retrospective study evaluated the diagnostic performance of ARFI with virtual touch tissue imaging and quantification in 83 patients with benign and malignant breast lesions. Results showed a sensitivity and a specificity of 82.4% and 80.4% respectively [200]. ARFI may be used as an adjuvant diagnostic tool to conventional imaging to differentiate malignant breast tumors from benign ones without extra radiation or invasive methods [199,201,202].

6.8 Diffusion-Weighted Magnetic Resonance Imaging

DW-MRI or DWI is a technique that generates images of water diffusion in the tissue to visualize the internal structure and its properties. The study of diffusion patterns is useful to identify and contrast healthy tissue from abnormal lesions [203]. In oncology, a DWI image is a useful diagnostic tool to improve detection and biological characterization of breast tumors. In addition, DW-MRI images can characterize benign from malignant lesions according to the apparent diffusion coefficient (ADC) which is lower in malignant tumors [203,204]. The application of DWI-MRI as an adjuvant technique to CE-MRI may also reduce false positive results. Thus, DW-MRI could be valuable to inform the malignancy likelihood of a lesion detected by conventional imaging techniques in order to prevent unnecessary biopsies [204].

Bickel et al. assessed retrospectively the ADC obtained from DW-MRI in 170 primary malignant breast tumors. The objective was to evaluate DW-MRI as a tool to discern invasive breast cancer from ductal carcinoma *in situ* (DCIS). They reported a sensitivity and specificity of 78.06% and 90.5% respectively, therefore this technique showed potential as an indicator for breast cancer invasiveness [205]. Additionally, ADC has become a marker of interest to evaluate neoadjuvant treatment response, since its values can be influenced by tumor cells and necrosis,

and can be monitored before lesion progression [206]. A retrospective study evaluated the predictive value of ADC in neoadjuvant CTX response in 118 women with locally advanced breast cancer. Results showed that ADC obtained before treatment can predict the response of breast cancer to neoadjuvant CTX if tumor subtypes are considered [207]. However, further studies are required to validate ADC as a predictive marker to assess therapeutic efficacy [204].

6.9 Ductoscopy and Ductal Lavage

Fiberoptic ductoscopy (FDS) is a type of breast ductoscopy which permits direct visualization of the ductal epithelium of mammary glands through the nipple orifice cannulation. To perform this technique, the duct orifices are dilated with a suitable probe such as Bowmann's lacrimal dilator to insert the sub-millimetre fiberoptic micro endoscope. Injecting a saline solution improves visibility and eases passage of the endoscope through the intraductal space [208,209]. Mammary ductoscopy is commonly practiced in women with spontaneous nipple discharge (SND), which can be produced by malignant or benign ductal lesions [210,211]. Liu et al evaluated the ability of the FDS technique in combination with cytological tests to diagnose SND in 1,048 women. They reported a sensitivity 94.2% for FDS versus 98.1% for FDS + cytology testing [212]. A study led by Zielinski et al. assessed the diagnostic value of FDS in 164 patients with intraductal proliferative lesions. Results showed sensitivity and specificity of 68.1%, 77.3% respectively [208]. This evolving technology still requires further research to establish a consensus criteria for diagnosis and the association of its findings with malignancy [211].

On the other hand, ductal lavage is a procedure which collects atypical epithelial cells from the milk ducts for cytologic analysis. This technique is commonly used to detect precancerous and cancerous cells in women with a high risk to develop breast cancer. A microcatheter is introduced in the ductal orifices after nipple aspiration and all the ducts are cannulated with a fluid (commonly 1-3ml of 1% lidocaine). Then the ductal system is infused with normal saline and the breast is compressed to collect ductal fluid with cellular material to analyze [213,214]. Matos Do Canto et al., studied the metabolomic profile of breast ductal fluids collected by ductal lavage from 43 women with confirmed unilateral breast cancer. The metabolic analysis showed a sensitivity of 90.7% and specificity of 88.4% for breast cancer detection [215]. The routine use of ductal lavage is still not recommended for BCS due to missing support from actual evidence [7]. Even though these techniques can provide additional diagnostic information, their limitations regarding the sensitivity and specificity are currently analyzed since they could vary depending on the classification scale and cut-off applied [216].

6.10 Radiomics

In the past two decades, the field of medical image analysis has grown exponentially due to the significant technological advancements in pattern recognition tools and high-throughput

computing which led to the development of radiomics [217]. Radiomics involves the extraction of hidden quantitative features from a single or multiple digital medical images which could be applied in cancer detection, diagnosis and prognosis [218–220]. In breast cancer, these features are related to morphological aspects of lesions (tumor size, shape, intensity and texture) and functional characteristics of tumors (blood flow and cell metabolism) [219,221]. Thus, radiomics data could provide information about the underlying physiological and molecular mechanisms which are reflected by the genotypic and phenotypic characteristics of the cancerous tissue [218,221].

Radiomics analysis is a complex process that begins with the acquisition of high-quality images from the different imaging methods available. The process continues with the selection of a region of interest (ROI) which is segmented either manually or automatically (i.e.. delineate the borders of the ROI that will be targeted for image analysis). Then, the selected region is converted into three dimensions to form volumes for the feature extraction step. In this imaging processing phase, candidate high-dimensional imaging parameters are extracted for classification and analysis. Finally, the data is analyzed and organized to develop models to predict outcomes in combination with clinical information, histology data and genomic profiles [217,219,221,222]. In breast cancer, radiomics has mostly been used in combination with MRI. However, recent studies have explored its potential use with other imaging techniques such as mammography, DBT and ultrasonography [221].

Radiomics has shown potential to serve not only as an emerging method of breast cancer detection but also as a tool for tumor characterization, prediction of tumor behavior, treatment response and recurrence which contributes to personalized monitoring, management and treatment. For example, a radiomics approach on ultrasonography imaging was proposed by Zhang et al. in 2017 to classify benign and malignant breast tumors. This consisted of the extraction of high-throughput features from sonoelastograms which quantified the shape, hardness and hardness heterogeneity of tumors for further analysis and clustering. This approach showed a sensitivity of 85.7% and a specificity of 89.3% [223]. Additionally, a retrospective study explored the ability of radiomics to predict cancer risk recurrence using MRI and multigene assays. This study enrolled 84 patients diagnosed with invasive ductal and lobular tumors. Tumor size, shape, margin morphologic appearance, enhancement texture, kinetic curve assessment, and enhancement-variance kinetics were the parameters extracted from MRI sequences. These MRI imaging features were then correlated with the risk of recurrence scores, estimated by the multigene assays MammaPrint®, Oncotype DX®, and PAM-50® (currently known as Prosigna®). The analysis showed promising results, combining the evaluation of both phenotypic and genomic data used to assess the risk of cancer recurrence [224]. Therefore, the integration of imaging, radiomics and GEP/S could provide a more accurate diagnosis and prognosis for personalized cancer management and treatment decisions. However, the use of radiomics by itself and its correlation with GEP/S or other prognostic tools needs to be further studied.

6.11 Internet of Things and Machine Learning Based Diagnosis Tool

Recently, electronic systems opened new perspectives on ergonomic and human-machine interfaces with the introduction of embedded technologies allowing applications such as Internet of Things (IoT) and more specifically connected sensor nodes [225]. The ongoing automation of processes uses modern smart technology, development of wearable medical devices with IoT and novel human-machine interfaces thanks to the recent possibilities offered by on-chip sensor devices or System-on-Chip (SoC) [226]. Furthermore, AI allows new approaches to understand multi-parameter systems processing different sources of biological data as input. Such a system could improve the diagnosis of breast cancer by implementing a continuous monitoring of specific parameters (e.g. temperature, glucose rate, stiffness, etc.) of the biological tissues involved in this illness.

As it is explained in the subsections throughout this review, different diagnostic methods rely on punctual measurements of biological parameters. However, to reduce the uncertainties coming from sparse measurement, improved monitoring methods should involve continuous data analysis over an extended period of time. This would generate a large amount of information (Big Data) that should considerably increase the patient history and lead to a more accurate medical evaluation. Indeed, AI concepts applied to Big Data have recently enabled the development of electronic systems able to interpret the data and to make decisions based on these interpretations, also called ML [227]. Additionally, this would build a contextualized history of the patient diagnosis. For example, and as already explained earlier, the temperature distribution inside the breast could change during the circadian clock or in response to TME selective pressure through metabolic adaptations [228]. Engineers could combine this physiological information with (non-invasive) wearable sensors and embedded electronics to detect the temperature pattern as a function of time. Such systems need to be on the patient's body (or at least in their clothes) and must be communication enabled (e.g. wireless communications) to transmit data to a cloud using concepts of connected objects like IoT. For safety reasons the cloud could send the data to ML based electronic systems for analysis after which, the conclusions will be sent to medical professionals [225]. To do so, it is important to consider different sensors that can be embedded into underwear like bras and electronic devices that are able to receive, send and store data. Eventually, these wearable systems could preprocess data and clinical history from breast cancer patients (e.g. age, weight, height, antecedents etc.) prior to sending them to the cloud after which more complex statistical analysis such as ML is employed [229].

At this time, the combination of ML and embedded systems for breast cancer detection needs to be further investigated. For the hardware, two key questions need to be answered considering different settings, such as thermal sensing. First, which type of temperature sensor provides the best compromise between accuracy, precision and ease-of-use? Possible options

include InfraRed (IR) [230,231], Millimeter-wave/THz [232,233] and flexible electronics [234]. Second, which electronic architecture of the embedded system for preprocessing and communication is the best suited? This discussion opens an interesting perspective to create a complete modern system for breast cancer diagnosis, incorporating the measurement of different biomarkers to achieve multiparameter sensing.

The established and emerging methods for detection and diagnosis of breast cancer are constantly improving. Established methods are being combined to provide a more accurate result and allow personalized treatments. The emerging methods are adapting new, wearable and connected technologies to analyze and store data, reducing the time of processing, delivery of results and the costs of medical devices. The interaction between patients, researchers and physicians is of great importance as the emerging technologies will need multidisciplinary communication and technical expertise to understand the biological complexity of breast cancer, and to meet patients' needs during breast cancer detection (**Fig. 3**). Women could be more willing to perform non-invasive methods such as optical imaging, DITI, ARFI, DW-MRI or DWI if they are comfortable and affordable. New ways of interpreting imaging data with emerging techniques such as Radiomics, as well as improving processing and delivery of results, will gain interest and applicability in the upcoming years.

7. Biopsy

After the detection of an anomaly in breast tissue by imaging techniques, biopsies are required to provide an accurate diagnosis. A biopsy is an invasive procedure where abnormal breast fluid or tissue is removed for cytological, histological and molecular analyzes. The test is recommended only in suspected cases of cancer based on the BI-RADS lexicon scale used by radiologists [235]. Tumor biopsy is still the gold standard technique that confirms if a tumor is benign or malignant [236]. There are three types of biopsies: 1) fine needle aspiration which is chosen to assess the liquid characteristics from cysts or abscess, 2) core needle biopsy which removes a small amount of the suspicious tissue, and 3) excisional biopsy that removes most or all of the abnormal tissue in conjunction with some healthy tissue [7,237]. Fine needle aspiration involves a small needle but does not provide information regarding the architecture of the tumor and requires experienced cytologists. Core needle biopsy requires a bigger needle equipment compared to the first one but is analyzed by standard histopathology which gives information about tissue structure and morphology. Excisional biopsy is also evaluated by standard histopathology but includes a surgical procedure for the excision of a bigger tumor sample [237]. In the past, performing excisional biopsy of breast lesions was commonly guided only by palpation. Nowadays, image-guided biopsy is preferred to sample the tissue as it showed better precision and accounts for cancer cases with nonpalpable tumors [238]. Biopsies are commonly guided by mammography, ultrasound and MRI which can also allow optimal preoperative workup. The core needle biopsy is preferred for evaluation of primary breast lesions while the fine needle aspiration

and sentinel lymph-node (LN) biopsy are useful for LN evaluation [113,183,239,240]. A correlation of imaging and pathology findings is imperative to provide an accurate diagnosis. Biopsies can also be analyzed by GEP/S where gene expression aberrations can be detected to determine the type of treatment recommended by physicians [241,242].

8. Staging and Grading of Breast Tumors

Anatomical staging and grading methods are used to determine tumor characteristics such as size, growth rate and spread. They are usually performed after diagnosis by established imaging methods and biopsies to select the most appropriate treatment (**Fig. 5**). Grading refers to the appearance of cancer cells compared to healthy cells and how quickly they may grow and spread. Breast tumors can be low-grade (uniform like appearance and slow-growing cells), intermediate-grade (bigger cells with variable shape and faster growth than normal cells) and high-grade (faster-growing cells with different sizes and shapes compared to healthy cells). Aggressiveness is associated with a higher grade breast cancer.

The American Joint Committee on Cancer (AJCC)'s Cancer Staging Manual uses the Tumor, Node and Metastasis (TNM) system to describe tumor size, involvement of LN and spread to other tissues. Stage 0 stands for a DCIS or Paget disease without nodal involvement or distant metastasis. Stage 1 is divided into A and B. IA involves tumors that are ≤20mm in size, but IB represents from no evidence of the primary tumor to a tumor size of ≤20mm. But independent of the tumor size, a nodal involvement (also called micrometastasis) of 0.2-2mm (or 200 cells). Stage II is also divided into A and B, the first of which comprehends tumors of ≤20mm (or without evidence of primary tumor) with ipsilateral level I or II axillary nodes, in addition to 20-50mm tumors without LN compromise. IIB are tumors that measure 20-50mm with I or II level axillary LN, or tumors with a size greater than 50mm without LN metastases [243].

Stage III is subdivided into three groups A, B and C. The IIIA group is mainly assigned to tumors with ipsilateral axillary nodes level I or II physically fixed or matted in the tissue at the examination, or tumors with ipsilateral internal mammary nodes. The IIIA group contains a range of tumor sizes, from no evidence of primary tumor up to tumor size greater than 50mm. IIIB are tumors of any size with direct extension to the chest wall or skin invasion by malignant cells. The group can or cannot have the same IIIA LN type of involvement to be included. On the contrary, IIIC are tumors of any size that satisfy one of the following criteria regarding LN: ≥10 nodes involvement; or infraclavicular level III; or ipsilateral internal mammary plus I or II axillary; or three axillary nodes plus micro or macro metastases by sentinel LN biopsy (in negative internal mammary nodes); or supraclavicular ipsilateral nodes. At last, Stage IV can have any kind of tumor size and LN entanglement but is the only group on which there must be evidence of proven metastasis by any means necessary (e.g. imaging). Even though anatomical staging is a crucial step for cancer diagnosis, it cannot provide an accurate prognosis. Nowadays, anatomical staging

is combined with prognostic staging which comprises tumor grading, receptor status and genomic testing for a more complete diagnosis, prognosis and cancer management (**Refer to Genetic Signatures & Clinical Guidelines Recommendations: Towards the Prognostic Staging** for an example of the current integrated staging system) [243].

9. Breast Cancer Classification

Breast cancer can be classified according to the area and cell types that are originally affected. Based on these criteria, the two main categories are carcinomas and sarcomas.

9.1 Carcinomas

Carcinomas are malignant tumors that originate in the epithelial tissue, compromising the cells that compose the lobules and breast milk ducts. Hence, they can be divided into ductal and lobular carcinomas. In addition, depending on the invasiveness of the cancer, carcinomas can be further classified as *in situ*, invasive and metastatic [41].

9.1.1 Ductal Carcinoma

Ductal carcinoma is a type of cancer that begins with the proliferation of malignant epithelial cells in the lining of breast milk ducts which are connected to the nipples (**Fig. 6**) [244].

9.1.1.1 Ductal Carcinoma *In Situ*

DCIS can be defined as the proliferation of ductal epithelial cells with morphological features of malignancy without invading further than the basement membrane (**Fig. 6**) [245]. The development of carcinomas is complex due to their multifactorial nature and the lack of precise risk information [244]. DCIS can measure from 1mm to ≤25mm in size, depending on their tumor grade [246]. In most cases, they are presented as non-palpable lesions which can be detected by mammography [247,248]. It has been observed that 30 to 50% of DCIS can progress to invasive cancer [249].

9.1.1.2 Invasive Ductal Carcinoma

Invasive ductal carcinoma (IDC) is the most common breast cancer type. Malignant epithelial cells in the lining of the breast milk ducts grow and surpass the basal membrane into the surrounding breast tissue and in late-stage cases they can migrate to other body tissues. IDC symptoms such as swelling, nipple discharge, lumps and pain are related to this stage of the tumor (**Fig. 6**) [41,250].
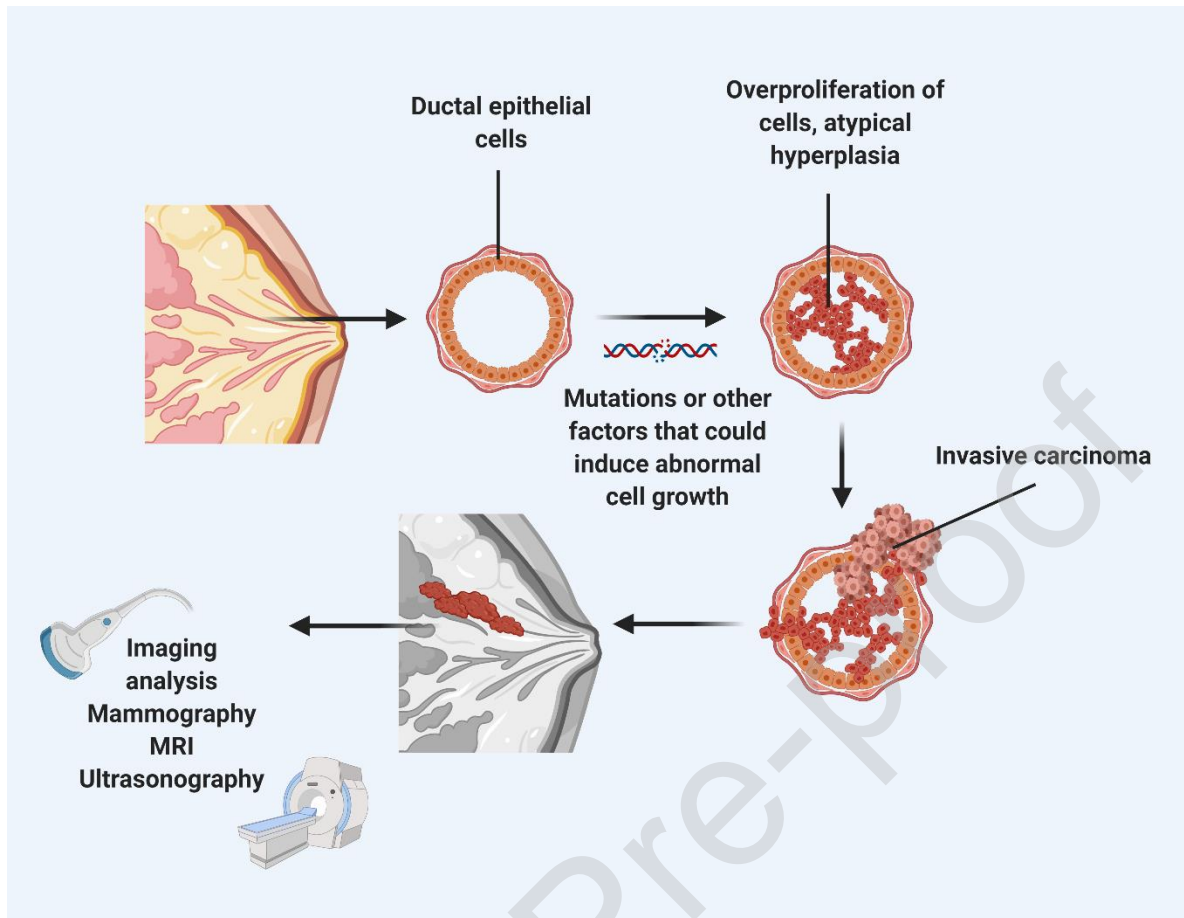
Figure 6. Development of ductal carcinoma *in situ* to invasive ductal carcinoma. Mutations in ductal epithelial cells can cause dysregulations in their growth and proliferation, leading to atypical hyperplasia. These cells can gain a different phenotype becoming malignant within the ductal walls. In turn, this lesion can infiltrate the surrounding tissue crossing the basal membrane, and thus progress to invasive cancer. Ductal carcinomas usually can be detected by imaging techniques such as mammography, MRI, and ultrasonography. Created with BioRender.com.

9.1.2 Lobular Carcinoma

Lobular carcinoma is a type of lesion that affects the lobules of the breast. They usually develop as non-cohesive dispersed cells or organized in single files with a linear pattern (**Fig. 7**) [41].

9.1.2.1 Lobular Carcinoma *In Situ*

Lobular carcinoma *in situ* (LCIS), also called lobular neoplasia, is a breast lesion associated with abnormal cell growth in the lobules. The AJCC eight edition no longer considers LCIS as breast cancer and is considered as a benign lesion [243,251].

9.1.2.2 Invasive Lobular Carcinoma

Invasive lobular carcinoma (ILC) refers to the spread of the tumor beyond the lobule to other parts of the breast tissue (**Fig. 7**). Depending on the cancer stage, it can further spread to other parts of the body. ILC is the second most common type of breast cancer comprising up to 15% of all cases. ILC can develop at any age but tends to affect women in the early 60s [41]. Even though ILC tumors have a good prognostic phenotype, cancer detection and long-term control of patients are still challenging. ILC tumors can be difficult to diagnose through current methods due to its growth pattern, which allows its infiltration in a diffuse manner, hiding its actual size [252,253]. Additionally the ILC has a more frequent multicentric, multifocal and bilateral presentation than IDC [253,254].
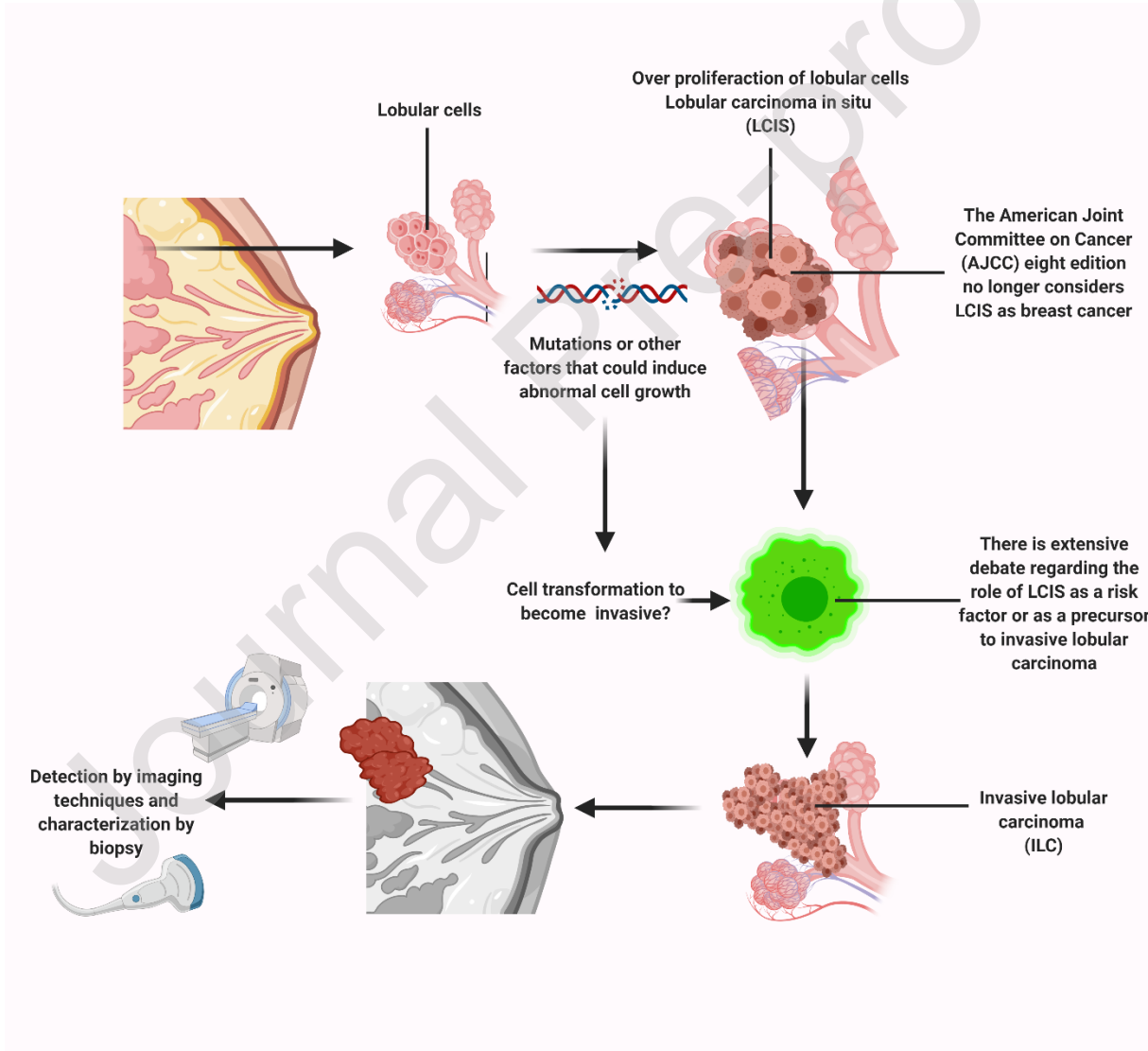
Figure 7. Development of lobular carcinoma *in situ*, a possible origin to invasive lobular carcinoma. LCIS is a breast lesion that is confined to the lobule limits and whose origin could be linked to mutations or other factors inducing abnormal cell growth. However, LCIS is not considered as breast cancer. The role of LCIS as a direct precursor of ILC is controversial since not all LCIS develop further into invasive cancer. ILC is characterized by the spread of cancerous cells beyond the lobules reaching distant breast tissue. ILC diagnosis may be challenging, usually requiring the combination of imaging techniques, biopsy, and molecular analyses. Created with BioRender.com.

9.1.3 Metastatic Carcinomas

Metastatic breast cancer, also called advanced breast cancer or stage IV, refers to the state in which tumor cells have spread beyond the boundaries of their location of origin, invading other organs (**Fig. 8**). Breast secondary tumors or metastases are commonly found in distant organs such as the bones, lungs, liver and brain [41]. It is estimated that about 30% of the women diagnosed with breast cancer at an early stage progress to recurrent or metastatic stages [255]. After cancer treatment, including mastectomy, recurrence is common due to microscopic tumor cells that may remain after surgery and metastasize again [41,250]. A study by [256]. that evaluated local recurrence of breast cancer after mastectomy showed a 6% of local cancer reappearance. Additionally, no correlation was found between age, tumor size and grade or LN involvement [256]. The risk factors affecting breast cancer relapse are poorly understood as it varies depending on the tumor stage and its specific molecular and genetic markers [41,250].

Stage IV Breast Cancer
Cancer cells spread to different tissues and organs

Invasive cancer

MIlk Duct

Lobules

Metastasis to the
axillary lymph nodes

Metastasis to distant organs
such as the brain, liver, bones and
lungs

Figure 8. Metastatic breast cancer or stage IV. This stage of cancer progression is characterized by cell heterogeneity and the spread of malignant cells beyond the limits of its original location. 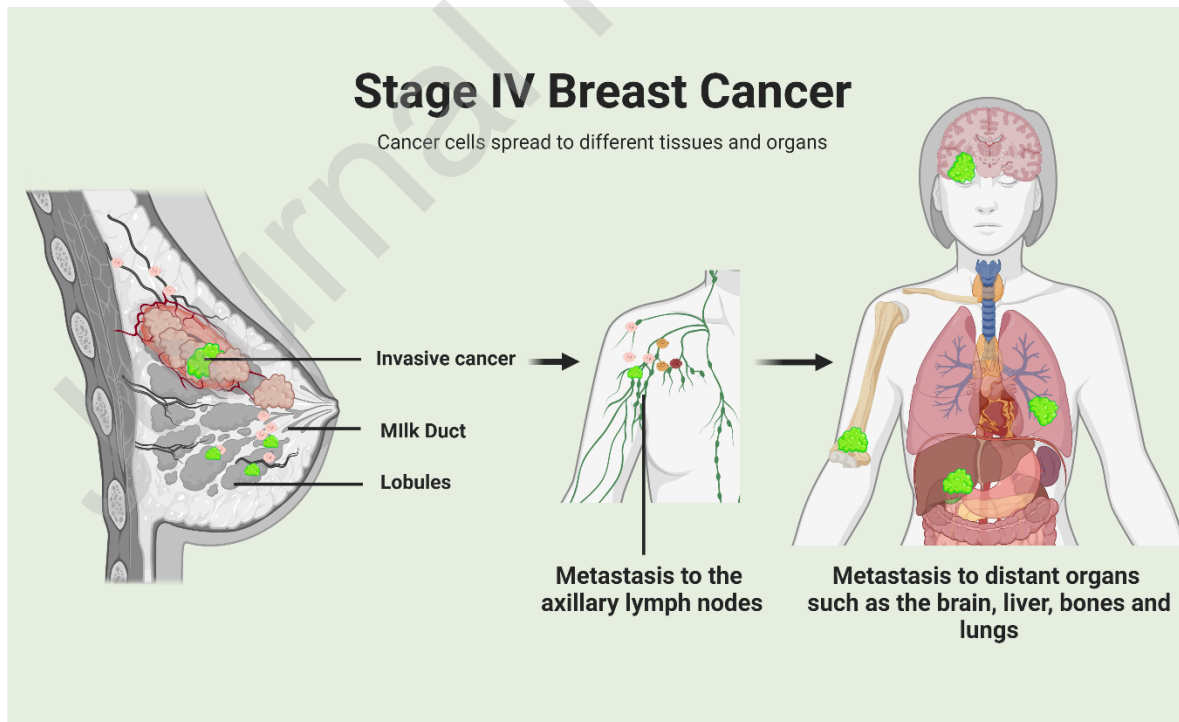At this stage, cancer cells metastasize to axillary lymph nodes and distant organs such as the brain, liver, bones, and lungs. Created with BioRender.com.

9.2 Sarcomas

Sarcomas are less common, constituting about 1% of malignant breast cancers. Sarcomas arise from the stromal tissue of the breast which consists of myofibroblasts, connective tissue and blood vessels. They can be divided into primary sarcomas which have *de novo* development or secondary sarcomas which are related to therapy radiation or chronic lymphedema [257]. Affected patients are usually women in their 50s or 60s with a painless mobile unilateral lump of variable size characterized by a faster growth rate, high risk of recurrence and worse prognosis compared to breast carcinomas [258]. Typically, the dissemination of the sarcoma does not involve LN, instead, it spreads through the blood to the bone marrow, lungs and liver. As breast sarcomas are rare and heterogenous, there is not a conclusive treatment strategy. However, current treatment guidelines include surgical excision as the first option, and CTX/radiotherapy for patients with a high risk of relapse [257].

10. Breast Cancer Biomarkers

Breast cancers are phenotypically diverse among patients in terms of growth rate, aggressiveness, hormone dependance and therapy response. Molecular biomarkers have been identified in an attempt to characterize such heterogeneity and define molecular subtypes for a better prognosis and cancer management [41,91,259,260]. The current established molecular biomarkers are those related to cell proliferation (Ki67) and receptor status: estrogen/estradiol receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) [41,261]. Estrogen-related receptors (ERRs) and extracellular elements such as microRNAs (miRNAs), exosomes (EXOs) and circulating tumor DNA (ctDNA) are emerging molecular biosignatures currently under research [261]. Genetic biomarkers for breast cancer include gene variants identified in genetic testing by sequencing, and GEP/S performed by multi-gene prognostic assays [262,263]. The use of suitable biomarkers for diagnosis could provide insights into breast cancer pathogenesis and contribute to personalized cancer management and therapeutic approaches (**Fig. 5 & 9**).
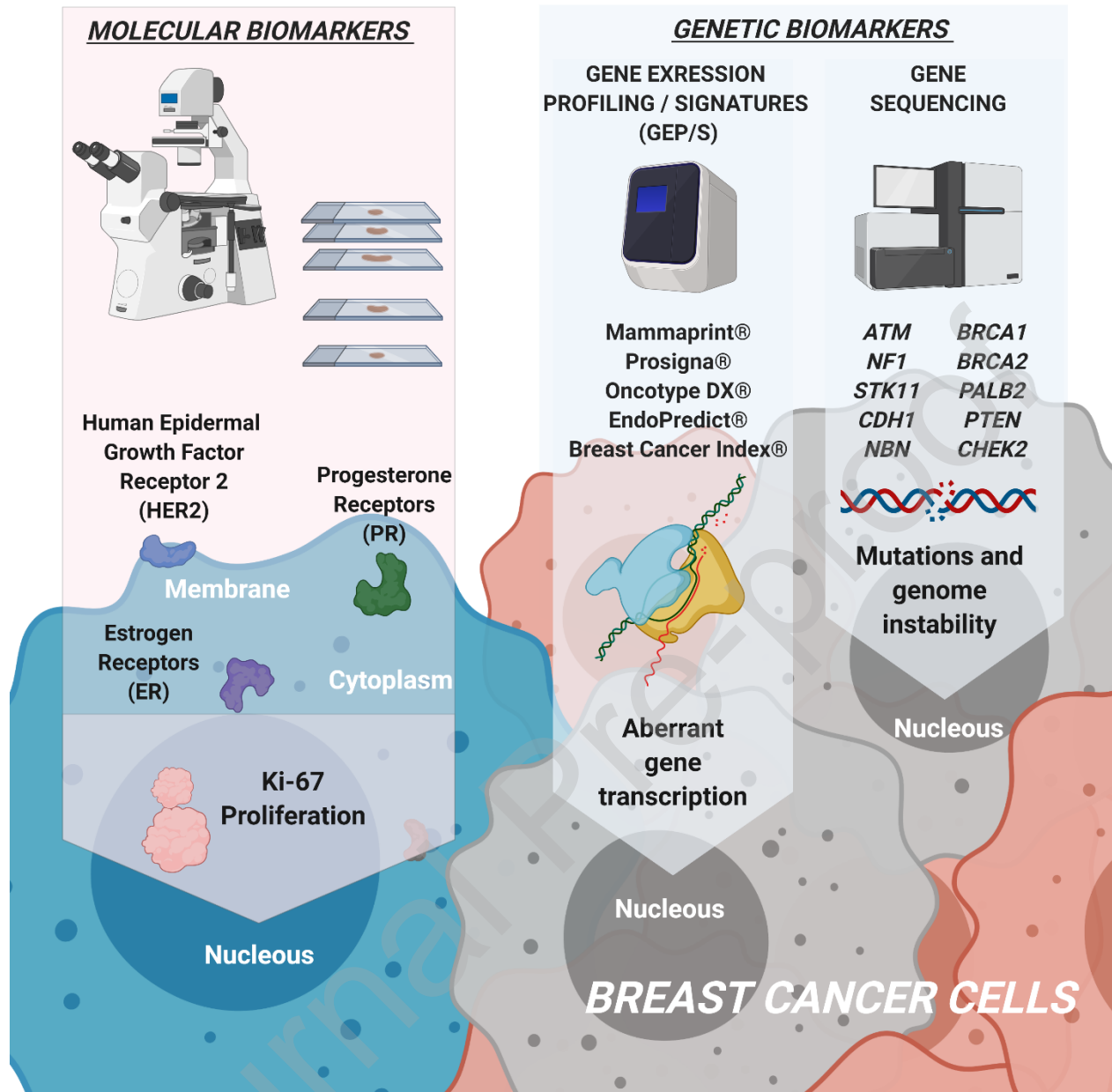
Figure 9. Molecular and genetic biomarkers, localization and tools of analysis. After detecting breast cancer, molecular and genetic biomarkers help the physician to choose the best therapeutic strategy. Molecular biomarkers are identified by IHC, from the cell membrane (HER2), the cytoplasm (PR and ER), and nucleus (Ki-67). These biomarkers provide information regarding hormone response and proliferative state of the tumor cells. Genetic biomarkers are key to characterize mutations that could increase the possibility to detect aggressive breast cancer. Gene expression profiles/signatures help to understand how a cancer behaves, allowing a better prediction of its future aggressiveness and response to therapy. Created with BioRender.com.

10.1 Established Molecular Biomarkers

10.1.1 Ki67

Ki67 is a nuclear protein that has been associated with cell proliferation in breast cancer pathogenesis. It is known that Ki67 is expressed during the cell cycle, from late G1, during G2 and S phase, and peaking at mitosis. However, Ki67 is missing in resting cells (G0) [264]. Studies have shown that high Ki67 levels are associated with a higher incidence of metastasis and recurrence in breast cancer, concluding that this protein could be considered as an important prognostic marker of tumor proliferation in breast cancer [83,264,265]. A study conducted by Soliman and Yussif, evaluated the prognostic value of Ki67 in breast cancer. Results showed that patients with less than 15% of Ki67 levels had better overall survival than those with higher Ki67 levels. Additionally, individuals with Ki67 levels higher than 15% displayed higher incidence of metastasis and recurrence. Finally authors conclude that Ki67 could be considered a valuable prognostic biomarker of breast cancer according to receptor status (e.g. molecular subtypes) [265]. However, the immunohistochemical profile of Ki67 and its implication in tumor aggressiveness could vary among populations with different ethnic backgrounds [266]. Hence, breast cancer biochemical markers should be interpreted with caution in terms of cancer heterogeneity by race and ethnicity.

10.1.2 Estrogen Receptor

The hormone estrogen/estradiol and its receptor (ER) are involved in breast cancer initiation and progression. It has been reported that some types of breast cancer initiate as estrogen dependent, therefore expressing ER, especially in the epithelial tissue [91]. Upon estrogen/estradiol binding, the ligand-activated receptor is translocated to the nucleus where it binds to a responsive element in the promoter region of genes related to tumor progression and metastasis [83]. Among ER subtypes, ERα has shown interaction with EMT regulators such as Snail and Slug which results in tumor invasion. ERα is thought to regulate Snail transcription by making a co-repressor complex along with histone deacetylase 1 (HDA1) and nuclear receptor co-repressor (N-CoR) [267]. ERβ is associated with tumor proliferation and metastasis. Its upregulation has anti-proliferative effects and decreased expression seems to induce a metastatic stage in breast cancer [83,268]. ER+ tumors comprise approximately 75% of breast cancer patients. In general, ER+ tumors are less aggressive and are associated with better clinical outcome after surgery when compared with ER- tumors. About 50% of ER+ patients have a positive treatment response to HT with anti-estrogen or aromatase inhibitors. Even though ER status is the most prevalent breast cancer biomarker, others such as PR and HER2 have also been considered for breast tumor subtyping and cancer management [91].

10.1.3 Progesterone Receptor

PR is a member of the nuclear HR family which is activated by the steroid hormone progesterone [269]. In breast cancer, progesterone-PR interaction can lead to the transcription of genes associated with cancer pathogenesis. Therefore, PR is a HR biomarker used in cancer subtype diagnosis [269,270]. PR+ tumors comprise 65% to 75% of breast cancers [91]. Since PR expression is regulated by estrogen signaling, more than 50% of PR+ breast tumors are also ER+ [269,270]. However, some cases have been reported in which breast tumors resulted PR+ but not ER- [271]. In addition, some studies found that ER+PR+ breast tumors are more susceptible to HT as compared to ER+PR- tumors [272]. Absence of PR expression in ER+ tumors may indicate abnormal growth factor signaling which could contribute to tamoxifen resistance. This emphasizes the importance of PR and ER testing in breast cancer diagnosis and treatment choice [91].

10.1.4 Human Epidermal Growth Factor Receptor 2

HER2 is a member of the epidermal growth factor receptor family whose homo or hetero-dimerization with HER1 or HER3 leads to the auto-phosphorylation of tyrosine residues which triggers a series of signaling pathways that could contribute to tumorigenesis, cell growth and proliferation [271]. Up-regulation of HER has been detected in about 15-30% of patients with breast cancer [83,273] HER2+ breast cancers are usually aggressive and its overexpression has been linked to unfavorable prognosis and good clinical outcome with systemic CTX [91,274]. Moreover, overexpression of HER2 is observed in 13% to 20% of IDCs [91]. HER2 status is also considered in clinical practice for treatment decisions regarding anthracycline-based chemotherapy and use of taxanes [91,275]. Clinical results suggest that HER2 overexpression contributes to resistance to HT in ER+ and PR+ tumors, thus ER+, PR+ and HER2+ cancers may not benefit from HT based on a single agent. In these cases, targeted anti-HER2 therapy in combination with HT may improve patient outcome [91,276]. Overall, ER+, PR+ and HER2- tumors have the best prognosis, whereas ER-, PR- and HER+ tumors are poorly differentiated, have more aggressiveness, poor prognosis and are least likely to respond to HT [91].

10.1.5 Molecular Subtypes Based on Immunohistochemistry Detection of Established Biomarkers

The use of immunohistochemistry (IHC) to detect molecular markers such as ER, PR HER2 and androgen receptor (AR) on tumor cells provides significant information on the pathophysiology of the tumor and treatment sensitivity [270] **(Fig. 9)**. Combinations between these markers can define molecular subtypes (**Refer to Table 1**). Luminal A, Luminal B, HER2+, Triple Negative and Basal-like subtypes are commonly described in clinical guidelines, whereas Normal-like and Molecular Apocrine are not usually included. It is also possible to find subclassification phenotypes in between subtypes such as the Luminal B-HER2-Like [41,91,277–279]. Molecular subtypes not commonly used in clinical guidelines are a concern because they are correlated to cases in which diverse diagnostic tools like histopathology and GEP/S fail to provide an accurate

prognosis [41,91,277–280]. Detection of molecular subtypes in combination with clinicopathological variables, such as tumor size and stage, provides a better prognosis determination and treatment selection.

Table 1.- Molecular subtypes of breast cancer

Subtypes	Molecular Profile	Properties
Luminal A	ER+ PR+ HER2- Low Ki67	● Most common subtype that characterizes about 50% of breast cancers. ● Best prognosis with HT and sometimes adding CTX.
Luminal B	ER+ PR+- HER2- High Ki67	● About 10-20% of breast cancers. ● Worse prognosis compared to Luminal A due to high proliferative capacity and lower response to treatment.
HER2-enriched	ER- PR- HER2+	● Poor prognosis compared to luminal subtypes due to its fast growth and invasion. ● Treatment with anti-HER2 (Herceptin), or CTX with anthracyclines.
Basal like	ER+ PR+- HER2- Low Ki67	● Dysfunction of the *BRCA1* gene (**Refer to Genetic Tests and Biomarkers**) by hereditary or spontaneous mutations have the worst prognosis. ● Categorized as Basal A when expressing basal markers or B when presenting less basal markers and more mesenchymal ones. ● Basal B subtype linked to a more metastatic phenotype
Triple-negative	ER- PR- HER2-	● Common in younger African American women. ● Highly related to *BRCA1* mutations. ● Poor prognosis ● Treated by CTX only.
Normal-like	ER+ PR+ HER2- KI67-	● Molecular and genetic profiles resemble normal breast tissue. ● Hardest to identify ● Intermediate grade tumors from 1 to 3 (**Refer to Staging and Grading of Breast Cancer**). ● Worst prognosis due to cancer diagnosis is made in late disease phases.

Molecular apocrine cancer	ER- PR- AR+	● Besides its molecular marker profile, Ki67 is usually found elevated, giving it a high proliferative characteristic. ● Better prognosis than ER+ and PR+ tumors because of the good response to taxanes. ● Poor prognosis is imminent if there is no good treatment response.

Source: [41,91,278,279].

10.2 Emerging Molecular Biomarkers

10.2.1 Estrogen Related Receptors

ERRs which share a high degree of homology with the canonical ERs, are associated with regulation of metabolic genes and cellular energy metabolism [281]. However, ERRs also modulate molecular pathways involved in breast cancer cell metabolism, growth and proliferation [282,283]. Unlike ERs, ERRs do not bind to ER endogenous ligands such as estrogen. ERRs are known as orphan nuclear receptors since to our knowledge no physiological ligand has been found to bind them yet [282,284]. The transcriptional activity of ERRs seems to depend on the interaction with co-regulator proteins such as peroxisome proliferator-activated receptor-γ (PPARγ), peroxisome proliferator-activated receptor gamma co-activator 1α (PGC1α) and PGC1β, and is regulated by HER2 (also called ERBB2) signaling pathway [283].

ERRα, ERRβ, and ERRγ are the three subtypes that constitute the ERR family. ERRα expression is often observed in tumors with poor prognosis. ERRα mRNA expression positively correlates to *HER2* and coactivator amplified in breast cancer 1 (*AIB1*) expression. However, it has a negative correlation to that of ERα and PR [285]. Moreover, ERRα activates or represses estrogen response elements (EREs) for transcriptional control based on ER status. In ER- breast tumors, it serves as an estrogen-independent activator by interacting with coactivators and binding to EREs of genes such as estrogen-regulated trefoil factor 1 *(TFF1)* and vascular endothelial growth factor *(VEGF)* [282]. In ER+ breast tumors, ERRα competes with ERα for DNA binding and acts as a modulator of estrogen responsiveness by interacting with corepressors and binding to negative EREs [286]. ERRα promotes progression and invasion of primary tumors contributing to bone metastasis, and is thus considered as an unfavorable prognosis biomarker [282,283].

ERRβ expression is controversial in breast tumors. ERRβ mRNA levels are positively correlated with that of ERβ and inversely correlated with the S-phase fraction measurement in the cell cycle suggesting inhibition of cellular proliferation or favoring cellular differentiation [282]. In addition, ERRβ signaling pathways have been found to inhibit EMT through Follistatin-mediated regulation of E-cadherin [287]. However, the precise role of ERRβ in breast cancer remains elusive and still needs to be investigated.

ERRγ is frequently found to be overexpressed in breast tumors. However, high ERRγ mRNA expression levels are related to less aggressive and steroid receptor cancer cells, which may indicate hormonal sensitivity and good prognosis in breast cancer patients [285,288]. Co-expression of ERRγ with ER and PR is associated with upregulation of E-cadherin which induces mesenchymal-to-epithelial transition, possibly decreasing tumor invasiveness [288]. An AAAG tetranucleotide polymorphism in the untranslated region of the *ERRγ* gene is related to breast cancer susceptibility and HT resistance in invasive lobular carcinoma [282,289].

10.2.2 MicroRNAs

miRNAs have also been identified as potential biomarkers for breast cancer. miRNAs are small non-coding RNA molecules that function as epigenetic regulators, since they are involved in gene expression control on a post-transcriptional level. Methods such as microarrays, Northern blot and qRT-PCR have been used to detect miRNAs levels [83]. Deregulation of miRNAs has been related to development and progression of some diseases including cancer [83,290]. Recent reports have shown that particular miRNA expression profiles correlate with tumor aggressiveness, drug resistance and clinical outcome in breast cancer. Increased levels of oncogenic miRNAs are thought to inhibit tumor suppressor genes, while downregulation of tumor suppressive miRNAs induces the expression of target oncogenes, overall leading to cancer initiation and progression [291]. In addition, studies have found that miRNA levels and functions vary among the different molecular subtypes of breast cancer [83,291].

In 2014, Park and his colleagues reported increased expression levels of certain circulating miRNAs including miR-1280, miR-1260 and miR-720 in patients with ER+ breast cancer. However, a downregulation of miR-1280 expression was observed after HT [292]. Other miRNAs such as miR-16, miR-34c, miR-183, miR-200c, miR-203 and Let-7 have also been associated with breast cancer pathogenesis, especially during cancer initiation [83]. Expression levels of miRNAs in breast CSCs have also been studied. Shimono et al. found different expression patterns in 37 miRNAs between breast CSCs and nontumorigenic cancer cells. In addition, the study reported the downregulation of three specific clusters (miR-200c-141, miR-183-96-182 and miR-200b-200a-429) in breast CSCs. Moreover, they demonstrated that miR-200c is able to regulate *BMI1* expression, which is involved in self-renewal of stem cell niche. Regulation of miR-200c over *BMI1* resulted in clonal expansion inhibition of breast cancer cells, hence preventing tumor formation [293]. However, deregulation of miR-200c, miR-141, miR-34c, miR-106b-25 cluster, miR-30a, and miR-30c has been related to metastasis initiation via EMT-related molecular processes [83,294–296]. Therefore, analysis of miRNA expression profiles could be useful for cancer diagnosis and prognosis.

10.2.3 Exosomes

EXOs are nano-size membrane encapsulated vesicles with 40-100 nm diameter that are candidates to be used as diagnostic biomarkers for breast cancer. They transfer various molecular signals such as proteins, DNA, mRNA, miRNAs. EXOs and their cargoes have modulating roles in the cellular activity of target cells [62,83,297]. Tumor-related EXOs have been implicated in the initiation and development of cancer including immunosuppression, angiogenesis, metastasis and drug resistance [298–300]. It is known that breast cancer cells produce EXOs containing miRNAs, which can induce normal breast cells to undergo malignant transformation, thus contributing to tumor growth and progression [301].

Various studies observed associations between alterations in expression levels of circulating EXO-encapsulated miRNAs and breast cancer. Hannafon et al. showed that exosomal miR-21 and miR-1246 were upregulated in plasma of breast cancer patients compared to healthy subjects [302]. Others have reported correlation between exosomal miRNAs and breast tumor subtype and pathological stage. High levels of exosomal miR-939 have been found in basal-like breast cancer and it is associated with unfavorable prognosis in triple negative tumor subtypes [303]. Moreover, miR-195 has been found to be a potential biomarker for non-invasive and early stage breast cancer whereas miR-21 levels have been found in early and advanced stages of the disease [304,305]. Singh et al. found high concentrations of exosomal miR-10b in metastatic MDA-MB-231 cells as opposed to non-metastatic or normal breast cells [306]. miRNA biological markers could serve as complementary diagnostic tools in breast cancer by analyzing them at different times during the disease progression (e.g. baseline, pre-treatment, follow-up) [300].

10.2.4 Circulating Tumor DNA

ctDNA refers to short cell-free DNA fragments that originate from tumors. These can be detected by molecular analysis of noninvasive liquid biopsies using PCR techniques or genome sequencing, thereby becoming promising biomarkers in BCS, disease monitoring and treatment response [236,307]. A 2019 study performed plasma ctDNA analysis for the detection of somatic mutation in *PIK3CA, ESR1, ERBB2* and *AKT1* in 234 metastatic patients. Mutations were identified in 63 patients (39.6%) showing potential gene variant patterns in this subpopulation of breast cancer patients which could be useful for clinical management [308]. Similarly, a prospective multicenter study evaluated the predictive factor of ctDNA for cancer relapse in a cohort of 101 patients. This study found that ctDNA detection during follow-up (every 3 months for the first year and every 6 months afterwards) is linked to a high risk of future relapse in early-stage breast cancer. Moreover, the study found an association between ctDNA detection at diagnosis, before treatment and relapse-free survival [309]. The potential of ctDNA to aid treatment decisions has also been evaluated. A study conducted by Schiff et al. identified three driver mutations (*RB1, PIK3CA, ESR1*) of resistance to fulvestrant and palbociclib and presented

promising results that support ctDNA analysis to monitor targeted drug resistance [310]. Moreover, Bidard et al are currently investigating the efficacy of palbociclib in combination with HT through ctDNA analysis of ESR1 mutation in ER+, HER2- metastatic breast cancer patients. [311]. Thus, ctDNA testing as a potential biomarker tool for breast cancer management has been elucidated in clinical studies. Nonetheless, more evidence is still required to validate the efficacy of this biomarker for cancer prognosis and treatment response.

10.3 Genetic Tests and Biomarkers

10.3.1 Gene Sequencing

Gene sequencing is used as a method for detecting the pathogenic variants of genes associated with high-risk breast cancer. Sanger sequencing is the classical method to detect these genetic variations. However, it has less sensitivity and sequencing volume than newer techniques. Such techniques as Next generation sequencing and Whole genome sequencing have enabled large-scale sequencing which allows the identification of novel mutations and candidate genes associated with breast cancer. Nevertheless, validation studies and co-segregation analysis of novel identified variants are required to determine their clinical significance [312–315]. Mutations in *BRCA1* and *BRCA2* genes have been associated with hereditary breast and ovarian cancer. Women with *BRCA1* or *BRCA2* mutation by the age of 70 could have an approximate breast cancer risk between 57% to 65% and 45% to 49% respectively, depending on the study [316]. Other pathogenic variants in genes such as *PALB2, PTEN, CHEK2, ATM, NF1, STK11, CDH1,* and *NBN* have also been associated with breast cancer risk. Individuals with a personal or/and family history of breast and ovarian cancer are recommended to seek counseling for genetic screening in order to endeavor appropriate preventive measures [262,317]. Genetic testing counseling should be considered before and after the genetic screening to provide appropriate information regarding test options, interpretation of results and following screening. Furthermore, genetic tests should be performed based on the analytical validity, clinical validity, clinical utility, ethical, legal and social implications (ACCE) test framework [262,318] **(Fig. 9)**.

Genetic tests are highly sensitive and accurate. For example, BRACAnalysis® has a sensibility of >99.98%, with a rate of errors of <1%. The accuracy of this test >99% in a patient with a 10% probability of being positive based on personal or family history [319]. However, there are some limitations that can occur when performing the genetic sequencing of *BRCA1* and *BRCA2* inheritable mutations. These situations will impede the detection during the sequencing: 1) some sequences can only be determined in one direction (forward or reverse); 2) sometimes the polymorphisms are less frequent and difficult to detect; 3) presence of certain inversions, insertions or regulatory mutations. For example, some insertions that do not result in duplications will not be detected by sequencing. Valencia et al. describes that the test result could be issued to the physicians between one to several weeks, and these are presented in three forms: 1) positive for

deleterious mutation, 2) no deleterious mutation, 3) genetic variant without an identified breast cancer risk. Genetic testing for non-*BRCA* mutations associated with hereditary breast cancer syndromes (e.g. Li-Fraumeni), have poor indications and interpretations. Moreover, they have variable costs (e.g. 400-3000) depending on the number of genes tested, laboratory pricing and insurance [319,320].

After a positive test result, preventive procedures and medication should be considered to reduce breast cancer risk of development. These preventive measures can be chosen depending on the age, medical history, prior treatments, past surgeries and other factors related to the patient. Risk-reducing strategies in *BRCA1/2* mutation patients include chemoprevention, oral contraceptives and risk reduction surgery [321,322].

10.3.2 Gene Expression Profiling/Signatures

After a confirmation of breast cancer by biopsy, there are some clinical recommendations (**Refer to section Genetic Signatures & Clinical Guidelines**) to perform a multigene expression analysis of the tumor biopsy to guide therapeutic decisions. GEP/S is a helpful tool for breast cancer prognosis and management as it identifies differences in the aggressiveness among tumors with the same anatomical staging, genetic predisposition or IHC markers [243]. For example, GEP/S could assist the therapeutic decision in low grade breast cancer which might become aggressive and resistant to CTX [323]. Among the commercially available assays, in this section we described the most used and discussed in clinical guidelines: MammaPrint®, Prosigna®, Oncotype DX®, EndoPredict® and Breast Cancer Index®. Most of the genes used for GEP/S are dissimilar making each signature not interchangeable. Clinical guidelines recommendations regarding each signature are mentioned at the end of this section. (Table 2) [97,243,263,324,325] (**Fig. 9**).

10.3.2.1 MammaPrint®

MammaPrint®, developed by the company Agendia®, is a 70-gene expression prognostic test used to determine breast cancer recurrence risk within the 10 years after diagnosis (Table 2). It was designed for patients with an early stage of breast cancer with LN+ (1-3 nodes) or LN- diagnosis. This test classifies results into two low-risk and high-risk groups. The use of MammaPrint® provides an accurate assessment of prognostic risk among ER+ tumors. This is not the case for ER- tumors because they are mostly classified as a high-risk group [263,326,327]. The signature was developed using an Agilent microarray platform of 25,000 genes that analyzed 78 frozen tumor samples with a complete medical history of the patients from the Netherlands Cancer Institute. The tested samples had to fulfill the following inclusion criteria: LN-, tumor diameter <5cm, no previous malignancies and issued from patients under 55-years-old. Then, using a supervised classification algorithm to find the fittest model, a significant correlation was found

between 231 out of the 25,000 genes and distant metastases within 5 years. Later, they ranked the 231 genes and chose the top 70 that most accurately classified the tumors into low or high risk groups. Finally, 19 external samples (from the same institute) were used to validate the signature. MammaPrint® predicted the risk group correctly in 17 of the 19 samples [328,329].

In 2019, Brandao and colleagues mentioned three key studies that assessed the clinical validation of MammaPrint®, including the TRANSBIG consortium study, the RASTER and the MINDACT trials [327]. The TRANSBIG consortium study (an international network conformed by physicians, scientists, patient advocates and biotechnology professionals) performed an independent multicenter retrospective validation in 302 patients under the age of 61 with T1-2, LN- and 70% ER+ tumors. The goal of the study was to compare the prognostic value between MammaPrint® and a clinicopathologic prognostic tool (Adjuvant! Online). Results showed that MammaPrint® determined a strong prognostic factor of cancer recurrences, distant metastasis, (hazard ratio of 2.32; α =0.05) and overall survival (hazard ratio of 2.79; α =0.05) for up to 10 years after diagnosis. Authors concluded that the signature could provide additional independent prognostic information from the data obtained by the clinicopathologic prognostic tool [330,331]. The microarRAy-prognoSTics-in-breast-cancER (RASTER) trial was the first prospective study that assessed 427 patients regarding MammaPrint®'s performance. The aim of the study was to analyze the recurrence prognostic factor of MammaPrint® as a guidance for adjuvant therapy decisions compared to clinicopathological tools (Adjuvant! Online). Patient inclusion criteria consisted of: ≤54 years of age, T1-3, LN- and adjuvant systemic treatment. The results confirmed an additional prognostic value of MammaPrint compared to the clinicopathological risk estimation in a 5 years period [332].

The Microarray in Node-Negative Disease May Avoid Chemotherapy Trial (MINDACT) sponsored by the European Organization for Research and Treatment of Cancer (EORTC) is a prospective randomized multicenter validation study that included 6,693 patients with early stage breast cancer. The preliminary results showed that the use of MammaPrint® at classifying patients with low genetic recurrence risk, may help to avoid CTX in patients previously identified in the high clinicopathological risk group. Therefore, using MammaPrint® could avoid CTX in patients with a high clinical but low genomic recurrence risk within a 5-year period. Additionally, MINDACT validated the use of adjuvant CTX in those classified in the high-risk group. The trial is still ongoing and will end in June 2022 [333–335].

10.3.2.2 Prosigna®

PAM-50®, the previous version of Prosigna®, was created by the NanoString® company to define an alternative option for conventional molecular subtyping (e.g. IHC) (Table 2) [336]. The current purpose of Prosigna® is to provide a risk of recurrence score (from 0 to 100%) up to 10 years after diagnosis, employing 50 genes plus clinicopathological indicators (e.g. tumor size)

[337,338]. The score is applicable in postmenopausal patients with HR+, LN- or LN+ (1-3 nodes) and stage I-II tumors treated with HT [339–341]. Prosigna® divides node-negative patients into low (≤40 score), intermediate (41-60 score), and high risk of recurrence (>60 score). For node-positive patients, the cut-offs for risk of recurrence vary depending on the number of node involvement [342].

Prosigna® was validated by using formalin-fixed paraffin-embedded tissue samples across multiple clinical laboratories, showing no significant variations on its analytical performance [341]. A retrospective validation study used 1,017 ER+, HER2- and LN+/- primary breast cancer samples [from the TransATAC (Arimidex, Tamoxifen, Alone or in Combination) trial)] treated with anastrozole or tamoxifen to assess the prognostic information from the score. This prognostic information was also compared to the prognostic data from Oncotype DX and immunohistochemical 4 (IHC4). The study found that the Prosigna® score provides more prognostic information than Oncotype DX, with a better differentiation in intermediate and high risk groups. ICH4 provided similar information to Prosigna® in all patients but lower in the HER2- and LN- patients [343]. Gnant et al. assessed 1,478 samples from the ABCSG-8 trial that used postmenopausal women with early breast cancer ER+ and treated with adjuvant HT alone (tamoxifen or tamoxifen followed by anastrozole), to see if the prediction score provides additional information than clinical variables [Clinical Treatment Score (CTS)]. The signature significantly (p<0.0001) adds prognostic information to the clinical variables. These results, in addition to the results from the ATAC trial, provide Level 1 evidence (one or more validation studies with consistent results) for the signature clinical validity [336,344]. A retrospective study comprehended 2,485 samples from both ATAC and ABCSG-8 trials. Patients were postmenopausal women that had early breast cancer with HR+ and LN- or LN+ (1-3 nodes). The patients were also treated with tamoxifen and/or anastrozole. The study aimed to know if Prosigna® could classify the patients providing additional information regarding their prognosis compared to clinical variables (CTS). Results showed that Prosigna® provided more information than clinical variables in LN- (p<0.0001) and LN+ ($p<0.0002$) patients, and classified them into the risk of recurrence subgroups for up to 10 years after diagnosis [345]. Additionally, this signature retrospectively analyzed 122 tumor samples that were HR+ and HER2- patients who had received multiple cycles of a standard anthracycline and taxane therapy before surgery. Prosigna® provided a significant prediction of recurrence risk for patient response to neoadjuvant CTX [346]. In 2015, Alvarado and colleagues prospectively compared the recurrence risk estimates of Prosigna and Oncotype from 52 patients showing a poor correlation between these signatures and none interchangeability [347].

10.3.2.3 Oncotype DX®

Oncotype DX Breast Recurrence Score®, provided by the Exact Sciences Corp., analyzes the expression of 16 cancer-related and 5 housekeeping genes using RT-PCR. These genes are

divided into six representative groups: 1) Estrogen group, 2) HER2 group, 3) proliferation group, 4) invasion group, 5) others and 6) reference group (Table 2) [348]. The test is able to report a quantitative recurrence score value for each patient, with a standard deviation of 2 recurrence score units in a 100-unit scale [349]. Oncotype DX® provides a cancer relapse score that divides patients into high-risk (score ≥31), intermediate-risk (score 18 to 30) and low-risk (score <18) groups. These scores predict disease recurrence for up to 10 years and the probable efficacy of using adjuvant CTX in high risk patients [263,350–353]. This test intends to benefit patients with an early stage of invasive cancer, ER+, HER2- and LN- or LN+ (1-3 nodes) breast cancer [354].

Based on a meta-analysis study performed in 2013, the signature showed an elevated prevalence of patients with an intermediate recurrence risk. A classification of intermediate risk could represent an unnecessary investment for the patient as results lack clinical utility [355]. The TAILORx trial compared patients receiving HT and chemo-endocrine therapy to find out if those with an Oncotype DX® intermediate score could avoid adjuvant CTX. This study concluded that patients with an intermediate risk score could not benefit from the administration of CTX. Approximately 85% of these patients could be spared from adjuvant CTX if the recurrence score is lower than 25 and the age is >50 years or if the recurrence score is lower than 15 and the age is <50 years [354]. The TAILORx results were consistent across multiple studies with thousands of samples. Among them; two studies with 38,568 and 6,768 samples respectively, both from the Surveillance, Epidemiology and End Results (SEER) databases [356,357]; a retrospective study with 709 samples from the Clalit Health Services registry [358]; and the prospective West German Study Group Phase III PlanB Trial with over 3,198 samples [359].

10.3.2.4 EndoPredict®

EndoPredict®, provided by the Myriad company, quantifies the mRNA levels of 8 genes and 4 reference genes in formalin-fixed paraffin-embedded tissue samples by RT-PCR (Table 2). When combined with clinical characteristics such as nodal status and tumor size, this signature becomes the EndoPredict Clinical® (EPclin®) analysis [360,361]. EPclin® is capable of predicting the risk of distant metastasis within 10-year period after diagnosis, in breast cancer of postmenopausal women with ER+, HER2-, LN- or LN+ (1-3 nodes) and, receiving HT or CTX. The score classifies the patients into a high (≥3.32867) and low (<3.32867) risk groups [362–365].

A retrospective 10-year study compared the prognostic classification between EPclin® and clinical guidelines, such as the NCCN, German S3 and SG. The study included 1702 postmenopausal woman from the ABCSG-6 (tamoxifen-only arm) or ABCSG-8 trials, that had ER+ and HER2- tumors and received only HT (either tamoxifen or tamoxifen followed by anastrozole). The genetic signature showed an absolute risk reduction of 18.7% for distant metastasis compared to clinical guidelines. EPclin® reclassified 58%-61% of the patients to a low risk group, from the high risk group originally determined by the clinical guidelines. On the

contrary, EPclin incorrectly reclassified 5% of women to a low risk group, when actually having a high risk of distant metastasis within the 10 years [360]. Buus et al. compared the assessment for the risk of distant metastasis of EndoPredict® and EPclin® with Oncotype DX®. The retrospective study evaluated 928 breast cancer patients (from the TransATAC monotherapy arm trial) with ER+ and HER2- tumors treated with anastrozole or tamoxifen for 5 years. EndoPredict® alone had a similar performance to Oncotype DX® when predicting metastasis in a 5-year period, but it was superior in the 10-year period. EPclin® outperformed Oncotype DX® when estimating the risk of metastasis in the 10-year period, with the exception of LN- patients in the 5-year period [366]. The ongoing Extended Endocrine Trial (EXET) is evaluating the benefit from receiving HT for longer than 5 years (extended therapy) in patients classified in the high risk group. Results will be available in 2022. [367].

10.3.2.5 Breast Cancer Index®

Breast Cancer Index®, created by Biotheranostics Inc., is a RT-PCR test designed for the prediction of early (0-5 years) or late (5-10 years) breast cancer recurrence after diagnosis, in patients with ER+, LN- or LN+ (1-3 nodes). The test uses two parameters to calculate the score: 1) the gene ratio of *HOXB13:IL17BR*, and 2) the molecular grade index of 5 proliferation-related genes (Table 2). Depending on the score, patients can be classified into low (<5.0825), intermediate (≥ 5.0825 to <6.5025), or high (≥ 6.5025) risk groups [263,368–372].

In a prospective study, 665 samples of postmenopausal patients with ER+ breast cancer from the TransATAC tissue bank were used to compare Breast Cancer Index® with Oncotype DX® and the IHC4 assay. The study found that Breast Cancer Index® was the only significant prognostic test for early and late distant recurrence. The test could also identify patients who can benefit from HT as indicated by a high risk score [373]. Sgroi et al. evaluated the prognostic performance of Breast Cancer Index® for distance recurrence and treatment benefit from extending letrozole 5 more years after a 5-year treatment with tamoxifen. The retrospective study assessed 5,157 eligible breast cancer ER+ and/or PR+ patients from the MA.17 trial. Results showed that a high risk score was statistically associated with decreased late recurrence in patients with extended letrozole treatment (2,575 patients) compared with placebo (2,582 patients) (adjusted OR: 0.33; 95% CI: 0.15-0.73; p=0.006) [374]. The prospective phase III trial Tras-aTTom randomized 6,953 HR+ patients, to stop or continue tamoxifen after 4 years of initial therapy. This trial showed a statistical association between the signature and a high risk score with a 9.8% benefit for a 10-year treatment with tamoxifen and no association with a low risk score. Therefore, this study provided a level 1B of evidence for Breast Cancer Index as a predictive biomarker for extending HT [375]. Overall, studies have provided evidence for the prognostic value of GEP/S in breast cancer. However, clinical guidelines are required to considerate the use of GEP/S in clinical practice.

10.3.2.6 Genetic Signatures & Clinical Guidelines Recommendations: Towards the Prognostic Staging

In 2017, the St. Gallen International Breast Cancer Conference (SG) panel gathered more than 3,000 experts from >100 countries to evaluate the use of the five genetic signatures covered above (Table 2) in clinical practice. Only 64% of the panelists agreed that molecular subtypes of breast cancer should be determined by a multigene test instead of conventional techniques used at the time (e.g. IHC). However, 86% agreed not to consider necessary the use of GEP/S in ER+, PR+, HER2-, low-Ki67, T1a-b, LN- and low-grade early breast cancer patients. Aside from this, all panelists concurred that gene signatures provide valuable information regarding prognosis in patients with high clinicopathological risk factors (e.g. HER2+). Additionally, SG accepted that these signatures could help in the therapeutic decision regarding the omission of CTX, especially for ER+, HER2- and LN- in early breast cancer. Panelists conceded that using gene signatures for ER+, HER2- and LN+ is not advisable at decision making for treatment. Finally, just 46% of the panelists agreed that the genetic signatures provide valuable information for the clinical decision, regarding extended HT (more than 5 years) for breast cancer [324].

Most of the panel (93.8%) endorsed the practice of Oncotype DX® for breast cancer prognosis for up to 5 years after diagnosis in patients with ER+, HER- and LN- tumors. While MammaPrint® and Prosigna® had an acceptance of 81.3% and 80% respectively in these cases. The least preferred options were EndoPredict® with 70% and Breast Cancer Index® with 60% of the experts' approval. Addressing the use of the signatures in LN+ cases, the percentage of approval decreased to 60% for Oncotype DX®, 42.9% for MammaPrint®, 75% for Prosigna®, 43.3% for Breast Cancer Index® and 55.6% for EndoPredict®. When considering CTX prescription in ER+, HER2- and LN+ patients, the acceptance percentage did not abate much for MammaPrint® and Oncotype DX®. This was not the case for Prosigna®, leading to an acceptance of 46.7%, and even lower for EndoPredict® and Breast Cancer Index® with 15.8% and 8.1% respectively [324].

In the SG panel of 2019, the discussion about genetic signatures was less debated compared to 2017. The 93% of panelists recommended the use of genetic signatures to endorse CTX in ER+, HER2- and LN- tumors whereas 74% suggested their application in T3 and LN- patients, and 78% in T3 and LN+ cases. For Luminal A-like tumors with ER+, HER2-, grade 1 and ≥4 LN+ involved, only 65% of the experts consented to use the genetic signatures. After the TAILORx trial, the experts preferred CTX for women younger than 50 years with LN- breast cancer and an Oncotype DX® recurrence score of 21-25. Such score was considered before the study as indeterminate, which did not provide prognostic information to guide clinical decision. Based on the MINDACT trial results, 80.9% of the experts decided to spare CTX in patients older than 50 years, LN+ and within the MammaPrint®-low risk group [376].

The 2019 recommendations from the American Society of Clinical Oncology (ASCO) regarding the use of Oncotype DX® stated that: 1) the test is suggested in ER+, PR+, HER2- and LN- tumors to guide adjuvant systemic CTX; 2) HT alone is recommended for patients with a recurrence score of <26 that are >50 year of age, or with a recurrence score of <16 that are ≤50-years-old, due to there is little to no benefit from CTX on these cases; 3) CTX is recommended for patients ≤50-years-old with a recurrence score between 16 and 25; 4) high quality evidence recommends that patients should be considered for a chemo-endocrine therapy if they have a recurrence score of >30. Based on the expert panel's consensus there is insufficient quality evidence to apply the same recommendation if the patient has a recurrence score from 26 to 30; 5) the signature should not be used to guide decision for adjuvant systemic CTX if the patient is ER-, PR-, HER2-, and LN+, or HER2+, or triple negative breast cancer [377].

ASCO recommendations for EndoPredict® include: 1) the signature is suggested to guide adjuvant CTX decision in patients ER+, PR+, HER2- and LN-; 2) the signature is not recommended to guide the decision within HER2+ and LN+, or triple negative. Regarding MammaPrint®: 1) the withholding of CTX can be suggested by a high-risk categorization, because of a potential but limited benefit from the treatment; 2) in patients with a low clinical risk, the genetic signature would not change treatment decisions, because even with a high genetic risk, CTX is not recommended; 3) a high genetic risk categorization recommends to withhold CTX if the patients are ER+, PR+, HER2- and LN+. However the patients should be informed that there is a chance to benefit from CTX in these cases, and even more if there are >1 LN+ involved; 4) the genetic signature is not recommended in low clinical risk, HER2+, or triple negative patients due to insufficient data [377]. For Breast Cancer Index®, ASCO stated that: 1) the signature is helpful for adjuvant systemic treatment decisions if patients have ER+, PR+, HER2- and LN- tumors; 2) the test is not recommended in LN+, HER2+, or triple negative patients to help in treatment decisions. A final recommendation is to avoid using Oncotype DX®, EndoPredict® or Breast Cancer Index® to decide on extending HT, if the patient is ER+, PR+, HER2- and has had at least 5 years of HT without evidence of recurrence [377].

The NCCN panel states that GEP/S provide prognostic information in addition to the anatomical staging and biomarker information. GEP/S could be considered for assessing the risk of recurrence, but not all of them foresee if systemic CTX could reduce the risk of recurrence. The panel avails Oncotype® as the only GEP/S that is capable of predicting the benefit of adding adjutant CTX to further reduce the risk of recurrence. For patients with axillary LN-, HR+ and HER2-, the panel has specific recommendations regarding GEP/S: 1) Oncotype® is suggested in patients with invasive or ductal breast cancer of >0.5cm diameter and LN- to estimate risk of recurrence and CTX predictive benefit; 2) Mammaprint® is being capable of identifying patients with low genomic risk but high clinical risk that could avoid CTX. The panel also recommends GEP/S prognostic information in axillary LN+, HR+ and HER2- to assist treatment decisions [6].

ESMO recommends the use of MammaPrint®, Oncotype DX®, Prosigna®, EndoPredict® or Breast Cancer Index®, as an additional guide for treatment decision in cases of uncertainty after considering all clinicopathological factors. MammaPrint® or Oncotype DX® could be applied in ER+ and HER2- patients to obtain prognostic information, to prescribe adjuvant CTX if high risk or high score is obtained. After considering the tumor size and LN status in their final risk assessment, Prosigna® or EndoPredict® are recommended to indicate adjuvant CTX in ER+ and HER2- patients [97]. ESMO do not recommended GEP/S to patients who have: 1) ER+, LN-, T1a-b and grade 1 tumors; 2) presence of a comorbid condition that limits to receive adjuvant CTX; 3) special cellular subtypes of luminal-like breast cancer (e.g. low-grade encapsulated papillary carcinoma) that are treatable with locoregional treatment only and have a good prognosis estimated by clinical factors; 4) presence of up to 3 LN+ and high risk factors (e.g. comorbidities); 5) ≥4 LN+ and adjuvant CTX eligibility [97].

There is a vast amount of published information that validates and compares the signatures reviewed above. Even though the scope of employability among patients is similar between signatures (**Refer to Table 2**), their performance and/or applicability could vary depending on the type of validations (e.g. prospective or retrospective studies, number of samples) and the population used in such validations (e.g. age, TNM anatomical stage, molecular subtype) [326,366,378–380]. Buus et al. presented an abstract at the "San Antonio Breast Cancer Symposium" that showed an ongoing study aiming to evaluate the concordance of prognostic information of the four genetic signatures (All of the above except for MammaPrint®) using a Spearman rank correlation. The preliminary results from 785 patients of the TransATAC sample set revealed the following: 1) EndoPredict® scores are moderately correlated to the other three signatures; 2) Oncotype DX® recurrence scores are divergent from those of Breast Cancer Index® and Prosigna® [380]. Therefore, besides the fact that all signatures give prognostic information of breast cancer, their use is limited by the wide spectrum of tumor's characteristics. It is crucial to identify which signature is the most appropriate for each patient, even if all have powerful validation studies. The key is to achieve a more personalized cancer management with the help of genetic signatures.

Clinical guidelines can avail the use of genetic signatures in clinical practice. Hence, guidelines recommendation serve as a powerful tool because they consider clinical expert opinion, the signature scope, patients characteristics and the quality of evidence available [7,243]. The latest AJCC's Cancer Staging Manual proposed "Prognostic staging" as a change from the previous edition, where only anatomical staging (TNM) was considered. The new edition combines TNM with tumor grade, receptor status and genomic tests to provide a more accurate prognosis. For instance, previously a "T0-2, N2, M0, ER+ and HER2-" tumor received a stage IIIa. If the patient also obtained an Oncotype DX® of <11 (low-risk), the stage would change from IIIa to Ib [243]. The updated prognostic staging can concede physicians a more holistic comprehension of the final

diagnosis prognosis. Thus, physicians are able to generate a more thoughtful treatment decision with the most favorable prognosis possible for each patient.

Table 2.- Genetic signatures for breast cancer prognosis

Characteristics	MammaPrint®	Prosigna®	Oncotype DX®	EndoPredict®	Breast Cancer Index®
Number of genes	70[1]	50[2]	21[3]	12[4]	7[5]
Molecular method	Microarray	RT-PCR	RT-PCR	RT-PCR	RT-PCR
Scope of employability	Early stage invasive breast cancer ER+, HER2-, and LN- or LN+ (1-3 nodes).	HR+, LN- or LN+ (1-3 nodes) and stage I-II breast cancer when treated with HT.	Early stage invasive breast cancer ER+, HER2-, and LN- or LN+ (1-3 nodes).	Breast cancer in postmenopausal women with ER+, HER2-, LN- or LN+ (1-3 nodes) and while treated with HT or CTX	Early stage invasive breast cancer ER+, HER2-, LN- or LN+ (1-3 nodes) and the benefit when extending HT.
Manufacturer	Agendia BV®	NanoString Technologies, Inc.	Exact Sciences Corp.	Myriad®	Biotheranostics, Inc.

FDA status	Cleared, submissions: K101454, K081092, K080252, K070675, K062694.	Cleared, submissions: K130010	Not cleared or approved	Not cleared or approved	Not cleared or approved
Guidelines inclusion*	ASCO NCCN ESMO EGTM SG	ASCO NCCN ESMO EGTM SG	ASCO NCCN ESMO EGTM SG	ASCO NCCN ESMO EGTM SG	ASCO NCCN ESMO EGTM SG
Cost±	≈ $3,200	≈ $4,000	≈ $4,000	≈ $2,100	≈ $3,450

Abbreviations: ASCO: American Society of Clinical Oncology; NCCN: National Comprehensive Cancer Center Network; ESMO: European Society for Medical Oncology; SG: St. Gallen International Breast Cancer Conference; EGTM: European Group on Tumor Markers.

*Recommendations from guidelines vary from the scope of employability, for more information refer to "Genetic Signatures & Clinical Guidelines Recommendations: Towards the Prognostic Staging" or to the guidelines cited web pages to see evidence-based levels.

±Prices listed are an approximate and may vary according to the region time and supplier. Any conversion was applied at the moment of consult.

1. *MS4A7, TGFB3, CENPA, Contig32185_RC, COL4A2, CMC2, CDC42BPA, HRASLS, RFC4, LPCAT1, ALDH4A1, FGF18, GMPS, CCN4, PRC1, NDC80, ECI2, CCNE2, DRS1, Contig48328_RC, AA555029_RC, ORC6, ESM1, SCUBE2, Contig51464_RC, MMP9, TSPYL5, ADGRG6, PALM2-AKAP2, Contig55377_RC, IGFBP5, BCL2, MCM6, NMU, MELK, Contig38288_RC, PITRM1, Contig55725_RC, NUSAP1, SLC2A3, Contig46223_RC, DTL, MTDH, Contig20217_RC, EBF4, RAB6B, Contig28552_RC, Contig40831_RC, DIAPH3, UCHL5, Contig63102_RC, EXT1, Contig63649_RC, TMEM74B, STK32B, Contig2399_RC,*

SMIM5, SERF1A, OXCT1, Contig35251_RC, Contig56457_RC, AP2B1, IGFBP5, FLT1, Contig46218_RC, GNAZ, Contig32125_RC, Contig25991, DCK, GSTM3.

2. *ACTR3B, ANLN, BAG1, BCL2, BIRC5, BLVRA, CCNB1, CCNE1, CDC20, CDC6, CDCA1, CDH3, CENPF, CEP55, CXXC5, EGFR, ERBB2, ESR1, EXO1, FGFR4, FOXA1, FOXC1, GPR160, GRB7, KIF2C, KNTC2, KRT14, KRT17, KRT5, MAPT, MDM2, MELK, MIA, MKI67, MLPH, MMP11, MYBL2, MYC, NAT1, ORC6L, PGR, PHGDH, PTTG1, RRM2, SFRP1, SLC39A6, TMEM45B, TYMS, UBE2C, UBE2T.*

3. The six gene groups analyzed are categorized as the Estrogen group (*ER, PGR, BCL2, SCUBE2*), HER2 group (*GRB7, HER2*), Proliferation group (*Ki67, STK15, Survivin, CCNB1, MYBL2*), Invasion group (*MMP11, CTSL2*), Others (*GSTM1, CD68, BAG1*) and Reference group (*B-actin, GAPDH, RPLPO, GUS, TFRC*).

4. *BIRC5, UBE2C, DHCR7, RBBP8, IL6ST, AZGP1, MGP, STC2;* Reference genes: *CALM2, OAZ1, RPL37A, HBB.*

5. Genetic index: *HOXB13, IL17BR*; Proliferation genes: *BUB1B, CENPA, RACGAP1, RRM2, NEK2.*

Source: [6,97,324,325,327,377,381–386].

11. Issues with Current Screening and Diagnostic Tools: The Need for New or Improved Techniques

Breast cancer diagnosis and treatment could involve repeated visits of patients to fully equipped medical centers. It has been shown that living in rural areas can affect the access to breast cancer diagnostic tools, potentially resulting in delays in cancer diagnosis [387]. Additionally, being far from primary health centers and hospitals, prevents the availability of treatment, surgery, or appropriate interventions, decreasing the possibility of positive outcomes and patient recovery [387–390]. Another issue is the discomfort and stress that is generated during screening and diagnostic procedures that reduce the willingness of patients to perform the tests [11–14]. There is a need for accurate, non-invasive data collection and comfortable equipment to perform screening and diagnosis. Hence, future screening and diagnostic tools should be developed aiming for portability, possible wearable features, comfort, and improved connectivity for data sharing. New devices should take into consideration the possibility to connect to wireless and global networks (e.g. Starlink) to share clinical data between patients and physicians. Data shared in wireless networks often pose a threat of privacy leakage during the process of communication for analysis [391,392]. Strengthening the security and protecting the privacy of participants, patients, and cluster centers should be considered when developing new technologies [392]. A tool with such attributes, in addition to well-planned screening and awareness campaigns for specific populations at risk, could increase accessibility for patients living in rural areas and thus overcome travel distance barriers [393].

The effectiveness of community campaigns for BCS is still under question as evidence is sparse and not accurately reported or reviewed [394–396]. Transmitting clear and accurate information regarding breast cancer prevention and improving the accessibility to screening and diagnostic equipment could influence how many women could be tested [397]. However, in our opinion, this could also lead to overdiagnosis and unnecessary examination if clinical recommendations regarding BCS and diagnosis are not followed **(Fig. 1)** [7,96,106]. For example, in one study Hispanic women at the Columbia University Medical Center in New York were found to be screened more frequently than non-Hispanic white women even if they had a lower risk. This could increase the possibility of false positives, unneeded recall for breast imaging, and biopsies for Hispanic women [398]. Interestingly, in another study it has been shown that Hispanic women living on the US border have knowledge about breast cancer, however, they were under screened due to the lack of accessibility to equipment [399]. Offering information regarding breast cancer awareness and the possibility of access to risk-stratified screening should come together for early diagnosis.

Screening could lead to overdiagnosis and then to overtreatment, including surgery, radiation, and HT. This problem could be prevented if screening and diagnosis are performed with a risk-factor stratification or more advanced methods for the classification of tumor aggressiveness [7,398,400,401]. Overdiagnosis is defined as when cancer is identified; however, it won't progress or cause symptoms or death [106,402]. Overdiagnosis should not be confused with false-positive results as this happens when a positive test in an individual is recognized subsequently as not cancer. It has been reported that the magnitude of overdiagnosis in breast cancer using mammography is estimated to be up to 25% [402]. This seems to be an inevitable effect of mammography screening as the objective is to diagnose breast cancer before clinical detectability [403]. Even if mammography is detecting smaller cancer lesions, the problem of overdiagnosis occurs when detected cancers are slow-growing or don't progress at all, even if they are treated [400]. GEP/S could help in the analysis of cancer samples and the distinction of indolent from aggressive tumors leading to the reduction of overdiagnosis and better clinical management [400]. Currently, most GEP/S are able to detect the molecular differences between noncancerous tissues and those with different degrees of aggressiveness [400]. GEP/S and the analysis of the three-dimensional structure of the tumor biopsy could improve the detection of cells and niches of cancer stem-like cells that could be associated with higher aggressiveness and resistance to therapy [404]. However, a biopsy is needed still to determine the aggressiveness of a cancer lesion by GEP/S analysis which is invasive and painful [405]. An ideal BCS and diagnostic tool should be non or minimally invasive with a high capacity to detect cancers and predict accurately its aggressiveness leading to an increase in the assessment of the aggressiveness of breast cancer and decrease of overdiagnosis. One of the most appealing diagnostic and screening methods in research is the use of liquid biopsies to analyze circulating cells or cell-free DNA as prognostic and predictive classifiers [307,406,407].

Analysis of the predisposition to cancer and prognosis in a specific population based on its genetics has shown to be necessary to improve treatment selection [23]. Genetic testing of hereditary mutations or gene expression analysis is becoming more accessible regarding its price and offering capacity [408]. However, physicians and patients' knowledge of these tools is still missing limiting the advancement of disease treatment [409]. It should be emphasized during medical training and awareness campaigns what is genetic testing and how to apply it [408]. It could be important to test GEP/S and NGS in populations with multiple ethnic origins and with variable predisposition to develop cancer. It is common to find variations in gene expression among different populations [410–412]. These variations should be taken into consideration when recommended or used for breast cancer prognosis and therapeutic decision [23,413]. Therefore, genetic tools should be evaluated and approved by considering how they were developed, tested, and validated in large and ethnically diverse groups.

Measurement methods and interpretation of diagnostic biomarkers vary among laboratories, thus possibly influencing the accuracy in cancer diagnosis [414]. For example, the threshold for determining Ki67 levels could vary between exams as it depends on the experience of the observer, biopsy quality, and representativeness of the chosen microscopy fields. It is still unknown whether choosing hotspot areas or averaging all areas in the microscopic field would provide a more accurate measurement of Ki67 [415]. A study carried by Jang et al. compared the prognostic impact of Ki67 labeling index in luminal/HER2- breast tumors between the average method versus the hot spot method. Results showed that when using the hot spot method, the most suitable cutoff was 22% with a sensitivity of 75% and a specificity of 60%, whereas the most appropriate Ki67 cutoff for the average method was 18% with sensitivity and specificity of 67.9% and 63.4% respectively. The authors concluded that both methods have good predictive performances for tumor recurrence in luminal/HER2- breast cancers. However, they acknowledge that the average method has a greater reproducibility [414]. Similarly, methodological factors in the IHC analysis such as tissue fixation, choice of antibody, and threshold for interpretation can vary, affecting test accuracy and reproducibility [416]. A study conducted by Dekker et al. evaluated the reproducibility of IHC ER and PR testing using tissue microarray (TMA) from nine pathology departments. Overall sensitivity and specificity for testing ER expression were 99.7 and 95.4% respectively whereas for PR testing were 94.8 and 92.6% [417]. In 2012, a study evaluated the feasibility of retesting HER2 status on TMA using SP3 and 4B5 antibodies, and mono color silver *in situ* hybridization, as a quality control approach for HER2 IHC testing. Results showed that out of 1,210 invasive breast carcinomas, 1.3% of the tumors were identified as false positives and 0.7% as false negatives. Finally, the study proposed this method to improve HER2-testing standardization [418]. Applying ML algorithms on data measured in a large worldwide population along the last decades from a prognostic tool would certainly improve data interpretation from mammography or IHC images. These key assets could increase the reproducibility of clinical diagnosis and standardization of methods even applied to a particular population.

The accessibility and effectiveness of current screening and diagnosis breast cancer tools still needs to be improved. Technical innovations in diagnosis tests must be proved clinically with a process that may take several years and could discourage research groups [419]. It is our belief that an agreement and coordination between multidisciplinary research team members, regarding the constraints to develop new screening and diagnostic tools should be considered before starting a project. Regulatory agencies, such as the FDA monitor the development and safety from established to emerging biomarkers, with requirements that should be included as milestones in the development of new projects. Newer methods such as next generation sequencing would help to find low-frequency genetic variants that increase the possibilities to develop breast cancer helping to risk-stratify patients [420]. Risk-stratification approaches could combine genetic and non-genetic risk factors in order to provide a personalized cancer management.

12. Conclusion

Even though breast cancer survival rate after 5 years of diagnosis is very high, it is still a disease difficult to diagnose and treat as its behavior differs from one person to another. Our capacity to detect cancer, define its stage and future aggressiveness is based on multiple tools for screening and diagnosis. This represents a multidisciplinary challenge for biomedical and technological scientists, as understanding tumor heterogeneity and physiological variations among patients requires multiple sources of information in order to provide newer and more accurate methods or improve existing ones. As current methods and tools to detect cancer are being improved, new technologies are on their way to provide an increase in portability, connectivity, price, and data management that will have important consequences for the maintenance or recovery of health from any individual and patient. Thus, medical practitioners have to be constantly updated on new practices, and interpretation of its results. This comprehensive and critical review fuses biological, technological and medical information in an attempt to provide physicians and people interested in the field, with the necessary knowledge to communicate needs and provide better support for the decisions during screening and diagnosis before the eventual treatment. The use of tools to screen and diagnose are part of a bigger strategy to fight cancer which can influence decisions for better treatments without recurring to invasive procedures or unnecessary CTX.

Many challenges need to be solved to achieve better screening and diagnosis tools in order to accurately predict cancer progression. Image-based techniques such as mammography, MRI and ultrasound are commonly used as primary detection methods in breast cancer. However, as mentioned in this work, these are expensive, have image resolution limitations and vary in diagnostic accuracy. On the other hand, biomarker-based methods are still under development with promising results. In addition, there is still a lack of evidence, consensus in guidelines and protocols for the generalized use of GEP/S in clinical practice, where more studies considering population and ethnic diversity are needed. Furthermore, advanced diagnostic tools such as genetic

testing and GEP/S are expensive and require highly trained physicians and technicians. Finally, the combination of image-based techniques such as mammography, biopsies and immunohistochemical tests is still the current applicable approach that can discriminate benign from malignant breast tumors. Beyond this current and accepted standard, the ideal detection method would be balanced between comfort, accessibility, cost, and effectiveness. This ideal detection technique would function as an automated device that identifies breast lesions and differentiates benign from malignant masses at early stages. This would also allow long term compilation of ongoing data that could be easily interpreted by the patient, health professional and scientists. The goal of these specifications would be to avoid unnecessary surgery, overdiagnosis and overtreatment.

Data Availability Statement

The corresponding author can answer or provide information on request to any comments or questions regarding this publication.

Author Contributions

DB & AL: methodology, investigation, writing, visualization, review, and editing.
PL & DS: methodology, investigation, writing, visualization, review, and editing.
FT: investigation, review and editing, funding acquisition.
FS: methodology, investigation, writing, review, and editing.
LT: methodology, investigation, writing, review and editing, project administration, funding acquisition.
AC: conceptualization, methodology, investigation, writing, visualization, review and editing, figures, supervision, project administration, funding acquisition.

Conflict of Interest

The authors declare that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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