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Tumor Heterogeneity in Breast Cancer and Its Implication in Drug Resistance

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Abstract

Breast cancer is one of the most common causes of death in women. Understanding the mechanism underlying the origin and progression of the cancer is important. Malignant cells spread from a primary tumor to distal organs in a process known as metastasis. There is a variety of interconnected cell types in solid tumors, including Mesenchymal Stromal/Stem-like Cells (MSCs) and Tumor-Associated Macrophages (TAMs). The stroma or tumor microenvironment has been shown to be significantly altered by breast cancer not only because of its neoplastic cells, but also because of alterations in the surrounding cells. Despite the fact that the tumor microenvironment plays an important role in cancer initiation, growth, progression, invasion, and metastasis, the molecular mechanisms underlying these tumor-promoting effects are unclear. Pathologic assessment of Tumor Microenvironments (TMEs) has become increasingly important for breast cancer treatment which contributes to tumor heterogeneity. Moreover, recent studies have demonstrated that TME has become increasingly recognized as a treatment target. Since breast cancer development and progression are severely impacted by the alterations which are also potential therapeutic targets. Herein, we examine the molecular changes of breast cancer microenvironments, the interactions between cancer cells and various types of cells, and the clinical relevance of these findings. This review discusses the TME of breast cancer, the pathologic assessments that are relevant to prognostication and treatment decision based on the TME, and the therapeutic options that interact with and/or exploit the breast cancer TME.

Introduction

Breast cancer remains the leading cause of cancer related deaths worldwide. Despite of significant biomedical research, breast cancer remains a health problem; contemporary the leading cause of breast cancer associated death is its spread to distant sites in the body, termed metastasis. During metastasis, cancer cell detaches themselves from primary tumor and activates survival pathways after undergoing invasion, enters into circulation and extravasate into surrounding tissue thereby creating a favourable environment for growth. Metastasized cells become highly aggressive and non-responsive to current therapies [1]. The phenomenon involved in metastasis are initiated both by genetic and epigenetic variations that causes cancer cells to reversibly transit among different states and vary in their competence to contribute to the tumor growth. For example, cancer cells cause reversible transition between epithelial-like and mesenchymal-like states [2]. The cellular process converting epithelial cells to mesenchymal cells with the ability to invade the adjacent tissues is known as Epithelial-Mesenchymal Transition (EMT). EMT results in the loss of epithelial polarity and acquisition of a mesenchymal-like phenotype associated with the expression of mesenchymal markers including vimentin [3], N- cadherin [3], α Smooth Muscle Actin (α-SMA) [3], fibronectin and fibroblast specific protein [3].These phenotypic changes are induced by several transcription factors like Snail, Slug, Zeb1 and Zeb2, the bHLH proteins Twist and TCF3, the fork head box proteins FOXC1 and FOXC2, as well as the homeobox protein goosecoid [4]. Recently, it has been evident that EMT is a phenotypic characteristics of cancer cell plasticity [5]. Cell plasticity allows tumor cells to change into a phenotypic identity independent of their drug target, without undergoing additional genetic mutations [6]. Over recent years, the heterogeneity on tumor cells had been focus of attention of several investigations and is emerging. The heterogeneity of the Tumor Microenvironment (TME) refers to the finding that various tumour cells can exhibit different physical and phenotypic characteristics, including cellular morphology, gene expression, metabolism, proliferation, and metastatic potential [7]. Further, TME releases paracrine factors from other tumor cells or from stromal cells and provide a hospitable niche for tumor dormancy. This is also known as Extracellular Matrix (ECM) which greatly influence behaviour of cell

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thereby contributing to the matrix remodelling or integrin receptors which are influenced by the ECM ligands. Besides, alteration of the biomechanical properties of the Extracellular Matrix (ECM) are sufficient to promote cancer cell invasion and migration [8]. A bidirectional interaction is established between the tumour cells and its surrounding stroma as an initial stage toward invasive development during metastatic spreading. Stromal changes like the alteration of extracellular matrix, activation of fibroblasts and myoepithelial cells and pericytes or smooth muscle cells as well as immunological and inflammatory cells are recruited to support the tumour formation [9]. In fact, TME components vary between and within each patient which contributes to discrete mode of progression of breast cancer in specific patients. Despite the mounting body of evidence for TME involving in the tumor behaviour and pathogenesis, it is still unclear that

how TME continues to change and evolve as a given tumor evolves in response to systemic changes or therapies. For instance, the host immune system is polarised toward particular phenotypes that impact tumour progression and immunomodulatory mediators that are present in the TME secreted by the inflammatory cells [10]. According to the studies, the microenvironment plays a crucial role in maintaining cancer stem cells and cancer-initiating cells as well as encouraging the seeding of cancer cells in metastatic sites [11,12]. Furthermore, recent study state that the connections between tumour cells and surrounding cells in the tumour which are dynamic and reciprocal, orchestrate events crucial to the progression of tumour toward metastasis [13]. As a result, several cellular and molecular components of the microenvironment are emerging as promising targets for treatment approaches. In the current review, we discuss the dynamic and different levels of heterogeneity which progresses tumor, the underlying mechanisms and also provide cue to develop strategies for inhibition of tumor malignancies to improve patient outcomes.

Tumor Heterogeneity in Breast Cancer

The heterogeneity of tumors is a characteristic of malignancy. A non-malignant cell may become malignant by sequentially acquiring alterations that increase cell proliferation, evade growth suppression signals, induce angiogenesis and eventually leading to tissue invasion and metastasis. The dynamic nature of cancer continues to evolve even after it has developed malignant transformation. Cancer cells harbouring distinct molecular signatures and sensitivity to anti-cancer treatments could arise from this ongoing process and produce a molecularly heterogeneous bulk tumour. There are two types of heterogeneity in tumours: intertumoral andintratumorally. Heterogeneity between patients with similar histological types of tumours is called intertumoral heterogeneity whereas intratumorally heterogeneity refers to distinct tumor cell populations within the same tumor specimen.

Intertumor heterogeneity

Clinical and histopathologic heterogeneity: Breast cancer heterogeneity is best demonstrated by physical examinations and imaging studies that assess the disease's clinical stage. Tumor size, regional lymph node status, and distant metastatic spread is all included in the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) TNM staging system [14]. There are a number of factors that influence standard breast cancer treatment, such as the patient's age, menopausal status, and general health conditions, as well as tumor characteristics, such as the clinical stage,

histopathological features, and biomarker profile [14]. Based on these traditional clinicopathological variables, the outcome of breast cancer patients can differ greatly. The histopathological classification of breast cancer is based on morphologic heterogeneity. The most common histologic type of invasive breast cancer (40–75) % is Invasive Ductal Carcinoma (IDC). In spite of its widespread nature, IDC is poorly defined, and the World Health Organization (WHO) explains it as "the heterogeneous group of tumors whose characteristics do not meet the criteria for classification into a specific histological type". According to WHO classification, there are 17 different subtypes of IDC, with invasive Lobular Carcinoma (ILC) being the most common (5–15) % [15]. The prognosis and response to adjuvant treatment differ considerably between other special subtypes of breast carcinoma. In contrast to IDC and ILCs, tubular, mucinous, and papillary carcinomas usually have excellent clinical outcomes [16]. Contrarily, systemic chemotherapy is frequently used to treat metaplastic carcinoma and poorly differentiated IDC since they have a considerably worse prognosis.

The grade of breast cancer also emphasises the heterogeneity of the 'umour. Three morphologic characteristics are evaluated, including the proportion of the tumour that is organized in glands and tubular structures, the level of nuclear pleomorphism, and the mitotic rate, to determine the grade, which is then divided into three categories (low, middle, and high) [17]. A strong predictive indicator, the grade of breast cancer is included in clinical decision-making tools like the Nottingham predictive Index and Adjuvant Online. Proteomic, genomic, and transcriptomic analyses of breast tumours of various grades reveal various patterns. Grade continues to be an independent predictive factor for ER-positive tumours in multivariate models that incorporate gene signatures. The development from low-grade to high-grade carcinoma is extremely uncommon, according to molecular studies, and grade 1 and 3 breast carcinomas likely represent two completely different illnesses [18].

Biomarker heterogeneity: In accordance with the recommendations of the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP), immunohistochemistry (IHC) is routinely used to evaluate the expression of Estrogen Receptor (ER), Progesterone Receptor (PR), and human epidermal growth factor receptor 2 (HER2). The aforementioned biomarkers are recognised prognostic and predictive variables, and understanding how they are expressed in breast carcinomas are crucial for determining how patients should be treated. Estrogen and progesterone receptors are expressed in about 80% and (60–70) % of breast carcinomas, respectively [19]. Some breast carcinomas are ER+/PR or, rarely, ER/ PR+, despite the fact that (70–80) % of ER-positive tumours also express PR (ER+/PR+). The effectiveness of hormone therapy varies with ER+/PR+ tumours responding to it most effectively (at a rate of about 60%), while ER+/PR and ER/PR+ tumours poorly respond [20].

By applying the approved reagents, testing methodologies, and scoring algorithm during IHC staining, it was possible to identify the HER2 oncoprotein in about (15% to 20%) of primary breast carcinomas. According to how HER2-equivocal (2+) staining is defined, In Situ Hybridization (ISH) has indicated that (10% to 20%) of HER2-equivocal breast carcinomas are HER2-amplified [21]. Positive (3+) HER2 staining is closely correlated with gene amplification. Although HER2-positive breast carcinomas have the worst prognosis

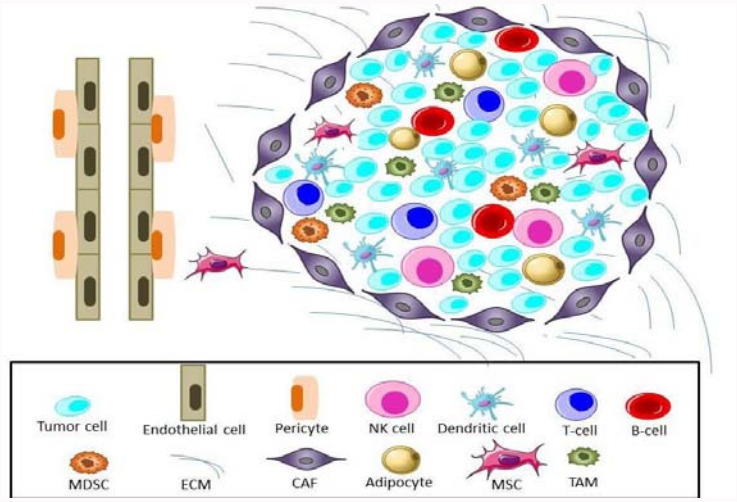

Figure 1: TME components: To support their own growth and survival in adverse environments, tumour cells sabotage many cellular and non-cellular non-malignant TME components. Immune cells from the haematological system such as B cells, NK cells, DCs, TAMs, T cells and MDSCs make up the majority of the cellular components and resident stromal cells (CAFs, ECs, etc).
Extracellular components are made up of ECM and proteins that are released by cells including cytokines and growth factors.

of all invasive breast cancers, they exhibit a high rate of response to anti-HER2 targeted therapy (e.g., trastuzumab, lapatinib), as shown by the pathologic complete response post-neoadjuvant treatment in roughly 50–60% of patients with HER2-positive tumours [22].

The term "triple-negative" breast carcinomas refer to breast cancers that lack the markers ER, PR, and HER2. These cancers are exceedingly diverse in terms of their histology, genetics, prognosis, and response to treatment. New evidence suggests that 12–55% of triple-negative (ER-PR-HER2-) breast cancers exhibit nuclear expression of the Androgen Receptor (AR) [23]. Although there is debate regarding the prognostic importance of AR expression in triple-negative carcinomas, if it is linked to better survival in other tumour subtypes. Clinical trials investigating AR antagonists (such as bicalutamide and enzalutamide) in triple-negative breast carcinomas that are AR+ (defined as nuclear staining in 10% of tumour cells by IHC) are now underway and showing encouraging results [24]. AR positive is linked to a reduced Ki-67 proliferation index, which suggests that AR may encourage a stem-like or mesenchymal character in this particular population of tumours. There are currently no standardised tests or recommendations for assessing the AR expression in breast cancer. Numerous other biomarkers have been researched for their potential to be used in the diagnosis, prognosis, and treatment of breast cancer. Growth and proliferation (Ki-67), invasion and metastasis (MMP-9, SK1), Epithelial–Mesenchymal Transition (EMT), immune response (PD-L1), therapy resistance (KLK10), survival (miR-574-3p, miR-660-5p) and so on, are categorised as functional properties of these biomarkers [25-29]. It is unclear how much tumour heterogeneity affects biomarker expression or how clinically significant it is. To more effectively target the tumor, direct therapy decisions, a methodological strategy and standardised quantitative reporting of biomarkers are needed.

Genetic heterogeneity: The four main intrinsic molecular subtypes of breast cancer, luminal A, luminal B, HER2-enriched, and basal like, are identified through gene expression analysis and have consequences for prognosis and treatment. The luminal A and luminal B subtypes exhibit tumour heterogeneity within ER-positive breast carcinomas and have higher survival rates than the HER2-enriched and basal-like subtypes [30]. Both luminal subtypes express ER, however luminal B tumours are more likely to express genes linked to proliferation and have a worse prognosis than luminal A tumours. The ER-/PR-/HER2+ and ER+/PR+/HER2+ tumours fall within the category of HER2-enriched subtypes, which are characterised by elevated expression of HER2 and proliferation genes [31].

In 70% of instances, the basal-like subtype is triple-negative and has an enrichment of genes expressed in basal epithelial cells. Claudin-low tumours with stem-like signature and molecular apocrine tumours that are AR-positive are further subgroups [32,33]. According to a meta-analysis of gene expression studies, the predictive significance of various signatures is correlated with the genes linked to proliferation. Clinical and molecular heterogeneity makes it difficult to classify breast cancer based on gene expression, even though gene expression profiles can predict treatment response and recurrence risk. Patients with breast cancer of the same molecular subtype who get the same treatments may experience variable clinical outcomes or develop drug resistance. In breast carcinomas, somatic mutations in TP53, PIK3CA, and GATA3 have been found to occur frequently (>10%) [34]. Additional molecular subgroups have emerged as a result of more recent research, such as a molecular classification based on integrated genomic and transcriptome profiling of 2,000 breast tumours that identified 10 new subtypes of breast cancer with unique clinical characteristics [35]. Additional research is required to assess the practical clinical relevance and therapeutic implications of driver-based breast cancer classifications.

In order to calculate the probability of recurrence in individuals who are ER-positive and/or lymph node-negative, RNA-based multigene expression tests have been created. Some multigene expression assays display significant evidence for therapeutic value, as per the ASCO clinical practice recommendations. There are four of them: the 50-gene assay Prosing based on the prediction analysis of microarray 50 model, the 7-gene based Breast Cancer Index (BCI), and the 21-gene assay Oncotype DX [36]. Tumours are divided into molecular subtypes by Prosing, BCI, and Endo Predict, which also forecast late recurrence. For patients with lymph node-negative, hormone receptor positive, HER2-negative breast cancer, oncotype

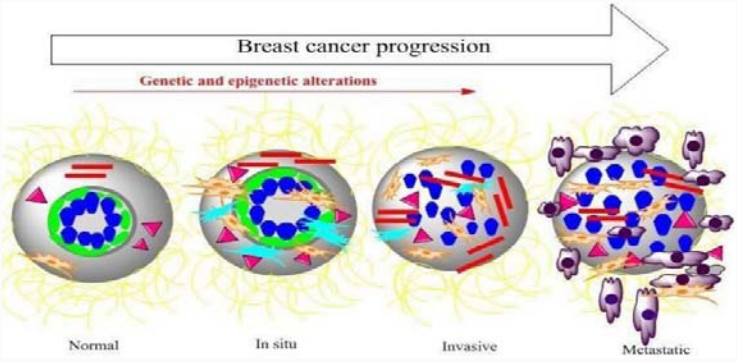

Figure 2: Breast cancer development stages: Tumour cell initiation and expansion within the mammary ducts progresses to ductal carcinoma in situ, which is defined as a complete filling of the mammary duct with tumour cells. The cancer is categorised as an invasive ductal carcinoma once the myoepithelium is damaged and tumour cells escape past the constriction of the mammary duct. Later the aggressive cells are selected and dissemination initiates in different organs.

DX, a reverse transcriptase polymerase chain reaction-based assay evaluates the likelihood of an early distant recurrence and the benefit of chemotherapy. The Recurrence Score (RS), which ranges from 0 to 100, is used to quantify the probability of recurrence. Tumours are divided into three risks categories: low risk (RS 17), intermediate risk (RS 18-30), and high risk (RS 31) [37]. Chemotherapy's potential benefits are estimated to be too limited (2%) to outweigh its potential risks in patients with tumours with RS 17 tumours. Contrarily, because of their higher (28%) recurrence risk, patients with RS 31 considerably benefit from treatment. Depending on the clinicopathologic criteria and personal preferences of the patient, the clinical management of intermediate risk patients is more diverse and may involve endocrine therapy with or without chemotherapy. Aiming to further categorise the advantages of chemotherapy for patients with intermediate RS who are clinically node-negative (TailorX) or node-positive (RxSponder) upon presentation, two ongoing clinical trials are now being conducted. IHC stains have been investigated as potential alternative approaches for indirect assessment of molecular subtype that may be employed in most laboratories because to the price, time, and technical skill necessary for molecular assays. The molecular subtypes of breast cancer can be distinguished with satisfactory and repeatable accuracy using the IHC staining panel composed of ER, PR, HER2, Ki-67, EGFR, and cytokeratin 5/6 (CK5/6): (1) Luminal A (ER+/PR±/HER2-/Ki-67−); (2) Luminal B (ER+/PR±/HER2−/Ki-67+; with Ki-67-positivity defined as ≥14%); (3) Luminal/HER2+ (HER2+/ER+/PR ±); (4) HER2+ (HER2+/ER−/PR−); and (5) Basal, including core basal (ER−/PR−/HER2−/EGFR+ or CK5/6+), and five-marker negative (ER−/PR−/HER2−/EGFR−/CK5/6−) subgroups [38]. Given that not all triple-negative tumours are basal-like and vice versa, and that ER-positive luminal tumours are remarkably diverse, genetic heterogeneity of breast cancer is probably far more complicated than the current molecular classifications. To better understand the interaction and clinical importance of prognostic and predictive molecular drivers in ER-positive breast cancer, assays incorporating multigene tests with mutational or genomic profiles must be developed.

Intratumor heterogeneity

Histopathologic heterogeneity: Morphologic intratumor heterogeneity can be understood as either variation in the tumor's various geographic locations (spatial heterogeneity) or as the tumor's evolution through time (temporal heterogeneity). In routine surgical pathology practice, spatial heterogeneity is easily seen within a single tumour, but it can also be found between original breast cancer and synchronous lymph node metastases, and even between synchronous metastases from different sites. Breast carcinomas that have a truly mixed morphology are made up of two morphologically distinct components (like IDC and mucinous carcinoma), but other tumours have ambiguous morphology (like IDC with lobular features) or have distinct differentiation foci (like IDC with focal squamous/basaloid or spindle cell differentiation) [39]. Individual tumours may contain clonal regions with particular genetic anomalies in morphologically different regions. Temporal heterogeneity encompasses the transition from in situ to invasive carcinoma, the evolution of an invasive tumour through time or in response to therapy, and the emergence of asynchronous metastatic disease exists.

Although the histologic, immunohistochemical, and molecular characteristics of the main tumour are now used to guide clinical therapy of breast cancer, changing morphologic and immunohistochemical features in metastases may have an impact on treatment success. Discordance rates for ER ranges from 16 to 33.6%, PR from 32 to 40%, and HER2 from (10% to 15.7%) [40]. In addition, one study found that women with primary and metastatic breast cancer who had inconsistent ER-staining had 48% higher probability of dying [41]. The difference in biomarker expression between primary and metastatic tumours may be brought on by therapy or unrelated to it. Additionally, chromosomal rearrangements, insertions, and deletions, single nucleotide or copy number alterations, and genomic heterogeneity have all been documented to have significant variances. The current practice guidelines suggest biopsying and retesting ER/PR/HER2 on accessible metastases because there is inadequate data to support the notion that altering treatment based on the altered biomarker status affects patient outcome.

Biomarker heterogeneity: Small biopsies can produce inconsistent results due to the extremely varied biomarker expression that can occur within a single tumour. It has long been known that different tumours will stain differently with ER/PR. The percentage of ER/PR expressing tumour cells varies from 1 to 100% depending on the specific tumour, and expression levels are strongly correlated with endocrine therapy response [42]. However, even tumours with extremely low levels (1% of tumour cells) may respond, supporting the ASCO/CAP recommendations adoption of the 1% limit for ER/PR positivity. The limited therapeutic value of identifying tumours with uneven distribution of ER expressing cells as ER positive is due to this approach's disregard for intratumor heterogeneity. IHC staining for the human epidermal growth factor receptor 2 (HER2) and gene amplification can be extremely diverse and have

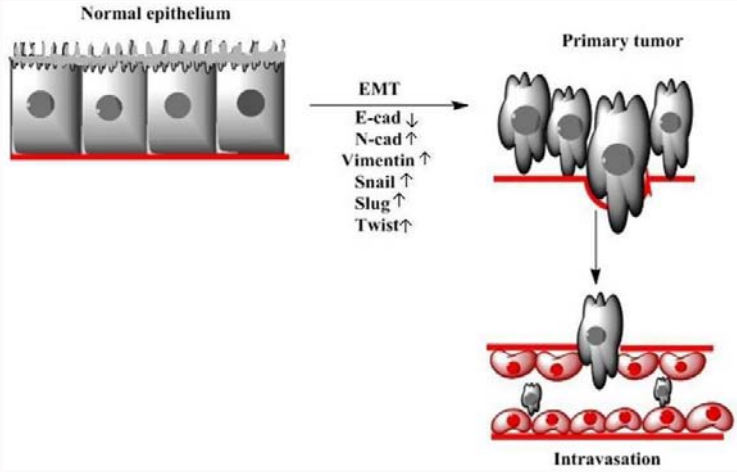

Figure 3: Epithelial-Mesenchymal Transition (EMT): Tight junctions, adherens junctions, and desmosomes hold together epithelial cells that exhibit apical-basal polarity, while hemidesmosomes attach them to the underlying basement membrane. These cells produce chemicals that support cell polarity maintenance and are linked to the epithelial state. The transcription factors ZEB, SNAIL, and TWIST are expressed in response to the induction of the Epithelial-Mesenchymal Transition (EMT), which inhibits the expression of genes related to the epithelial state and concurrently activates the production of genes related to the mesenchymal state. Tumor cell migration and intravasation are made possible by EMT at an early stage of the metastatic cascade.

an impact on survival without disease. While the prevalence of gene amplification heterogeneity is 5% to 30%, the discrepancy in HER2 IHC results ranges from 1 to >50% [43]. HER2 positive tumours exhibit full, strong circumferential membrane staining in 10%t o100% of tumour cells (3+ staining) when analysed by IHC. When examined by IHC, certain tumours show incomplete and/or weak to moderate circumferential membrane staining in >10% of cells but gene amplification when examined by ISH shows complete and intense circumferential membrane staining in 10% of cells (2+ staining) [21]. Protein overexpression without gene amplification, amplification without protein overexpression, or significant intratumor heterogeneity can all occur in some circumstances. Detecting gene amplification in one location is sufficient to classify a tumour as HER2-amplified, despite the fact that the ASCO/CAP recommendations recognise heterogeneous amplification and advise different areas. The clinical implications of intratumor heterogeneity are not taken into account in this strategy, which increases patient eligibility for targeted therapy. EGFR, p53, c-myc, and proliferation markers like Ki-67, cyclin-D1, and PCNA are additional biomarkers with diverse expression. In both ER-positive and ER-negative breast carcinomas, it has been demonstrated to have prognostic and predictive significance. However, Ki-67 expression levels might frequently be higher around the edges of tumours, and hot patches with varying staining can be found all across the tumour [25]. Furthermore, breast carcinomas of distinct histologic subtypes and grades might have intratumor heterogeneity of Ki-67 expression. A homogenous distribution of Ki-67 expression has been observed in lymph node metastases, in contrast to primary tumours. Furthermore, the strongest Ki-67 expression hotspots in original tumours were associated with highly proliferative metastatic tumour cell populations [44]. The clonal proliferation of the main tumour growth fraction with metastatic potential may be a reflection of the temporal heterogeneity in this case. Intratumor heterogeneity may be an actual physiological phenomenon or a technical artefact brought on by poor fixation and/or processing. Nevertheless, it is usually advisable to conduct in-depth sampling and IHC testing with sufficient negative and positive controls.

Genetic heterogeneity: Regarding chromosomal and genomic changes, breast cancer exhibits significant intratumor heterogeneity. These changes have an impact on a variety of processes and activities, including metabolic pathways, signalling pathways, antitumor immunity, cell senescence, migration, and metastasis. Different cell clones can either cluster in distinct regions of the tumour or disperse and mix in the same region. The complexity of intratumor genetic heterogeneity was best demonstrated by a study of 100 tumours that found driver mutations in >40 cancer genes,

including AKT2, ARID1B, CASP8, CDKN1B, MAP3K1, MAP3K13, NCOR1, SMARCD1, and TBX3, as well as 73 combinations of mutant genes [38]. Single-cell or single-molecule sequencing as well as bulk sequencing can be used to identify the genetic heterogeneity inside a tumour. Single-cell sequencing cannot provide information on the remaining cell population, whereas bulk tumour sequencing cannot pinpoint the location inside tumours or the degree of heterogeneity, limiting their clinical value in clinical practice. Molecular evolution and clone selection of tumour cells in response to targeted therapy were documented in an autopsy study comparing the molecular alterations in multiple synchronous breast carcinoma metastases.

Non-Genetic (epigenetic) heterogeneity: Epigenetic heterogeneity is characterised by changes in gene expression without alterations to the DNA sequence. Breast cancer tumour suppressor genes like p16INK4A and RASSF1A, as well as ER/PR/HER2, can be affected by epigenetic silencing through histone modification or DNA methylation. The biochemical processes that take place inside of cells can undergo stochastic variations that might result in transient phenotypic variances that can impact a cell's receptivity to treatment. These changes may affect chromatin states or mRNAs. Non-geneticheterogeneity's relevance in terms of clinical outcomes is still unknown.

Evolution Based on Clonal Selection in Heterogeneity of Breast Tumors

An individual tumour's genetic diversity may be dynamically changed over time, which is described as spatial heterogeneity when it

occurs across a number of tumour sites or within a single tumour site. Temporal heterogeneity, however, describes the uneven distribution of genetically diverse subpopulations within an individual tumour. There are several factors that can contribute to cancer heterogeneity, including tumorigenicity, treatment resistance, and metastasis potential [45]. Clinical behaviour and treatment response have been attributed to differences in ER expression among tumors and cell populations within tumors. In the context of cancers with established oncogenic drivers, an understanding of intratumoural heterogeneity can inform rational antineoplastic strategies. Current research focuses on being aware of the cellular and molecular mechanisms underlying the variety of tumours that contributes to diagnosis, prognosis, and therapy.

The vast majority of tumours are heterogeneous due to genomic instability, regardless of its source [46]. Additionally, dynamic chromosomal instability has the potential to create copy-number imbalances, and can also cause non-uniform chromosomal loss of genes with specific alterations, which can indirectly contribute to tumor heterogeneity based on mutations. It is suggested that increased levels of genomic instability promote the emergence of more competitive subclones within certain regions of individual tumours and across different metastatic sites, as well as those at the subclonal level. It is possible, however, for excessive levels of genomic instability to negatively impact the survival and fitness of cancer cells.

Heterogeneity may not be maintained by genomic instability alone. The development of tumour heterogeneity and tumour progression may be promoted by genomic instability in conjunction with other factors. In contemporary studies, clonal evolution and/or selection framework models are commonly used to explain how clonal diversity develops and maintains. In 1976, Peter Nowell proposed a stochastic model of tumour initiation based on the hypothesis that induced changes in non-malignant cells that resulted in selective growth advantages, thereby leading to neoplastic proliferation [47]. The expanding tumor population then undergoes evolutionary selection pressures as a result of the genomic instability, leading to genetically abnormal and heterogeneous subpopulations to emerge. The linear evolution of tumors is described by the sequential acquisition by successive clones of mutations conferring a growth and/or survival advantage, with successive clones outcompeting ancestral clones. In contrast, branching evolution describes multiple subclonal tumour cell population emergence and divergent propagation. An increased level of heterogeneity is possible due to branched evolution. In phylogenetic depictions of certain haematological malignancies, a linear pattern of evolution is invoked, whereas many solid tumours display a branched evolution. In an interesting development, the assumption that clonal subpopulations must always be in competition has been challenged by the results of studies suggesting that the propagation of tumours in cancers that initiate without cell autonomy may require cooperation between distinctly different subclones and metastatic sites can be seeded polyclonally. This cluster might be generated by geographic killing rather than treatment-induced clonal evolution in tumors with high heterogeneity between distant regions but low heterogeneity within local regions [48]. Such a scenario would result in a residual post-treatment tumor that is homogeneous and similar to what existed before treatment. Moreover, several genetic heterogeneities have been identified in human tumour samples as a result of whole genome and exome sequencing. Darwin's principle of 'survival of the fittest' will be observed as a result of this somatic heterogeneity.

Temporal evolution of cell-intrinsic traits

A genomic analysis of human tumours was eventually possible because of advances in DNA sequencing technologies. It was possible to reconstruct distinct phylogenetic trees, describing the evolution of clonal populations in pancreatic cancer, by sequencing DNA from primary and metastatic tumour samples. Additionally, Luond Fabiana and colleagues analyzed 21 breast cancer cases in order to reconstruct the evolution of the disease indicating that genetic variation arises due to changes in cancer genes and driving mutations [49]. For instance, the same study has revealed that cancer genes particularly TP53, have mostly been the focus of research of mutation patterns to look into underlying DNA damage and repair processes in human malignancies. Moreover, these investigations have shown that DNA repair procedures and carcinogen exposure can both affect mutation patterns. For instance, G>T/C>A transversions predominate in smoking-associated lung cancer, a pattern consistent with DNA damage brought on by tobacco carcinogens like benzo[a] pyrene diol epoxide [50]. Therefore, the variability of sub clonal populations is confirmed by DNA barcoding and sequencing of mouse samples from breast cancer xenografts: certain subclones stay stable while others expand or disappear. The evolution of new subclones both within and between primary and secondary tumours are therefore the result of dramatic changes in clone number and size while breast cancer is developing. DNA barcoding has been demonstrated in tests indicating that dominant clones of original tumours do not always result in metastasis allowing scientists to distinguish between cells shed by primary tumours that have not yet metastasized (shedders) and cells that have metastasized (speeders), indicating that therapeutic strategies should focus on specific subpopulations. For example, a barcoding-based mouse model of breast cancer heterogeneity has been created to study the molecular basis of metastasis that is driven by tumor heterogeneity [51]. In order to do this, mammary cancer cells were retrovirally infected with a molecular barcode and administered orthotopically to recipient mice with weakened immune systems. Tumor cells were taken after metastatic dissemination to the brachial lymph nodes, blood, lungs, livers, and brains, and barcode populations within each tissue were measured. The ability to show that various populations contributed to lymph node and hematogenous metastases as a result of this. Additionally, it was shown that there was a distinct overlap between the many clones in the circulating tumour cells and the hematogenous metastases. From underrepresented subpopulations in the initial tumour, it was also possible to recreate the evolution of circulating tumour cells. Only a portion of these cells possessed the extra ability to colonise secondary sites. Further explanations include the possibility that the development of metastatic tumour cells in the blood and their capacity to do so were directly related to vascular mimicry, which is the increased expression of the secreted proteins serpine2 and slpi [52]. Vascular mimicry is a process by which tumour cells create tubular structures for the transportation of blood and nutrients independently of conventional angiogenesis. The same study states how Serpine2 and Slpi function as anticoagulants to maintain perfusion. Through these methods, Serpine2 and Slpi help tumours grow by increasing their blood supply while also giving them more chances to metastasize.

Three distinct evolutionary patterns of primary tumours and metastasis were inferred from genomic profiling experiments performed on cancer cells from primary tumours and distant sites. Assuming either a single or multiple subclone(s) seeding late within

the primary tumour, we can model a simple linear evolution model, a parallel evolution model, and a parallel evolution model with early metastatic spread either from a single or multiple subclone(s) within the primary tumour. Additionally, a more recent study showed that 1293 single cells from 10 patients with ductal carcinoma in situ exhibited topographical genomic copy number patterns, leading to the discovery of a fourth model, called the "multiclonal invasion model" [53]. Multiple tumour subpopulations are generated in the mammary ducts, which ultimately migrate into nearby tissues to form invasive carcinomas. The emergence of genetic heterogeneity and subclonal diversification in a tumour, irrespective of its evolutionary course, might enhance its robustness and complicate the prognosis, therapy response, and clinical outcome of a patient.

Spatial evolution of cell-intrinsic traits

In many different cancer forms, spatial intratumor heterogeneity has been thoroughly characterised. For instance, a thorough heterogeneity landscape of Esophagus Squamous Cell Carcinoma (ESCC) has recently been shown by numerous groups who have done multi-regional deep sequencing. By examining 51 sub-tumor areas from 13 ESCC patients, Hirose et al. hypothesised that about 40% of driver mutations, including oncogenes such as KIT, and members of the PI3K/mTOR pathway and NFE2L2 pathways (NFE2L2 and KEAP1) were patially diverse [54]. EGFR amplification and CDKN2A/B deletions were two copy number changes that showed notable regional variability.

Another studies states that copy number changes and somatic mutations that are regionally segregated in ESCC have significant clinical implications. The representability of tumour regions submitted to pathological assessment is increasingly thought of as a critical aspect due to potential sample bias caused by spatial variability. Furthermore, spatial genetic variation plays a significant role in determining treatment responses. While most malignancies initially respond to treatment, however invariably relapse when new cancer cells emerge are no longer responsive to the medication. The preexisting heterogeneous cells may contribute to resistance against certain targets. Examples include the decreased effectiveness of EGFR inhibitor for individuals with heterogeneous driver status who has lung cancer. Targeted therapy resistance can develop in lung tumours with rare EGFR mutations, such as T790M, or MET amplification that occurs infrequently [55]. Chronic myeloid leukaemia is another well-known case in which mutant versions of the BCR-ABL fusion protein have been linked to the relapse of the illness after imatinib treatment. It has been observed that EGFR, FGFR1 and PD-L1 heterogeneous amplifications occur in ESCC, which contributes to the inadequate effectiveness of addressing such genomic lesions. Evidences from Luond and colleague's states that in breast, a primary tumour as well as its secondary lesions exhibit cell-intrinsic traits that change both temporally and spatially during tumour progression [49]. Luond and colleagues used multiple biopsy approaches to excise primary breast tumours to investigate the topographical distribution of subclones [49]. One to three neighbouring tissue regions were affected by at least one mutation, indicating that subclones were locally restricted. According to the findings of a related study by Green et al. when multiple breast cancer lesions with the same tumour grade, ER and HER2 status have been sequenced clonal evolutionary outgrowth occurs. This is because genetically similar breast cancer lesions are topographically closer to one another than genetically distinct lesions. The most common breast cancer driver mutations PIK3CA, TP53, GATA3 and PTEN, despite their histopathological homogeneity, had considerable interlesion heterogeneity in one-third of the patients [56,57]. Hence, spatial genetic heterogeneity presents significant obstacles to accurate cancer diagnosis and effective cancer treatment. In addition to genomic alterations, the presence of fibroblasts, extracellular matrix, and immune cells (macrophages and infiltrating lymphocytes) in the tumour microenvironment adds another layer of heterogeneity. The tumour microenvironment can affect the phenotypes of tumour cells by influencing both the intrinsic variability of cancer cells (such as through creating stress responses and genomic instability) and the extrinsic diversity of microenvironmental settings (e.g., different densities of blood and lymphatic vasculature, different numbers and types of infiltrating cells). Recent research on the intratumor heterogeneity of tumor-infiltrating T and B cells has revealed that the breast tumour microenvironment is indeed very heterogenous (including cell plasticity to EMT) which may have therapeutic consequences lesions (Figure 1).

Changes in the Microenvironment During Tumor Progression

Allinen et al. found extensive epigenetic changes in all breast cell types during the progression of cancer after isolating normal breast tissue, DCIS, and invasive carcinoma cells [58]. There was a substantial difference in gene expression between normal and DCIS myoepithelial cells, supporting dramatic differences in the microenvironment between normal and in situ lesions.

Researchers have also shown that most differentially expressed genes are associated with secreted proteins and receptors. In cancer cells, chemokines CXCL14 and CXCL12 bind to CXCR4 and promote their proliferation and migration. The transition from normal breast tissue to DCIS causes the most significant changes in gene expression in the stroma, as Chen et al. [59]. Moreover, the presence of several ECM-degrading proteases was also higher during the transition from DCIS to invasive carcinoma, indicating that these proteases may contribute to the destruction of the normal bone marrow. A study that detected significant changes in DNA methylation patterns in stromal cells supports the hypothesis that aberrant gene expression in cancer cells may be due to epigenetic changes, since genetic modifications have only been detected in cancer cells. A large number of these aberrantly methylated genes encode transcription factors involved in development and differentiation. The developing tumor microenvironment might be directly modulated by factors that are produced by tumor-associated myofibroblasts and fibroblasts derived from bone marrow-derived stem cells [60]. Additionally, active cytokine and chemokine signaling plays a crucial role in shaping the microenvironment of tumors. Munkacsy et al. [61] compared gene expression profiles of tumor stroma from breast cancer patients to create a 26-gene prognostic predictor that predicts clinical outcome independent of clinical subtype [61]. Their study identified two distinct sets of genes associated with hypoxia and angiogenesis, which were either linked to poor outcomes or indicated a Th1-like immune response. The expression signature of stromal genes associated with reactive stroma has also been identified in breast cancer as a predictor of response to chemotherapy. According to these results, the expression of genes in tumor-associated stroma directly affects the progression and outcome of disease. Breast cancer treatment includes therapies specifically targeting epigenetic changes in the microenvironment, including histone deacetylase inhibitors, which are currently being clinically investigated for treatment, thereby impeding the tumor.

A CGH (Array Comparative Genomic Hybridization) and SNP (Single Nucleotide Polymorphism) array analysis was performed to assess clonally selected genomic alterations in light of the dramatic changes in gene expression patterns during tumor progression in all cell types. Using these strategies, we identified clonally selected genetic aberrations such as amplifications and homozygous and heterozygous deletions (LOH-loss of heterozygosity) only within tumor epithelial cells but not within any non-transformed stromal cells. A novel genome-wide unbiased sequence-based DNA methylation profiling method, MSDK (Methylation-Specific Digital Karyotyping), has been developed to investigate the role of DNA methylation in regulating the phenotypic abnormalities present in the tumor microenvironment [62]. DCIS and invasive tumor cells showed DNA methylation changes in comparison to their normal counterparts. An analysis of selected genes by quantitative RT-PCR also found that DNA methylation had a consistent impact on mRNA expression levels, though the effect of DNA methylation was positive or negative depending on where the modified CpGs were located in relation to the transcription start site. In tumor stromal cells, epigenetic modifications are therefore responsible in part for the observed phenotypic alterations. In HER2+ breast cancers and prostate tumors, different methylation levels were also observed in the tumor epithelium and the surrounding stroma [63]. The phenotype and epigenetic features of tumor stromal cells are abnormal, and investigators need to investigate whether clonally selected somatic genetic changes are present.

Implication of Tumor Heterogeneity in Metastatic and Progression of Breast Cancer

Intratumour heterogeneity has yielded novel findings about the evolutionary drivers of cancer metastasis. Metastasis is the spread of cancer cells to distant tissues and organs and forms new tumor in the body resulting death of cancer patients. There is evidence that different convergent evolution drives metastatic sites with distinct resistance-causing genomic alterations thereby attaining clonality. Remarkably, recent studies state higher mutational burden in metastases which is driven by clonal rather than subclonal mutations [64]. Notably, the protein expression of the three receptors (estrogen, progesterone and HER2), clinical subtyping and treatment choices are influenced by phenotypic variance in breast cancer metastasis. Estrogen, HER2, and progesterone receptor discordance between the main and secondary tumours has been estimated to be between 10 to 25% and 40 to 50%, respectively [65]. Nguyen et al. assert that a biopsy of the first distant recurrence is necessary to ascertain the receptor status [66]. Moreover, discordance of the HER2 receptor is seen in chemotherapy, but not trastuzumab which states that treatment may have an impact. Therefore, both genetic and phenotypic change is associated with metastatic niche or the intrinsic metastatic potential and is related to the impact of treatment.

The spatiotemporal dynamics of cells with varying Epithelial-Mesenchymal Plasticity (EMP) can lead to the formation of distinct patterns of phenotypic and functional heterogeneity of the CSCs within the tumor microenvironment. A recent study by Mavrommati et al. showed that the sub clonal driver mutations was an independent risk factor for disease progression in comparison to the patients treated with cytotoxic chemotherapy as they are more likely to undergo clonal evolution in a way that the extent of heterogeneity is evolved during treatment [67]. In another study, whole-exome sequencing and copy number was used to calculate the percentage of cancer cells containing each somatic mutation to examine intratumoral heterogeneity in Chronic Lymphocytic Leukaemia (CLL) cases [68]. In accordance with earlier and later events in the evolution of CLL, the authors classified driver mutations as mostly clonal (e.g., MYD88, trisomy 12, and del (13q)) or subclonal (e.g., SF3B1, TP53). Similarly, another study by Turner et al. showed the rise of tumor subclonal in four different breast cancers post treatment [69].

Further, the bulk and RNA sequencing revealed that following the treatment, the acquisition of malignant phenotypes increased mesenchymal and growth factor signaling which may promote drug resistance, and decreased antigen presentation and TNF-alpha signaling. Likewise, a study by Marusyk et al. [70] reported that subclones could drive tumor growth by inducing changes in the microenvironment in a non-cell autonomous manner [70]. Cell autonomous gene action states that the product may be involved in signal receiving, signal transduction, or it may not be involved in a process involving cell-cell contacts. On the other hand, cell non-autonomous gene action implies that the end product is a signalling molecule or takes part in its synthesis. In

addition, greater proliferative potential is seen in non-cell autonomous subclones which is outcompeted by other subclones resulting in tumor collapse. Therefore, the subclonal alterations may have an impact on tumor biology and phenotype and will further require functional genomic studies. More detailed studies in this direction will aid in understanding how synergistic and antagonistic relationships between subclones during tumor evolution may promote or impede cancer progression and contribute to drug resistance. However, another property of cancer cells is drug resistance, which exhibit resistance to a variety of therapeutics, reversibly forming resistant progeny (Figure 2) [71].

Clinical Repercussions of Breast Cancer Heterogeneity

The heterogeneity of breast cancer increases a person's ability to adapt to rapidly changing circumstances, making diagnosis and therapy difficult. During both diagnosis and relapse, it is necessary to monitor the disease evolution at the entire tumour level in order to mitigate the risk of sampling bias. For example, in metastatic illness, CTCs are thought to originate from both the primary and metastatic lesions. Breast cancer, colon cancer, prostate cancer and melanoma all frequently develop metachronous metastases. Recent studies of independent metastatic locations within a single patient report that each metastatic site can evolve separately and develop de novo mutations [72]. Therefore, through the comparison of multiple, spatially separated sections of breast tumors and the non-invasive analysis of CTCs, along with the evaluation of individual metastatic lesions throughout the tumour progress, we will probably be able to gain a more comprehensive understanding of intratumor heterogeneity and should be able to improve patient diagnosis and treatment. As a result, high mutation rate and plasticity induces drug resistance in cancer cells. Stromal cells, in contrast, appear more resistant to drugs and recurrence is likely due to their genetic stability. A reduction in specific cell-cell interactions or eradication of distinct cancer cell populations may lead to changes in the microenvironment that may drive a resistance to therapy due to extensive cooperation between different subclones of cancers. Similarly, our group has shown that doxorubicin induced alterations of mitochondrial function and oxidative stress markers, were reduced in MDA-MB-231 cells, but not in MCF-7 cells (unpublished data- Bhumika Wadhwa- BW and

Rajesh Singh- RS).

It is known that as cancer progresses, metabolic requirements and vulnerabilities change. Nutrient intake and biosynthesis are necessary throughout the early phases of tumour formation and additional subtype-specific metabolic requirements manifest in locally invasive malignancies. The hypothesis that cancer-causing mutations allow cells in developing tumours to acquire metabolic features that favour cell survival, evade immune monitoring, and promote hyperplastic growth is consistent with the view that metabolic reprogramming is a hallmark of cancer. This idea is well established for oncogenic agents (such as MYC and KRAS) that have the ability to control metabolism in a cell-autonomous manner [73]. Thus, metabolic changes that occur in Tumor Microenvironment (TME) as well as fundamental adaptations to overcome the immune surveillance and cell death are examples for the development of resistance of drugs.

An important aspect of therapy resistance is the interplay between cancer cells and TME cells. Identification of adaptations in interactions between cancer cells and TME cells will enable the identification of critical molecular pathways and processes that are essential for a cancer cell's survival. A study by Nolfi et. al described that Extracellular Vesicles (EVs), which transport particular tumor-promoting substances able to activate survival pathways and immune escape mechanisms which are frequently used by cells inside the TME to communicate with one another to maintain tumour progression and therapeutic resistance [74]. However, to sustain tumour growth, modifying cell phenotype and triggering metastatic switch, the interaction between cancerous and surrounding cells in TME via EVs is crucial. In addition, EVs take part in a number of activities such as the direct removal of medications, inclusion of efflux pumps, and distribution of miRNAs and long non-coding RNAs (lncRNAs) [75]. Francesco et al. reported on the direct removal of medicines from intracellular space after observing the release of doxorubicin-containing vesicles in doxorubicin-treated MCF-7 breast cancer cells and proving that vesicles passively accumulated the drug [76]. Moreover, Nolfi and associates discovered that the tumor's acidic microenvironment facilitates EVs-mediated drug clearance and detected cisplatin-enriched exosomes in human metastatic melanoma cells [74]. Other study states that EVs may contain factors that cause efflux pumps to express. In that regard, Adriamycin-resistant breast cancer cell line MCF-7/ADM was shown by Zhang et al. to exhibit higher levels of the Ca2+-permeable channel TRPC5, and it was also shown that TRPC5 expression is necessary for P-gp activation [77]. In the same study, both in vitro and in vivo, the suppression of TRPC5 activity and P-gp expression decreased drug resistance and tumour growth, indicating that inhibiting either P-gp or TRPC5 could be a promising approach to overcome drug resistance. Moreover, in combination therapies, synthetic lethal interference therapies aim to identify therapy-resistant cancer cells, thereby killing only these cells, but not non-transformed cells in the body. Thus, to create personalised, effective combinatorial treatment regimens for each patient, it is essential to comprehend how each cell in the TME interacts with the others as well as the crucial pathophysiological, biochemical, and molecular factors that influence the development of tumours.

A high degree of cell plasticity allows individual cancer cells to adapt to microenvironmental constraints, which results in heterogeneous tumors and resistance to therapy. Drug resistance has been linked to EMT in numerous studies [78]. It is still unclear how distinct EMT states, cell plasticity, and therapy resistance interact. Siyuan-Qin and colleagues reduced primary tumour invasion and lung metastasis by transdifferentiating highly aggressive and metastatic breast cancer cells into genuine post-mitotic adipocytes by taking advantage of the plasticity of hybrid EMT states [79]. In order to overcome therapy resistance, it may be possible to target cell plasticity directly. Additionally, there is direct evidence linking the EMT phenotype to treatment resistance. For instance, EMT generated by EGFR signalling has been connected to tamoxifen resistance and enhanced invasiveness in the breast cancer cell line MCF-7 [80]. Moreover, the mesenchymal-like basal subtype of breast cancer, which has a greater mortality rate, is highly resistant to chemotherapy. Nevertheless, further development of these novel treatment strategies will require a better understanding of cancer cell plasticity and how these mechanisms affect tumour progression (Figure 3).

Therapeutic Failure, Resistance to Drugs and Future Strategies: Novel Approaches for Assessing Heterogeneous Tumors

The detection of somatic mutations in cancer genomes has undergone a revolutionary change in recent years due to high-throughput DNA sequencing [81]. Whole-genome sequencing shows all somatic mutations, but whole-exome sequencing, however is less expensive only detects coding mutations without allowing for non-coding area analyses or the identification of Structural Variants (SVs) [82]. These technologies enable the discovery of novel recurring somatic mutations, a fraction of which presents new targets for cancer diagnostics and treatment, when used for numerous samples of the same disease type.

Many methods have been developed to interpret the generated data due to the quick development of high-throughput DNA sequencing technology and their application to cancer genome sequencing [83]. Furthermore, different computational methods employ various combinations of the many signals that might be used in sequencing data to address the aforementioned problems. As a result, the authors are only able to include a small number of potential strategies. The analysis of additional high-throughput sequencing data, such as RNA sequencing data, which also provide essential components for precision medicine, is not covered because the study focused only on methodologies for DNA sequencing data [84]. Regardless, clinical translation and interpretation of these results remain a major challenge. It is important to note that to target colonisation in other organs, cancer cells may regulate the expression of molecules such as molecules including serine–threonine kinase 11 (STK11, also known as LKB which is a metastasis-suppressor gene regulating NEDD9 in lung cancer) [85], and the gene encoding ezrin (an intracellular protein needed for early survival of metastatic osteosarcoma cells in the lung) [86], and genes in 18-gene breast-to-lung metastatic gene-expression signature including the COX-2, MMP-1, the EGFR ligand EREG, ANGPTL4, and other mediators of infiltration and colonization by cancer cells in the lung [87]. Each gene associated with metastasis is theoretically a possible therapeutic target. Two metastatic virulence genes, RANK ligand (e.g., denosumab, in phase 3 studies), and TGF- β (e.g., monoclonal antibody GC1008, in phase 1 trials) are the ongoing therapeutic studies of metastasis [88,89]. Another clinical trial is ongoing for metastatic initiation gene c-MET (e.g., the small-molecule inhibitor ARQ 197, which is in phase 1-2 trials) [90]. Another example of surviving cancer cells in the metastatic microenvironment is SDF-1 chemokine in the bone

marrow, which recruits and improves the survival of breast cancer and prostate cancer cells [91].

To combat the inherent biologic redundancy in metastasis and to focus on the many stages of the process, combination therapy may be required. Celecoxib and cetuximab, two medications designed to block the progression of metastatic disease, were only effective in combination therapy-no single agent-therapy in one series of preclinical experiments for preventing lung metastases by breast cancer cells with a high propensity for lung metastasis [92,93]. Treatment with these medications might stop further reseeding and expansion of metastatic sites if cancer cells are continuously migrating between lung metastasis sites. Several anti-metastatic medications along with cytotoxic chemotherapy may be required for cancer treatment. In the adjuvant setting for colorectal, ovarian, and non-small-cell lung malignancies, bevacizumab, an antibody that targets vascular endothelial growth factor, is being evaluated in combination with chemotherapy [94]. Similarly, the combination of different molecules used to target different levels of signalling pathways by synergistically blocking cancer cell escape routes and minimizing the emergence of survival mechanisms, could prove to be a promising way forward, keeping in mind that specific molecular profiling particularly for metastatic relapses should be carried out to elucidate further resistance phenotypes and allow for the design of specific new targets.

Besides, future biomarker and clinical trial studies might consider longitudinal characterisation of tumour evolution using advanced sequencing technology and characterisation of the distinct mechanisms of tumour heterogeneity through the disease course. In order to develop successful immunotherapies for cancer, it is crucial to understand the cellular composition and interactions of the tumor microenvironment.

Conclusion

Recently tremendous growth is seen in understanding the breast tumor heterogeneity and evolution. It is demonstrated that the ecology of the breast tumour, which includes genotypic and phenotypic tumour clones as well as immunological, stromal, and normal breast cells, is shown to be affected by tumour heterogeneity, which also affects tumour growth and metastasis. A deeper insight is required of how the immune system can be rendered within the tumour cells to tackle clonal alterations, to identify high-risk subclones of tumor that might be eliminated prior to, or at the time of metastatic seeding. Nevertheless, developing novel biomarkers that can more accurately identify patient subgroups who can benefit from individualised therapeutic approaches will be one of the challenges facing the breast cancer research community. This requires integrating our knowledge of the ecosystem of breast tumours and how it evolves over time. Thus, in-depth understanding of new avenues to therapeutic approaches, for instance, tumor microenvironment manipulation and targeting tumor heterogeneity will lead to the eradication or even curative treatment. Furthermore, this may allow clinicians to make informed therapeutic choices treating patients with drugs tailored to their tumor profile based on therapeutic response, disease relapse and progression.

Increasingly, breast cancer therapies are targeting the tumor microenvironment, which is important in tumor development. Breast cancer progression and metastasis are facilitated by immune cells that suppress immunity, soluble factors, and altered extracellular matrix (ECM). Breast cancer expansion may be hindered by the surrounding inflammation caused by the natural progression of the tumor. In order to combat cancer, new therapeutic strategies are being developed to enhance antitumor activity while 'normalizing' the surrounding stroma. Our understanding of invasive breast cancer's disparate clinical behaviour has been enhanced by investigations into the molecular pathology and biology of the disease. Traditional histopathological evaluations of breast cancer are clearly valuable and can be used to classify them based on their basic phenotypic properties, such as ER expression status and HER2 level. However, without considering the breast cancer genome, transcriptome, and proteome, it is impossible to fully evaluate the complexity of the disease. Regardless of whether these measures are considered individually or in combination, there is substantial variation among breast cancers. There is evidence that each breast cancer has a unique genomic, transcriptomic, and proteomic makeup based on available data. Molecular signatures of breast cancers vary depending on whether they are compared with those of their closest neighbours (based on clustering), whether they are compared to other breast cancers of the same molecular classification, or whether they are compared across all cases, according to the Cancer Genome Atlas Network. Gene evaluations with a greater number of genes produce a greater degree of molecular diversity. Proteomics, pathway activation, and gene mutations can all be analysed with the same principle. Molecular alterations observed in breast cancer are either driver events or passenger events, depending on whether they are required or secondary to other changes. According to the available data, breast cancer could be driven by numerous drivers (rather than only a few major pathways), with only a small percentage of cases attributed to each driver pathway. By activating a positive mediator of neoplastic development (e.g. proto-oncogenes) or inactivating a negative mediator of neoplastic development (e.g. tumor suppressor genes), driver events and affected driver genes are assumed to impart some advantage to the emergent neoplasm. It will take more research to fully understand the intricate connections between a given breast cancer's molecular biology and how that cancer reacts to a particular therapeutic approach. Additionally, thorough molecular data from patients who were treated with particular therapeutic approaches (drugs or drug combinations) may allow correlation analysis to spot molecular lesions that could either guide treatment (by increasing sensitivity) or complicate it (by conferring resistance). Future therapy decisions and the selection of suitable therapeutic approaches may benefit from information obtained through next-generation sequencing or equivalent technologies of breast tumours from specific patients. The prevalence of cellular and molecular heterogeneity across breast tumours, however, always offers a considerable hurdle for any molecular technique applied in this way. As our understanding of complete breast cancer molecular signature advances, we will be able to use prospective trials to gradually introduce tailored therapy for specific patient subgroups, adding to the body of data for the pharmacogenomics of breast cancer.

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Declaration of Competing Interest

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