EDUCATIONAL HANDBOOK

Heterogeneity in breast cancer: Current evidence and clinical impact

Development supported by
GE HealthCare, Medical Affairs

GE HealthCare

Contents

COVER IMAGE: GETTY

Foreword by the Guest Editor

Adam Brufsky MD PhD
Professor of Medicine and Co-Director, Comprehensive Breast Cancer Center, UPMC Hillman Cancer Center, University of Pittsburgh, Pittsburgh, PA, USA

Cancer is intrinsically heterogenous, a characteristic that "complicates our diagnoses, confounds our prognoses, and challenges our therapies."

In this educational handbook, authored by a distinguished faculty, we consider the concept of heterogeneity, its various manifestations and the impact on diagnosis and management, as well as implications for future clinical practice.

In the first chapter, Mythili Shastry and Erika Hamilton from Sarah Cannon Research Institute, Nashville, TN, USA, describe the concept of heterogeneity. Tumor heterogeneity describes the coexistence of different biological, morphological, phenotypic and genotypic profiles between tumors and within tumors. The National Cancer Institute defines tumor heterogeneity as the differences between tumors of the same type in different patients, the differences between cancer cells within a single tumor, or the differences between a primary (original) tumor and a secondary lesion.

The authors discuss how heterogeneity has been described in various types of tumors, including breast, lung, ovarian, pancreatic, kidney, colorectal, brain, and prostate cancers, as well as hematologic malignancies, such as chronic lymphoblastic leukemia and acute lymphoblastic leukemia. They comment that breast cancer is highly heterogeneous – around 20 morphologically distinct subtypes have been identified – and consider the types and mechanisms of heterogeneity.

In chapter 2, the focus is on breast cancer and the emerging picture of the way the complex and wide variation of some of the characteristics of tumor cells manifests in this disease. Rohit Bhargava (University of Pittsburgh Medical Center, Pittsburgh, PA, USA) describes the many forms of heterogeneity that exist within breast cancer, and between primary and metastatic tumors and the extensive factors that influence these phenomena.

Zoé Guillaume and Thomas Grinda from the Department of Cancer Medicine, Gustave Roussy, Villejuif, France, review how discordance in receptor expression between primary and metastatic breast tumors is a common occurrence and can have a significant impact on overall survival as well as on treatment management. Although the mechanism of discordance is not fully understood, several hypotheses exist and work is ongoing to determine its prevalence and impact on patient survival.

Carlos Barrios of the Latin American Cooperative Oncology Group (LACOG), Brazil, considers the impact of heterogeneity on diagnosis, treatment, and implementation of modern precision medicine in the final chapter of the handbook. Unquestionably, patient selection strategies and our ability to set apart different subgroups of patients each requiring specific therapeutic strategies represent the most important and revolutionary advance in cancer care in the last two decades. However, paradoxically, tumor heterogeneity is perhaps one of the greatest barriers to personalized or precision medicine, where treatment aims to address specific molecular abnormalities or differences from one individual to another. In view of tumor heterogeneity, cancer cells can be seen as dynamic moving targets.

The authors also consider efforts to understand cancer heterogeneity and provide insights into the potential impact a greater understanding of this characteristic of tumors may have on future diagnosis and management of a disease with an estimated 19.3 million new cases and 10 million associated deaths in 2020.

This impactful resource therefore provides readers with a comprehensive overview of the current state of knowledge on the phenomenon of heterogeneity in cancer, with a particular focus on breast cancer.

I hope you will find the content educational, engaging and enjoyable.

The concept of heterogeneity and heterogeneity in cancer

Cancer is intrinsically heterogenous, a characteristic that confers complexity and adds to the challenges faced by clinicians diagnosing and managing the disease. In the first article of this handbook, the concept of heterogeneity and how it manifests in different types of cancer and within cancers is discussed.

Mythili Shastry PhD
Sarah Cannon Research Institute, Nashville, TN, USA

Erika Hamilton MD
Tennessee Oncology, Nashville, TN; Sarah Cannon Research Institute, Nashville, TN, USA

In an effort to distill the vast complexity of cancer, hallmarks attempt to 'rationalize the complex phenotypes of diverse human tumor types and variants in terms of a common set of underlying cellular parameters'. These hallmarks of cancer are:

- Evading growth suppressors
- Avoiding immune destruction
- Enabling replicative immortality
- Tumor-promoting inflammation
- Activating invasion and metastasis
- Inducing or accessing vasculature
- Genome instability and mutation
- Resisting cell death
- Deregulating cellular metabolism
- Sustaining proliferative signalling[1]

Cancer can, therefore, be regarded as the summation of many different aberrant mechanisms and is driven by acquired intra- and intertumoral variations.

Definition of heterogeneity

Tumor heterogeneity describes the coexistence of different biological, morphological, phenotypic and genotypic profiles between tumors and within tumors.

The National Cancer Institute defines tumor heterogeneity as: the differences between tumors of the same type in different patients, the differences between cancer cells within a single tumor, or the differences between a primary (original) tumor and a secondary tumor.[2] These differences may involve the tumor's genes and / or proteins. For example, some cancer cells in a tumor may have genetic mutations that are not present in other cancer cells in that tumor. Another example is heterogeneity of human epidermal growth factor receptor 2 (HER2) protein expression within tumor lesions and between different metastatic sites. Tumor heterogeneity can play an important role in how cancer is diagnosed and treated and how it responds to treatment.[2]

Types of heterogeneity

There are several types of tumor heterogeneity, including:[3]

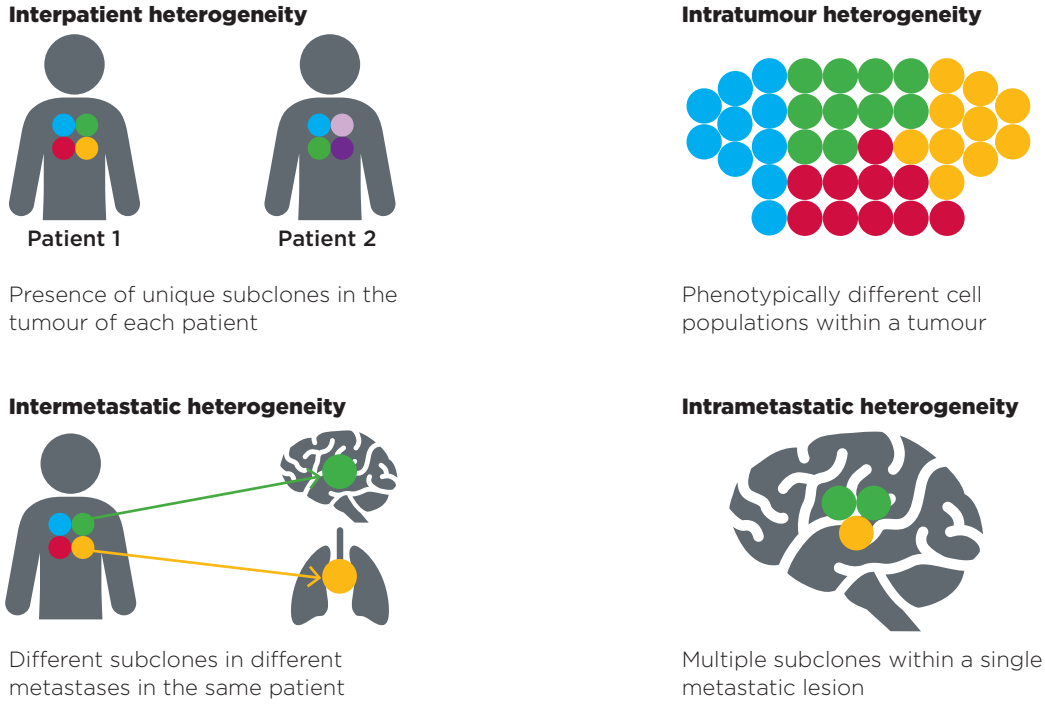
- Interpatient heterogeneity: the presence of unique subclones in the tumor of each patient that may be due to patient-specific factors such as germline genetic variations and environmental factors
- Intertumor heterogeneity: the coexistence of different biological, morphological, phenotypic and genotypic profiles between tumors in different parts of the body
- Intratumor heterogeneity: the presence of multiple subclones within one discrete tumor resulting in heterogeneity within one cancer lesion
- Intermetastatic heterogeneity: different subclones in different metastatic lesions can exist in the same patient; some subclones may have been derived from the primary tumor and some may have emerged due to acquired alterations within each metastatic lesion
- Intrametastatic heterogeneity: the presence of multiple subclones within a single metastatic lesion (see Figure 1)
- Spatial heterogeneity: heterogeneity occurring in different regions in the same tumor or in different tumors, seen in both primary cancer and metastases
- Temporal heterogeneity: the genetic heterogeneity that occurs over time and is usually a consequence of treatment.[4,5]

Indeed, the classification of discrete tumor subtypes, characterized by distinct molecular genetic profiles, morphology, and expression of specific markers (either concurrently or at different points in time), demonstrates intertumoral heterogeneity. Within a tumor there are cells with a range of functional properties and different biomarker expression patterns – reflecting intratumoral heterogeneity.[6]

The tumor microenvironment also plays a part in intratumor heterogeneity as a result of the interaction between cancer cells and other cells in the complex ecosystem, including proliferating tumor cells, the tumor stroma, surrounding blood vessels and immune cells.[7]

FIGURE 1

Types of heterogeneity in cancer



References 3,33

Mechanisms of heterogeneity

Different aspects of tumor heterogeneity have been researched, including genomics, transcriptomics, histopathologic features, and characterization of the inflammatory infiltrate.[8]

Mechanisms responsible for intratumoral heterogeneity can be broadly categorized into cell-intrinsic mechanisms and cell-extrinsic mechanisms.

Cell-intrinsic mechanisms include variability from one cell to another in: genotypic alterations and non-genetic or phenotypic variations, which are due to epigenetic modification; plastic gene expression, and signal transduction. Extrinsic mechanisms are a result of unequal microenvironments.[9]

Genomic instability is the best known and most studied intrinsic mechanism. Genomic alterations happen in the pathways of nucleotide excision repair, base excision repair, DNA mismatch repair, telomere maintenance, double-strand break repair, DNA replication, and chromosome segregation; they result in extensive and stochastic changes across the genome. Epigenetic changes – stable or heritable changes in genetic information without changes in DNA sequences – also play a significant part in intratumoral heterogeneity.[9]

Cancers can be caused by chromosomal instability (CIN), microsatellite instability (MSI), or through the serrated neoplasia pathway.[10]

Branched evolution can lead to intratumoral heterogeneity.[11]

Extrinsic factors contributing to heterogeneity include the tumor microenvironment and stress inducing elements. The microenvironment surrounding cancer cells impacts intratumoral heterogeneity by influencing the genotypes and phenotypes of cancer cells. Perhaps the most obvious example being variation in the blood supply that provides nutrients, growth factors and oxygen, and removes metabolic waste. For example, variations in the distance between tumor cells and blood vessels may result in a variation in supply. Such inequality may be a factor in heterogeneous signal transduction, gene expression and genomic instability in cancer cells either directly through systemically supplied growth factors or hormones, or indirectly through oxidative stress, hypoxia, or acidosis.[9]

Another extrinsic source of tumor heterogeneity can be the selective pressure from cancer treatment. Resistance to therapy can develop due to selection of specific clones that have acquired an alteration enabling them to survive in the local environment leading to heterogeneity.[12] During periods of stress including drug treatment, non-genetic processes such as epigenetic modifications can lead to phenotypic changes in cancer cells leading to drug tolerance.[13] This status was shown to be transient,

>

GETTY

allowing for dynamic regulation of this heterogeneity enabling drug tolerance.[13]

Which cancers exhibit heterogeneity?

Heterogeneity has been described in various types of tumors, including breast, lung, ovarian, pancreatic, kidney, colorectal, brain, and prostate cancers, as well as hematologic malignancies, such as chronic lymphoblastic leukemia and acute lymphoblastic leukemia.[3]

The WHO classification of lung tumors recognizes several types of lung cancers, including epidermoid carcinomas, adenocarcinomas, small cell lung carcinomas, large cell carcinomas, large cell neuroendocrine carcinomas, adenosquamous carcinomas, sarcomatoid and pleomorphic carcinomas, along with several other types, thus acknowledging the histological heterogeneity of lung cancer.[14] Heterogeneity at the cellular level is demonstrated by the example of adenosquamous carcinomas (a relatively rare subtype of non-small-cell lung cancer)[15] where cells with adenocarcinoma differentiation markers like CK7 and TTF1 as well as with squamous differentiation markers such as CK5/6 or other high-molecular-height cytokeratins can be found.[14]

The main subtype of renal cell carcinomas is clear cell; the other subtypes are chromophobe, collecting duct, translocation, medullary and mucinous tubular, and spindle cell carcinomas.[16] Rarer non-clear cell renal cell carcinoma have been found to have four subtypes.[17] Intratumor heterogeneity also exists.[11]

Epithelial ovarian carcinomas are classified by WHO into five major subtypes: high-grade serous (HGS) carcinoma; low-grade serous (LGS) carcinoma; mucinous carcinoma; endometrioid carcinoma; and clear-cell carcinomas.[18] The distinctions are based on histopathology, immunohistochemistry and molecular genetic analyses.[19] The relative frequency of each of these varies with HGS accounting for 70–80% of ovarian cancers (OC) and endometrioid and clear cell carcinomas comprising ~10% each, with LGSOC and mucinous being the rare subtypes.[18]

Pancreatic ductal adenocarcinoma (PDAC; the most common type of pancreatic cancer) exhibits intra- and intertumoral heterogeneity. The WHO classification describes several PDAC subtypes. Ductal adenocarcinoma is the most common (85%), followed by adenosquamous carcinoma (0.4– 10%), colloid carcinoma (2–5%), and medullary, hepatoid, signet ring, undifferentiated anaplastic, and undifferentiated with osteoclast-like giant cell carcinomas (all <1%). PDAC also often has different patterns (clear cell, foamy cell, large duct, intestinal, micropapillary, and cystic papillary), which may coexist within the same tumor.[20]

Four distinct molecular subtypes of colorectal cancer have been described: adenocarcinoma; medullary carcinoma; mucinous carcinoma, and signet ring cell carcinoma.[8]

Intrinsic intratumor heterogeneity is one of the factors behind the aggressiveness of glioblastomas, one of the most frequent brain tumors. WHO classification still uses the histopathological grading system, but now molecular markers such as isocitrate dehydrogenase (IDH) are incorporated. There are three main glioma classes: IDH mutant, 1p/19q codeleted (oligodendrogliomas), IDH mutant, 1p19q intact (astrocytomas), and IDH wild-type gliomas.[21]

More than 90 different categories of B- and T-cell lymphomas are distinguished in the WHO classification; morphologic, immunophenotypic, and genetic heterogeneity are seen in lymphomas.[22]

Breast cancer is highly heterogeneous. A variety of distinct genetic changes in mammary epithelial cells mean that each patient can have a vastly different disease from another. Breast cancer can be classified into a number of molecular subtypes based on the expression or lack thereof of select biomarkers. These include HR+, TNBC, HER2-positive, and now a new designation HER2- low. Within these categories, there are unique mutations and biomarkers – PD-L1 +/- in TNBC, PIK3CA mutations and ESR1m in HR+ disease etc, that may inform treatment options. In addition to these intertumoral differences, intratumoral heterogeneity can be present in the same patient in tumor cell subpopulations within a primary tumor and in metastases.[23] The different breast cancer subtypes with distinctive morphological features and the grading of tumors based on the percentage of the tumor arranged in glands and tubular structures, the degree of nuclear pleomorphism, and the mitotic rate also illustrate the heterogeneity of breast cancer.[24]

Heterogeneity in hematologic malignancies

Inter- and intratumoral heterogeneity in hematologic cancers is exemplified by lymphomas, which exhibit morphologic, immunophenotypic, and genetic heterogeneity; intratumoral heterogeneity and subclonal evolution; as well as transformation and transdifferentiation. Transformation – the evolution of low-grade lymphoma into a high-grade lymphoma – is the most common example of intratumoral heterogeneity.[22]

Multiple myeloma shows heterogeneity at the genetic level with chromosome numbers, genetic translocations and genetic mutations, and at the clonal level with significant clonal heterogeneity evidenced by multiple clones coexisting in the same patient. Also, there is a hierarchy of clonally related cells that seem to have different clonogenic potential.[25]

Determinants of heterogeneity in solid tumors

Darwin's evolutionary principle of "survival of the fittest" appears to be at work within tumors

– described as clonal evolution whereby somatic heterogeneity gives rise to subclones with differing biological capabilities. Select subclones may be conferred with a growth advantage enabling them to survive and ultimately expand, while other subclones are unable to compete and eventually die.[23] This genomic instability may perpetuate in the expanding tumor population generating additional diversity that is subject to evolutionary selection pressure leading to further heterogeneity. This may follow a linear evolution model where a subclone acquires successive advantageous mutations and sequential clones outnumber the original ancestral clone or a branched evolution model where divergent subclones arise due to different mutations and branch out into hetergenous populations although they all share a common ancestor.[26]

The cancer stem cell model posits that a unique subset of cells referred to as cancer stem cells (CSCs) initiate and sustain tumor growth. These cells have a strong self-renewal capability as well as the ability to differentiate into multiple cell types.[27] CSCs also express multidrug resistance proteins that protect them from chemotherapeutics and induce drug resistance.[28,29] It is believed that these CSCs serve as "seeds" for tumor initiation and growth as well as metastases and recurrence.[30] Epithelial to mesenchymal transition has been linked to generation of breast cancer CSCs. However, some studies have demonstrated plasticity between mammary epithelial cancer cells and epithelial CSCs challenging the notion of a strictly defined subset of CSCs in breast cancer.[31] Furthermore, breast cancer cells can transition between luminal, basal and progenitor-like states highlighting the potential for these changes to affect cancer cell phenotype and malignancy.[23]

Conclusions

Heterogeneity is a feature of many different types of cancer. Tumor heterogeneity "complicates our diagnoses, confounds our prognoses, and challenges our therapies."[32] The term heterogeneity can also be used to describe various ideas: heterogeneity within a single tumor, from a primary to a metastatic site, heterogeneity among patients, etc. Our understanding of a patient's cancer oftentimes is based on a biopsy or excision, equating to a mere glimpse into select tissue in one lesion at one time point. These variables translate to many things we do not know about the full picture of a patient's cancer. Blood-based 'liquid' biopsies can help some but do not eliminate this problem. Ultimately, temporal and spatial heterogeneity add to the complexity of the disease and, as we shall discover in the next article, can manifest as a number of subtypes in a single cancer type.

References

1 Hanahan D. Hallmarks of Cancer: New Dimensions. Cancer Discov 2022;12(1):31–46.
2 National Cancer Institute. Tumor heterogeneity. www.cancer.gov/publications/dictionaries/cancer-terms/def/tumor-heterogeneity (accessed April 2023)
3 Jamal-Hanjani M et al. Translational implications of tumor heterogeneity. Clin Cancer Res 2015;21(6):1258–66.
4 Fumagalli C, Barberis M. Breast Cancer Heterogeneity. Diagnostics 2021;11:1555.
5 Guo L et al. Breast cancer heterogeneity and its implication in personalized precision therapy. Exp Hematol Oncol 2023;12:3.
6 Gerdes MJ et al. Emerging understanding of multiscale tumor heterogeneity. Front Oncol 2014;4:366.
7 Russano M et al. Liquid biopsy and tumor heterogeneity in metastatic solid tumors: the potentiality of blood samples. J Exp Clin Cancer Res 2020;39:95.
8 Sagaert X, Vanstapel A, Verbeek S. Tumor Heterogeneity in Colorectal Cancer: What Do We Know So Far? Pathobiology 2018;85(1–2):72–84.
9 Sun Xx, Yu Q. Intra-tumor heterogeneity of cancer cells and its implications for cancer treatment. Acta Pharmacol Sin 2015;36:1219–27.
10 Chan DKH, Buczacki SJA. Tumour heterogeneity and evolutionary dynamics in colorectal cancer. Oncogenesis 2021;10:53.
11 Zhang J et al. Intratumor heterogeneity in localized lung adenocarcinomas delineated by multiregion sequencing. Science 2014;346(6206):256–9.
12 Crucitta S et al. Treatment-driven tumour heterogeneity and drug resistance: Lessons from solid tumours. Cancer Treat Rev 2022 Mar;104:102340.
13 Sharma SV et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell 2010;141(1):69–80.
14 de Sousa VML, Carvalho L. Heterogeneity in Lung Cancer. Pathobiology 2018;85(1–2):96–107.
15 Li C, Lu H. Adenosquamous carcinoma of the lung. Onco Targets Ther 2018;11:4829–35.
16 Fisher R, Larkin J, Swanton C. Inter and intratumour heterogeneity: a barrier to individualized medical therapy in renal cell carcinoma? Front Oncol 2012;2:49.
17 Chen WJ et al. Heterogeneity of tumor microenvironment is associated with clinical prognosis of non-clear cell renal cell carcinoma: a single-cell genomics study. Cell Death Dis 2022;13:50.
18 Kossaï M et al. Ovarian Cancer: A Heterogeneous Disease. Pathobiology 2018;85(1-2):41–49.
19 Prat J, FIGO Committee on Gynecologic Oncology. Staging classification for cancer of the ovary, fallopian tube, and peritoneum. Int J Gynaecol Obstet 2014;124(1):1.
20 Cros J et al. Tumor Heterogeneity in Pancreatic Adenocarcinoma. Pathobiology 2018;85(1–2):64–71.
21 Becker AP et al. Tumor Heterogeneity in Glioblastomas: From Light Microscopy to Molecular Pathology. Cancers (Basel) 2021;13(4):761.
22 Schürch CM, Federmann B, Quintanilla-Martinez L, et al. Tumor Heterogeneity in Lymphomas: A Different Breed. Pathobiology 2018;85(1–2):130–45.
23 Lüönd F, Tiede S, Christofori G. Breast cancer as an example of tumour heterogeneity and tumour cell plasticity during malignant progression. Br J Cancer 2021;125:164–75.
24 Turashvili G, Brogi E. Tumor heterogeneity in breast cancer. Front Med 2017;4:227.
25 de Mel S et al. Implications of Heterogeneity in Multiple Myeloma. BioMed Research International 2014;2014:232546.
26 Dagogo-Jack I, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. Nat Rev Clin Oncol 2018 Feb;15(2):81–94.
27 Yang L et al. Targeting cancer stem cell pathways for cancer therapy. Sig Transduct Target Ther 2020;5:8
28 Peiris-Pages M et al. Cancer stem cell metabolism. Breast Cancer Res 2016;18:55.
29 Garcia-Mayea Y et al. Insights into new mechanisms and models of cancer stem cell multidrug resistance. Semin Cancer Biol 2019;S1044-579X.
30 Cojoc M et al. A role for cancer stem cells in therapy resistance: cellular and molecular mechanisms. Semin Cancer Biol 2015;31:16–27.
31 Chaffer CL et al. Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. Proc Natl Acad Sci USA 2011;108:7950–7955.
32 Tu SM et al. Stem Cell Theory of Cancer: Origin of Tumor Heterogeneity and Plasticity. Cancers (Basel) 2021 Aug 9;13(16):4006.
33 Januškevičienė I, Petrikaitė V. Heterogeneity of breast cancer: The importance of interaction between different tumor cell populations. Life Sci 2019;239:11700.

Heterogeneity and subtypes in breast cancer

In the second article in this handbook, we now focus on breast cancer and look at the emerging picture of the way the complex and wide variation of some of the characteristics of tumor cells manifests in this disease.

Rohit Bhargava MBBS
University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Breast cancer is highly heterogeneous – around 20 morphologically distinct subtypes have been identified. Each subtype is characterised by a distinctive molecular and/or biochemical signature, clinical course and prognosis, which differ from other subtypes.[1]

Subtypes of heterogeneity

Phenotypic heterogeneity

Phenotypic heterogeneity can be influenced by epigenetic, proteomic and metabolic differences between cells.[2] Such heterogeneity can exist even among cells possessing the same genetic changes. Researchers have observed that different cell phenotypes are separated spatially within a tumor, suggesting that it is perhaps differences in local environment that are responsible for much of the phenotypic heterogeneity seen in these cells rather than genetic changes per se (although differences in the presence of driver genes in different regions may also have a role).[2]

Epithelial–mesenchymal transition and, less commonly, the reverse of the process – mesenchymal–epithelial transition – is one of the main mechanisms contributing to phenotypic plasticity and heterogeneity of breast cancer cells. The process is thought to be one of the fundamental ways in which cancer spreads through metastases. The ability of cancer cells to switch between epithelial and mesenchymal phenotypes, and indeed adopt characteristics of both, allows them to exist in a range of hybrid phenotypes – an example of the cell plasticity that tumor cells possess.[3]

Molecular heterogeneity

As knowledge increases, the classification of breast cancer continues to evolve. In the fifth edition of the World Health Organization classification of tumors published in 2019 (an update of the fourth edition published in 2012), the classifications of breast cancer are based on clinically relevant morphological observations – along with factors such as tumor size, lymph node status and Nottingham grade – that serve as prognostic indicators.[4]

Characterization

Immunohistochemistry (IHC) is used to assess invasive breast cancer for biomarkers, including expression of estrogen receptors (ER), progesterone receptors (PR) and human epidermal growth factor receptor 2 (HER2) and Ki-67 proliferation index.[5]

IHC classes of breast cancer correlate with the four intrinsic molecular subtypes:

- Luminal A;
- Luminal B;
- HER2-enriched;
- Basal-like/triple negative.[5]

These characteristics, along with transcriptomic profiling, can be combined to further categorize breast cancer into additional molecular subtypes (Table 1).[6]

Again, these different subtypes have prognostic implications.[5] Figure 1 demonstrates how prognosis links with receptor expression.

Intertumoral versus intratumoral

Intertumoral heterogeneity in breast cancer is evident in the results of physical examination and imaging that are used in the clinical staging of the disease. The three-tier grading system (low, medium and high) for breast cancer also underlines the disease's tumor heterogeneity.[5]

As detailed, tumors also vary in the extent to which they express ER, PR, and HER2. Around 80% of breast cancers express ER and around 60–70% express PR; co-expression of ER/PR is common in breast tumors. The HER2 oncoprotein is overexpressed in about 15–20% of breast tumors. The semi-quantitative coordinate expression of receptors coupled with cellular proliferation in breast cancers not only determine prognosis but also a response to systemic therapies. This heterogeneity of biomarker expression by IHC can determine tumor molecular class and provide useful prognostic/predictive information, especially for luminal-type tumors.[7–13] Tumors in which there is no expression of ER, PR, or HER2 (that is, triple negative) are again highly heterogeneous in terms of histology, genetics and

TABLE 1

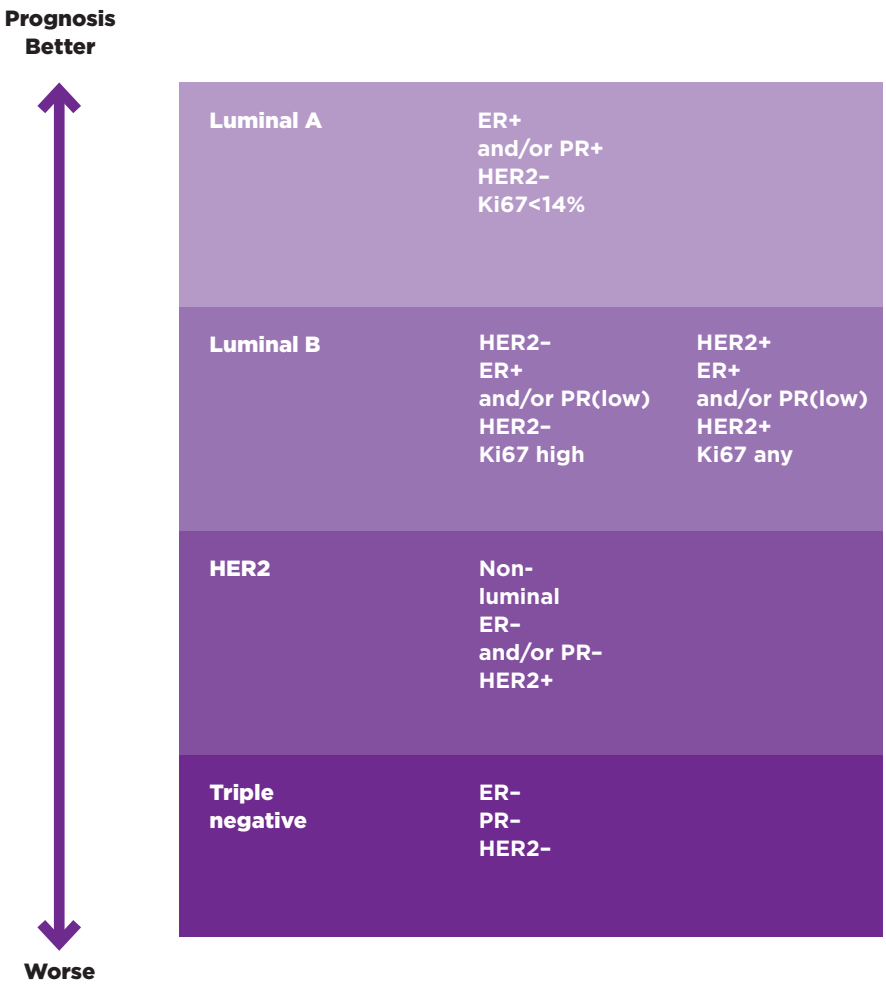
Breast cancer molecular subtypes

Subtype	Characteristics
Normal breast-like	Likely an artifact of tumor sampling
Luminal A	ER+/PR+, HER2- and Ki67-low
Luminal B	ER+/PR+ (low) and HER2+ or HER2-, and Ki67-high
HER2-enriched	HER2+, often ER-/PR- or low
Basal-like/triple-negative	ER-, PR-, HER2-
Claudin-low	ER-, PR-, HER2-, low expression of cell–cell adhesion molecules, including claudins 3, 4, and 7

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; Ki, marker of active cell proliferation; HER2, human epidermal growth factor receptor 2

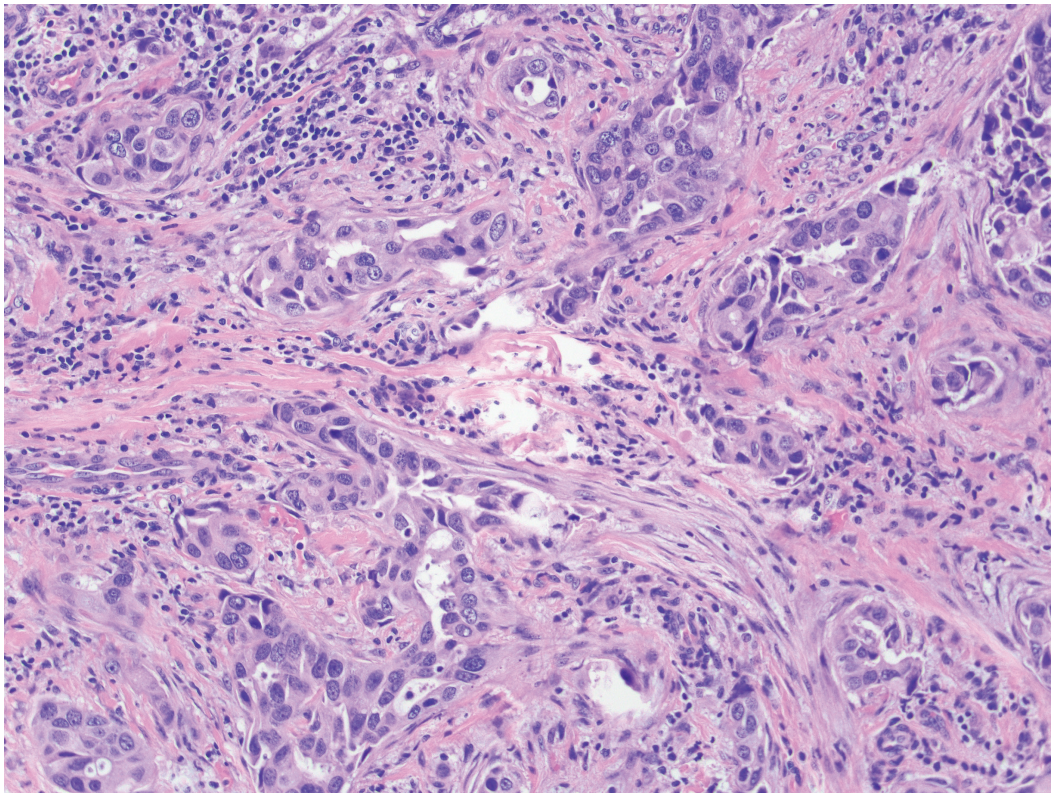
FIGURE 1

Breast cancer subtypes and prognosis



Abbreviations: ER, estrogen receptor; PR, progesterone receptor; Ki, marker of active cell proliferation; HER2, human epidermal growth factor receptor 2

Reference 14



Invasive breast carcinoma of no special type with scattered stromal tumor infiltrating lymphocytes. This high-grade tumor was negative for ER, PR and HER2 (triple negative breast cancer)

prognosis as well in their response to treatment.[5]

Individual tumors can vary in their characteristics within the tumors themselves – so-called intratumor heterogeneity. Morphologically this can be seen in different areas of the same tumor (spatial heterogeneity) or over time as the tumor progresses (temporal heterogeneity). Spatial heterogeneity also exists between primary and metastatic tumors.[5]

Biomarker heterogeneity can also be found in the same tumor with variation in estrogen and progesterone receptors as well as HER2 from one region of a tumor to another.[5]

Genetic heterogeneity in the form of chromosomal and genomic alterations can be detected in individual breast cancers.[5] Indeed, genetic mutations and / or epigenetic changes are the source of intratumor heterogeneity, and genome-wide sequencing technology can be used to define breast cancer subtypes based on copy number variation, DNA methylation, exome, RNA, microRNA sequencing and reverse-phase protein array data.[14] Variation in mutations, copy number alterations or structural variants accumulate with cell divisions and result in a tumor that has distinct subclonal populations. These subclonal populations can expand and contract because of the effects of selective pressures such as treatment or a change in environment that can happen as a consequence of metastasis.[2]

Heterogeneity also exists in the microenvironment of cancer cells.[15] Table 2 shows the components of the tumor microenvironment (TME) and their functions. This includes immune cells, endothelial cells, adipocytes and adipose tissue, fibroblasts and extracellular matrix proteins. Cell interactions mediated by the components of the TME release environmental cues to communicate with surrounding and distant cells. These interactions are critical in aiding the metastatic process at both the primary and secondary site. They also introduce a greater intratumoral heterogeneity and complexity through selective pressures on the cancer cells.[15]

Metastases can differ from their primary tumor – another example of intratumoral heterogeneity. Receptor status can vary between primary tumors and their metastases or circulating tumor cells. Differences in the genomes of clonally related primary tumors and their metastases have been shown in breast cancer. Genetic alterations are often similar in synchronous metastases and their corresponding primaries; however, almost a third (31%) of primary breast cancers and their metachronous metastases have significant differences in gene copy number by comparative genomic hybridization and fluorescence in situ hybridisation.[16] It has also been discovered through single-cell analysis that gene expression varies significantly between early and late

metastases. Early metastatic cells have a basal/stem cell-like signature and express genes associated with a de-differentiated, epithelial-to-mesenchymal transition-like phenotype. In contrast, cells in established metastases show more differentiated, luminal-like and proliferative characteristics.[6]

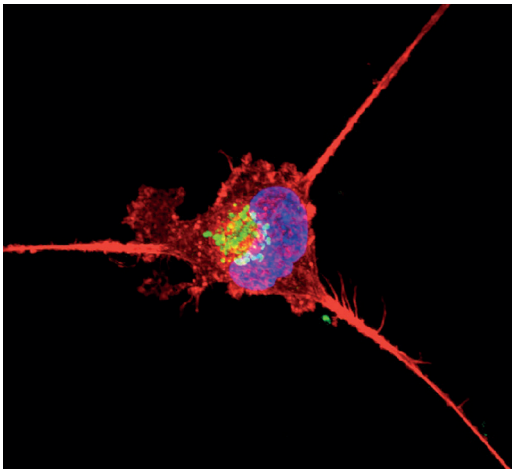
Chronology

Primary versus across treatment versus across metastasis

Cell characteristics in breast tumors can change spatially and temporally. Therefore, although ER, PR, and HER2 – fundamental in clinical subtyping, prognostication, and treatment selection – may stay largely unchanged throughout the treatment course, there are exceptions.[17] For example, changes have been recorded after neoadjuvant chemotherapy in loco-regional breast cancer and in matched primary and metastatic cancer lesions. Overall studies show that there is a 16–30% change in receptor status after neoadjuvant treatment with a change in ER and PR being more common than a change in HER2 status.[17] A discordance rate between primary and metastatic breast cancer of 10.3% for HER2, 19.3% for ER and 30.9% for PR was found in a meta-analysis of 39 studies.[17] The change in hormone receptors is more frequent after endocrine therapy and for tumors that initially showed lower expression levels (focal, patchy or heterogeneous expression by IHC). Receptor conversion (positive to negative) is more frequent when 1% cutoff is used compared with the traditional 10% threshold.[18]

Treatment may also induce some genetic changes, possibly through selective pressure. In a matched comparison of primary tumor and metastatic tumors new clonal mutations were detected mainly after treatment. A higher mutational burden is often seen in metastases compared with primary tumors across breast cancer subtypes and sites of metastases, whereas copy number alteration burden has been observed to be similar across primary tumors and metastases.[2]

Large numbers of cancer cells are released into the circulation every day but fewer than 0.1% from metastases. To do so requires that cancer cells avoid death, adapt to a new environment at the site of the metastasis and thrive.[19] Many of these are properties very different to those needed in the cancer cells that establish a primary tumor.[20]



Fluorescent light micrograph of triple-negative breast cancer cell

SCIENCE PHOTO LIBRARY

Cells, either singularly or in clusters, from a primary tumor site travel via the circulation to distant sites to colonize other organs and are then triggered at some point to later acquire specific functional properties to form macroscopic metastases. Genetic changes drive the development of metastases; IL-11, CTGF, CXCR4 and MMP1 genes have been found to promote bone colonization in breast cancer.[21]

In humans, it is thought that clusters of cancer cells are needed to form metastases, which requires the transformation of epithelial cells – the epithelial–mesenchymal transition – a process that happens under the influence of a number of growth factors and signalling pathways.[22] It involves malignant epithelial cells losing their junctional structures, expressing mesenchymal proteins, and remodeling their extracellular matrix.[22] The mesenchymal >

TABLE 2

Components of the TME and their functions

Component	Function
Immune cells	Provide an immunosuppressive environment
Endothelial cells	Vessel formation
Fibroblasts	Paracrine signalling to influence the tumor cells
Adipocytes and adipose tissue	Release of adipokines
Extracellular matrix proteins	Provision of biomechanical/biochemical support

phenotype is characterized by fibroblast-like morphology and these cells have more migratory and invasive properties.[3] Hypoxia, metabolic stressors and matrix stiffness are thought to be the triggers for epithelial–mesenchymal transition in cancer cells. For metastases to progress, the opposite process – mesenchymal–epithelial transition – must happen.[19]

Metastatic relapse can happen in breast cancer months or decades after initial diagnosis, although it is thought that metastases may already be present (but undetectable) at the time of diagnosis in most cases of patients who will experience recurrence of their disease. The difference in time between initial diagnosis and metastatic recurrence, when disseminated tumor cells that form the metastases are in a dormant state, is probably related to the molecular differences seen in the different subtypes of breast cancer. For example, patients with basal-like and HER2– enriched subtypes tend to have early relapse, within the first five years after diagnosis, compared with those who have luminal cancers.[23] There may also be some effect of pharmacotherapy itself with evidence suggesting that endocrine therapy may force disseminated tumor cells to become dormant rather than killing them. It might also be that in some cases the length of the dormant period relates to how long it takes for tumor cells that are resistant to endocrine therapy to promulgate metastatic disease.[23] A study of 107 patients with HER2+ breast cancer showed that a loss of HER2 expression occurred among more patients treated with neoadjuvant chemotherapy alone compared with those treated with chemotherapy and targeted anti-HER2 agents. An increased rate of relapse was associated with loss of HER2 expression, which the authors say demonstrated a dynamic conversion to a chemoresistant phenotype.[24]

Heterogeneity is present in metastases in much the same way that it is in primary tumors. Although genomically similar to the primary from which they are derived, metastases have significant phenotypic differences; those differences may continue to develop as metastases evolve as a result of tissue-specific environments, cellular plasticity and pharmacological pressures.[23] Indeed, there is evidence for dynamic switching between molecular subtypes from the primary to the metastatic tumor; patients with HER2– primary tumors may have HER2+ brain metastases.[24] Conversion in estrogen and progesterone receptor expression has also been documented. It seems that the metastatic tumor environment and therapy may influence these changes. Estrogen receptor expression conversion rates have been found to be higher in bone and central nervous system metastases and lower in the liver.[24]

Conclusion

Heterogeneity in breast cancer exists in many forms within, and between, primary and metastatic tumors and is influenced by a wide range of factors. A greater understanding of this complex picture promises to provide insights into the prognosis, diagnosis and management of this disease.

References

1 Hsiao YH, Chou MC, Fowler C, et al. Breast cancer heterogeneity: mechanisms, proofs, and implications. J Cancer 2010;1:6–13.
2 Caswell-Jin JL, Lorenz C, Curtis C. Molecular heterogeneity and the evolution of breast cancer. Annu Rev Cancer Biol 2021;5:79–94.
3 Kvokačková B et al. Phenotypic Heterogeneity of Triple-Negative Breast Cancer Mediated by Epithelial-Mesenchymal Plasticity. Cancers (Basel) 2021;13(9):2188.
4 Tan PH et al; WHO Classification of Tumours Editorial Board. The 2019 World Health Organization classification of tumours of the breast. Histopathology 2020;77(2):181–5.
5 Turashvili G, Brogi E. Tumor Heterogeneity in Breast Cancer. Front Med (Lausanne) 2017;4:227.
6 Lüönd F, Tiede S, Christofori G. Breast cancer as an example of tumour heterogeneity and tumour cell plasticity during malignant progression. Br J Cancer 2021;125(2):164–75.
7 Flanagan M et al. Histopathologic variable predict Onco*type* DX™ Recurrence Score. Modern Pathology 2008;21:1255–61.
8 Klein M et al. Prediction of the Onco*type* DX recurrence score: use of pathology-generated equations derived by linear regression analysis. Modern Pathology 2013;26:658–64.
9 Farrugia D et al. Magee Equation 3 predicts pathologic response to neoadjuvant systemic chemotherapy in estrogen receptor positive, HER2 negative/equivocal breast tumors. Modern Pathology 2013;30:1078–85.
10 Bhargava R, Clark B, Dabbs D. Breast Cancers With Magee Equation Score of Less Than 18, or 18-25 and Mitosis Score of 1, Do Not Require Oncotype DX Testing. Am J Clin Pathol 2019;151:316–23.
11 Bhargava R et al. The healthcare value of the Magee Decision Algorithm™: use of Magee Equations™ and mitosis score to safely forgo molecular testing in breast cancer. Modern Pathology 2020;33:1563–70.
12 Bhargava R et al. Magee Equations™ and response to neoadjuvant chemotherapy in ER+/HER2-negative breast cancer: a multi-institutional study. Modern Pathology 2021;34:77–84.
13 Bhargava R, Dabbs D. The Story of the Magee Equations: The Ultimate in Applied Immunohistochemistry. Appl Immunohistochem Mol Morphol 2022;Sept 21.
14 Januškevičienė I, Petrikaitė V. Heterogeneity of breast cancer: The importance of interaction between different tumor cell populations. Life Sci 2019;239:117009.
15 Tan K, Naylor M. Tumour microenvironment-immune cell interactions influencing breast cancer heterogeneity and disease progression. Front Oncol 2022;12:876451.
16 Martelotto LG et al. Breast cancer intra-tumor heterogeneity. Breast Cancer Res 2014;16(3):210.
17 Li A et al. Characterizing advanced breast cancer heterogeneity and treatment resistance through serial biopsies and comprehensive analytics. npj Precis Onc 2021;5:28.
18 Hoefnagel L et al. Receptor conversion in distant breast cancer metastases. Breast Cancer Res 2010;12:R75.
19 Fares J et al. Molecular principles of metastasis: a hallmark of cancer revisited. Signal Transduct Target Ther 2020;5(1):28.
20 Suhail Y et al. Systems Biology of Cancer Metastasis. Cell Syst 2019;9(2):109–27.
21 Zeeshan R, Mutahir Z. Cancer metastasis – tricks of the trade. Bosn J Basic Med Sci 2017;17(3):172–82
22 Aleskandarany MA et al. Tumour Heterogeneity of Breast Cancer: From Morphology to Personalised Medicine. Pathobiology 2018;85(1–2):23–34.
23 Riggio AI, Varley KE, Welm AL. The lingering mysteries of metastatic recurrence in breast cancer. Br J Cancer 2021;124:13–26.
24 Turner KM et al. Heterogeneity within molecular subtypes of breast cancer. Am J Physiol Cell Physiol 2021;321(2):C343–54.

GETTY

Discordance in breast cancer: origin and frequency

In this article, we review how discordance in receptor expression between primary and metastatic breast tumors is a common occurrence and can have a significant impact on overall survival as well as on treatment management.

Zoé Guillaume
Thomas Grinda MD
Department of Cancer Medicine, Gustave Roussy, Villejuif, France

Breast cancer is the most prevalent form of cancer and the primary cause of cancer-related mortalities in women.[1] However, it is a diverse disease categorized by three biomarkers: the estrogen receptor (ER); progesterone receptor (PR); and human epidermal growth factor receptor 2 (HER2). The role of these biomarkers is crucial, as they are used to classify the cancer into five subtypes based on its histology and immunohistochemical (IHC) expression. These subtypes not only have different prognoses but also require distinct treatment strategies.[2]

Over the past few decades, studies have demonstrated that the expression of these markers can change during the natural history of cancer, for example, between initial diagnosis and metastatic relapse or successive progression – a phenomenon known as phenotypic discordance. The detection of any discordance in the expression of markers is crucial for managing patients with metastatic breast cancer because the efficacy of personalized treatments relies heavily on the dynamic changes of these markers over time. That is why the latest recommendations strongly advocate for performing a biopsy at presentation or first recurrence of metastatic lesions.[3–5]

Mechanism of phenotypic discordance

Phenotypic discordance is currently being studied to determine its prevalence and impact on patient survival. These questions are crucial, and ongoing research is working to address them. The mechanism behind this phenotypic discordance is not yet fully understood, although several hypotheses have been proposed. One possible explanation could be attributed to the analysis techniques employed. Variability in sampling techniques such as cytopuncture, biopsy, and surgical resection,[6–9] as well as differences in immunohistochemistry techniques can lead to interpretation biases. Moreover, decalcification methods required for studying bone samples can decrease the reliability of immunohistochemistry and increase the risk of false negatives. In fact, a decrease in the staining intensity of ER and PR by 15–20% has been shown in the first 6 hours of treatment.[10] As breast cancer often progresses with isolated metastatic bone disease, these factors could partially account for the observed discordance.

Another hypothesis pertains to tumor heterogeneity, which can occur spontaneously due to the tumor's ability to generate clones and sub-clones with different genetic expressions that are selected during cancer evolution. It can also result from selection pressure during treatment.[11] For example, anthracycline-based chemotherapy has been associated with a switch in ER status, and the use of trastuzumab or adjuvant endocrine therapy can be linked to the loss of HER2 and hormone receptors, respectively, at the metastatic sites.[12] A study on lung cancer has prospectively investigated the evolution of intratumoral heterogeneity, finding that clonal expansion plays a crucial role in this type of cancer and that different subclones can emerge within untreated tumors and cause cancer to spread. These subclones may also contribute to relapse[13,14] (Figure 1).

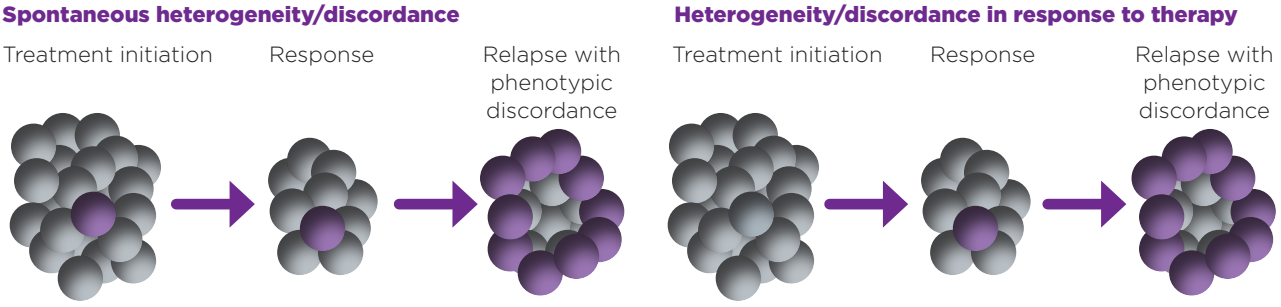
The initial receptor expression status was also found to be associated with a higher rate of discordance. In multivariate analysis, after adjustment for age, histological grade, number and type of metastatic site, hormone receptor (HR)+/HER2– status (OR = 0.05, [95% CI 0.03–0.08], $p < 0.001$) and HER2+ status (OR = 0.37, [95% CI 0.23-0.59], $p < 0.001$) were linked to hormone receptor discordance, as compared with HR-/HER2– status.[7] Similarly, patients with an initial PR+ status had a higher rate of discordance than PR– patients (hazard ratio, 1.71; 95% CI, 1.19–2.47; $p = 0.004$).[15] Metastatic sites have also been studied as a factor in discordance, but the findings have been inconsistent, and no significant differences have been consistently observed.

Frequency

Several studies have investigated the rate of discordance between the primary lesion and metastatic site(s) in breast cancer, but most of

FIGURE 1

Mechanism of phenotypic discordance



Different clones and subclones can be generated within untreated tumors or through selection pressure during treatment and can contribute to relapse

them have small sample sizes and report inconsistent results. Moreover, meta-analyses that combined these studies have yielded similar findings (Table 1 and Figure 2).[7–9]

A recent retrospective study on the ESME population, which is a nationwide population-based database of patients with metastatic breast cancer in France, investigated the discordance in receptor expression between the primary breast tumor site and first metastatic sites in 1677 patients.[7] The study found that the rate of change for HR status was 14.2% [95% CI 12.5–16.0], with a loss in expression in 72.5% of cases and a gain in expression in 27.5% of cases; for ER status, 15.1% [95% CI 13.3–17.0] of cases showed a change, with a loss in expression in 67.7% of cases and a gain in expression in 32.3% of cases; as for PR status, the study observed a modification in 31.1% [95% CI 28.7–33.5] of cases, with a loss in expression in 75.3% of cases and a gain in expression in 24.7% of cases; finally, regarding the HER2 status, the modification rate was 7.8% [95% CI 6.3–9.6], with an absence of overexpression/amplification in 45.2% of cases and a gain in expression in 54.8% of cases.[7] However, please note the timing of the metastatic biopsy, as well as differences in immunohistochemistry techniques, may explain the slight difference between these results and those of other studies.

Discordances in receptor expression can also occur during the evolution of metastatic disease. A study of 103 patients showed that discordance between the first and second metastases for ER receptor was observed in 18.8%, PR receptor in 19.8%, and HER2 in 10.7%, thus emphasizing the need for continuous monitoring of metastatic lesions throughout the course of the disease.[16]

Assessment

Currently, guidelines recommend performing a biopsy at the time of metastatic diagnosis to adapt treatment according to the new results.[3–5] Given the frequent discordance in receptor expression between primary tumors and metastatic sites, a new biopsy, while technically challenging, can improve patient management. It confirms the diagnosis of metastatic disease and, most importantly, guides therapeutic decisions by providing access to targeted therapies and avoiding the use of treatments that have become ineffective.

Successive biopsies during metastatic disease are also recommended in case of abnormal progression, such as primary resistance to treatment or acceleration of tumor growth, to detect potential discordance and adapt treatment accordingly.

However, repeat biopsies have some limitations. They are invasive, costly, and can be painful for the patient. In addition, repeat biopsies are not risk-free, as they may cause serious adverse events. For example, the SAFIR01 study reported serious adverse events in 9 out of 423 patients who underwent biopsy.[17] Therefore, it >

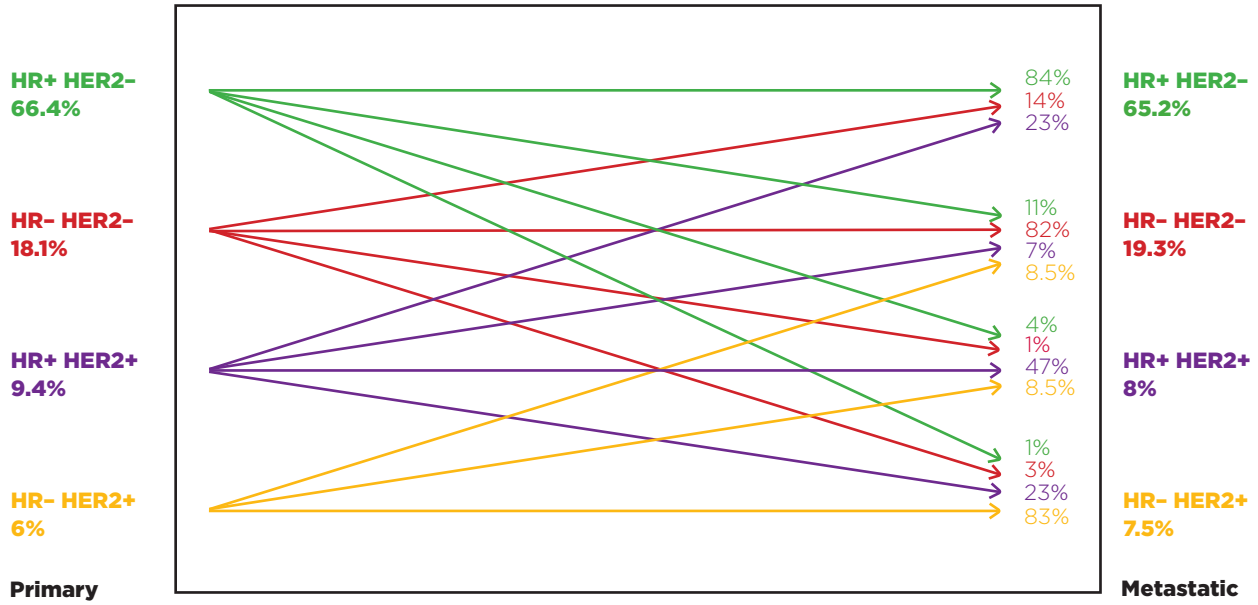
TABLE 1

Summary of studies evaluating phenotypic discordance in breast cancer

	Aurilio et al (2014)[8]	Schrivjer et al (2018)[9]	Grinda et al (2021)[7]
ER discordance	20%	19.3%	15.1%
PR discordance	33%	30.9%	31.1%
HER 2 discordance	8%	10.3%	7.8%

FIGURE 2

Discordance rates between primary breast cancer lesions and metastatic sites



Reference 7

is crucial to explain the reasons for repeat biopsies to patients and inform them of the benefits of this procedure in managing their cancer.

Liquid biopsy presents an interesting and non-invasive alternative to better reflect the heterogeneity of these cancers during their evolution. Numerous studies are currently ongoing to determine its potential role in the management of patients.[18]

FES PET/CT, which uses a fluorinated analogue of estradiol with a good binding affinity to ER, has shown promise in efficiently categorizing temporal and spatial disease heterogeneity and characterizing known or suspected metastatic lesions as expressing ERs, according to recent studies.[19] A similar PET scan targeting trastuzumab and HER2 is currently being evaluated. In the future, these molecular scans could have the potential to replace the need for biopsy.

Impact on survival / impact on prognosis and treatment management

Considering the potential change in phenotype during the disease, it is unclear how this may impact patient survival. The loss of hormone receptors has been identified as a poor prognostic factor associated with poorer overall survival.[20] Loss of ER or PR alone has also been linked to worse survival.[20] By contrast, the gain of hormone receptors did not appear to affect survival. Similarly, no difference in overall survival was observed in patients with discordant HER2 status (loss or gain). However, it should be noted that these findings may not consider the impact of therapeutic management changes resulting from the change in cancer status. To our knowledge, no study has yet investigated the impact of treatment modifications on survival in relation to changes in cancer status.[12]

Yi et al conducted a study on 1583 patients with metastatic breast cancer to evaluate the impact of changes in hormone receptor and HER2 status on treatment decisions.[15] The results showed that patients with receptor gain were more likely to receive a change in therapy. Specifically, 7.4% and 27.3% of patients with hormone receptor gain received hormone therapy as first or second line treatment, respectively. Furthermore, 76% of patients with HER2 expression gain received anti-HER2 therapy as first or second line treatment. Conversely, loss of hormone receptor expression was associated with an 83.1% likelihood of receiving chemotherapy as first-line treatment.[15]

The recent introduction of antibody-drug conjugates (ADCs) has further expanded treatment options for metastatic breast cancer patients.[21] For example, trastuzumab deruxtecan is now approved for the HER2-low subtype, which emphasizes the significance of biopsy confirmation of HER2 status. In Lin's study, 17 out of 42 HER2-0 tumors were found to have converted to HER2-low, which enabled these patients to access previously ineligible treatment

options.[12] In another study by Almstedt involving 191 patients, the discordance rate between the HER2 status of primary tumors and corresponding distant metastases was found to be 49.6% (n=63; Kappa −0.003, 95%CI −0.15–0.15). A HER2-low phenotype was observed most frequently (n=52, 40.9%), particularly with a switch from HER2-zero to HER2-low (n=34, 26.8%).[22] These findings underscore the significance of precise assessment and monitoring of receptor status to inform treatment decisions and enhance outcomes for patients with metastatic breast cancer.

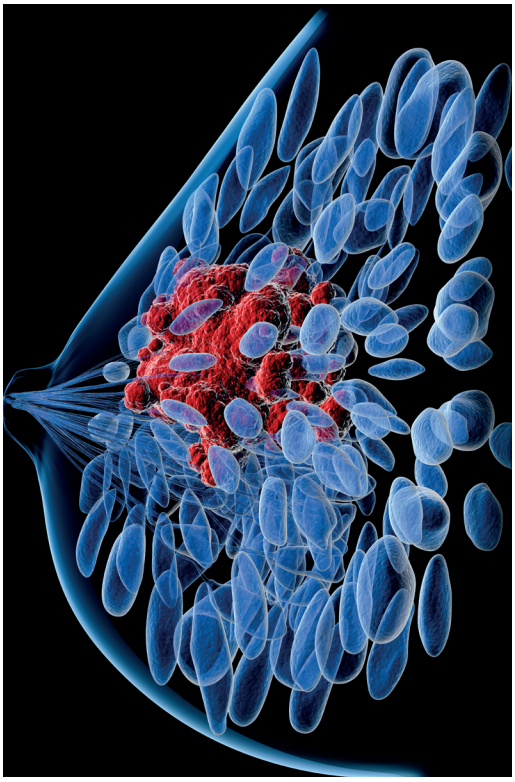
Real-world evidence

The ESME multicenter cohort study provides valuable insights into the general population of women with breast cancer. Of the patients included in this study, 17.6% had a histologically confirmed result from a biopsy of a metastatic site. Interestingly, 53% of primary HR+/HER2+ tumors exhibited a change in receptor status.[7]

In Yi's study, which included patients from a later time period, 48% of patients underwent biopsy of metastatic lesions. The study found a significant discordance in receptor expression, with 37.7% of cases showing a discrepancy between primary and metastatic tumors, regardless of molecular subtype. The higher rate of rebiopsy can be attributed to the fact that guidelines at the time recommended systematic rebiopsy for metastatic disease.[15]

Conclusion

Discordance in receptor expression between primary and metastatic breast tumors is a common occurrence and can have a significant impact on overall survival as well as treatment management. Identifying changes in receptor status is therefore essential to develop tailored management plans for each patient.



Female breast with tumor

GETTY

References

1 Sung H et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71(3):209–49.
2 Harbeck N et al. Breast cancer. Nat Rev Dis Primer 2019;5(1):1–31.
3 Henry NL et al. Biomarkers for Systemic Therapy in Metastatic Breast Cancer: ASCO Guideline Update. J Clin Oncol 2022;40(27):3205–21.
4 Gradishar WJ et al. Breast Cancer, Version 4.2017, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Cancer Netw JNCCN 2018;16(3):310–20.
5 Gennari A et al. ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer. Ann Oncol 2021;32(12):1475–95.
6 Mellouli M et al. Discordance in receptor status between primary and metastatic breast cancer and overall survival: A single-center analysis. Ann Diagn Pathol 2022;61:152044.
7 Grinda T et al. Phenotypic discordance between primary and metastatic breast cancer in the large-scale real-life multicenter French ESME cohort. NPJ Breast Cancer 2021;7(1):1–9.
8 Aurilio G et al. A meta-analysis of oestrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 discordance between primary breast cancer and metastases. Eur J Cancer 2014;50(2):277–89.
9 Schrijver WAME et al. Receptor Conversion in Distant Breast Cancer Metastases: A Systematic Review and Meta-analysis. JNCI J Natl Cancer Inst 2018;110(6):568–80.
10 Gertych A et al. Effects of tissue decalcification on the quantification of breast cancer biomarkers by digital image analysis. Diagn Pathol 2014;9:213.
11 Hu Z et al. Multi-cancer analysis of clonality and the timing of systemic spread in paired primary tumors and metastases. Nat Genet [Internet] 2020;18 May.
12 Lin M et al. Incidence and prognostic significance of receptor discordance between primary breast cancer and paired bone metastases. Int J Cancer 2023;152(7):1476–89.
13 Frankell AM et al. The evolution of lung cancer and impact of subclonal selection in TRACERx. Nature 2023;616(7957):525–33.
14 Al Bakir M et al. The evolution of non-small cell lung cancer metastases in TRACERx. Nature 2023;616(7957):534–42.
15 Yi ZB et al. Profile and outcome of receptor conversion in breast cancer metastases: a nation-wide multicenter epidemiological study. Int J Cancer 2020;148(3):692–701.
16 Lower E et al. Discordance of the estrogen receptor amd HER-2/neu in breast cancer from primary lesion to first and second metastatic site. Breast Cancer (Dove Med Press) 2017;9:515–20.
17 André F et al. Comparative genomic hybridisation array and DNA sequencing to direct treatment of metastatic breast cancer: a multicentre, prospective trial (SAFIR01/UNICANCER). Lancet Oncol 2014;15(3):267–74.
18 Ignatiadis M, Sledge GW, Jeffrey SS. Liquid biopsy enters the clinic — implementation issues and future challenges. Nat Rev Clin Oncol 2021;18(5):297–312.
19 Chae SY et al. Diagnostic accuracy and safety of 16α-[18F]fluoro-17β-oestradiol PET-CT for the assessment of oestrogen receptor status in recurrent or metastatic lesions in patients with breast cancer: a prospective cohort study. Lancet Oncol 2019;20(4):546–55.
20 Shiino S et al. Prognostic significance of receptor expression discordance between primary and recurrent breast cancers: a meta-analysis. Breast Cancer Res Treat 2022;191(1):1–14.
21 Modi S et al. Trastuzumab Deruxtecan in Previously Treated HER2-Low Advanced Breast Cancer. N Engl J Med 2022;387(1):9–20.
22 Almstedt K et al. Discordance of HER2-Low between Primary Tumors and Matched Distant Metastases in Breast Cancer. Cancers 2023;15:1413.

Impact of heterogeneity and emerging challenges

Being one of the main hallmarks of cancer, the fact that different tumors show unique characteristics with very distinct profiles has an impact on how we recognize tumor biology, prognosis, and response to treatment. In the final article of this handbook, we consider the impact of heterogeneity on diagnosis, treatment, and implementation of modern precision medicine.

Carlos Barrios MD
Latin American Cooperative Oncology Group (LACOG), Brazil

The heterogenous nature of cancer brings significant challenges both to clinicians and researchers. As noted earlier in this handbook, the multiple genetic aberrations, epigenetic modifications, metabolic reprogramming and microenvironment changes influencing the development and progression of tumors result in differences not only among individuals but within the same individual as well. Not surprisingly, this leads to diverse clinical outcomes and different responses to treatment, occasionally in different sites within the same patient.

Importantly, and with a major impact in our evolving personalized approach to the disease, we need to recognize that cancer changes with time, progressing differently in different sites, raising the challenge of diagnosing different underlying molecular abnormalities at different times during the disease course.[1] Therefore, both temporal and spatial heterogeneity do have significant impact on treatment decisions and influence disease outcomes. At the present time, managing a distant recurrence based on a single primary tumor biopsy from 5 years before does not make biological sense.[2]

Metastatic process and drug resistance

While localized forms of cancer can be effectively managed by available local therapies, the dissemination of cancer cells and the development of drug resistance are the main reasons for treatment failure and the main barriers compromising our ability to cure disseminated forms of the disease.[3]

These characteristics can be variably found in primary tumors at the time of an initial diagnosis and, frequently, develop with time as the tumor evolves and receives different treatments. As we cannot clearly clinically define the real "age" of a tumor at the time of diagnosis, we do not know how long that cancer genome has had the chance to evolve. Other than just time, genomic instability, highly variable among tumors, is another factor that contributes to the accumulation of molecularly defined characteristics.

As part of the evolution of the cancer genome, cells that have acquired the appropriate molecular capabilities and undergone the so called epithelial-to-mesenchymal transition process, among other enabling properties, are able to disseminate and develop metastases.[4] In parallel, they become more resistant to conventional chemotherapy. The observation that patients with treatment-resistant tumors often have highly aggressive phenotypes is consistent with the epithelial-to-mesenchymal transition contributing to drug resistance.[5]

The metastatic process is a complex and biologically defined tumor characteristic allowing a cancer cell to acquire the capacity to migrate from its site of origin, survive in the circulation and ultimately establish a metastatic focus at a distant organ. A number of molecular abilities are required for a cell to be able to complete the whole process. Not all cancer cells are able to metastasize and, while some are able to migrate and survive in the circulation for some time, not all have the full capacities to generate metastases.[6]

To some extent, the importance of treating cancer early, before significant heterogeneity develops, is supported by the idea that heterogeneity and the acquisition of molecular characteristics leading to metastasis and resistance develops over time.[7] Guideline recommendations and the effective early detection strategies applied to most cancer types remain as a clear demonstration that we are dealing with a tumor's dynamic quest for immortality.

Tumors can be intrinsically resistant to chemotherapy (primary resistance) or they can develop resistance over time – that is, secondary or acquired resistance.

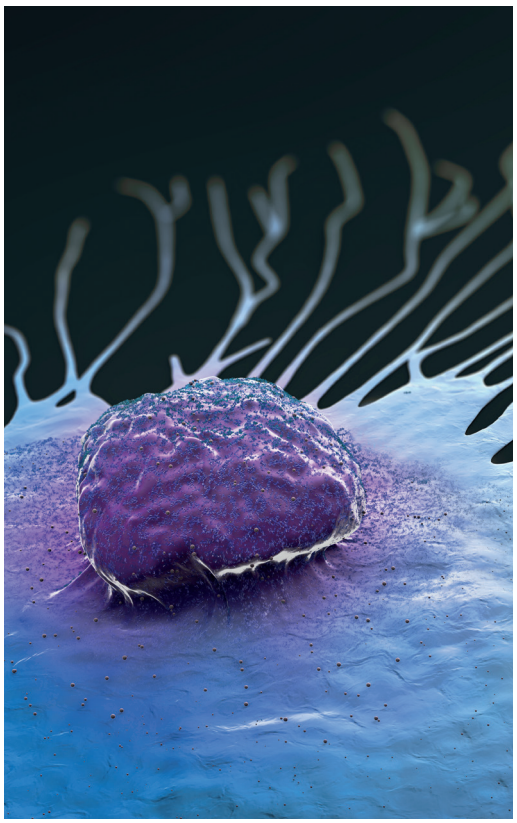
Intrinsic resistance is the result of the existence of resistant clones in the tumor present before treatment. Occasionally, only a small proportion of cells within a heterogeneous tumor may have resistance properties. With evolution, tumor progression and repeated treatments, resistant clones are selected out as treatment eliminates the co-existing sensitive clones.[8]

Acquired resistance can occur during treatment

and can be due to acquisition of molecular alterations (such as mutations), activation of bypass signalling pathways and cell lineage changes (that can occur due to tumor evolution or as a result of changes induced by treatment). After all, we should recognize that the main objective of a cancer cell is its own survival, and, as such, the cancer genome is constantly attempting to devise mechanisms that allows for continuous growth and cell division. For that, it needs to devise capabilities to counteract a sometimes-hostile microenvironment and face the different chemical and radiation strategies we impose with our treatment strategies.

Drug resistance is a complex biological process that can include increased drug transporter expression, changes in drug metabolism, enhanced DNA repair and changes in apoptotic pathways.[9] Clinical identification of resistance is a challenge and clearly has an impact on treatment selection.

Change in biomarkers induced by chemotherapy for example, can be associated with response to treatment. Change in biomarker status has been documented with neoadjuvant treatment. A change from HR+/HER2– to HR–/HER2– is associated with worse prognosis.[10] There is still incomplete information from available trials and clinical data on the optimal clinical approach to address changes in receptor status observed during treatment. Overall, it is fair to say that the phenomenon may be attributed to a manifestation of heterogeneity.[11]



GETTY

Migrating breast cancer cell

Other changes that can be induced by treatments include: triggering selection of resistant clones; inducing new mutations, genetic and chromosomal rearrangements; recovering functionality of previously inactivated genes whose potential had been exploited in synthetic lethal interactions; activating cellular dedifferentiation and trans-differentiation programs; potentiating the development of specific populations by non-cell-autonomous mechanisms.[12]

It is naïve to think about a tumor just as a collection of cancer cells. Clearly, there is a rich, dynamic and constant interaction between tumor cells and cells around them. As seen earlier in this handbook, the tumor microenvironment (TME) includes cancer cells themselves and the stroma, which is comprised of structures such as the basement membrane, extracellular matrix, vasculature, and various other types of cells (immune cells, fibroblasts, endothelial cells, microbiota, etc). The TME does not have a stable composition and is continuously changing during tumor progression, thereby influencing the response to drugs and the biological behaviour of tumors.[3] The recent development of immunotherapy, largely directed to specific antigens in immune cells and not to tumor cells, has called attention to the importance of a variety of cell populations infiltrating the TME.

The TME has been shown to influence treatment resistance.[9] Furthermore, treatment may alter the composition of the TME. Among other consequences, changes in the composition and degree of tumor immune infiltrates may have implications on the efficacy of treatments.[13] The recruitment of pro-tumor, angiogenesis-promoting macrophages can limit the effectiveness of certain therapies.[14] In a study by Caswell-Jin and colleagues, it was shown that although there was an increase in tumor-infiltrating lymphocytes (TILs) at the beginning of neoadjuvant chemotherapy in patients with HER2+ breast cancer, levels were found to have fallen at the completion of therapy.[15]

Heterogeneity in tumors can also result in variation of the genomic and transcriptomic profile of cancer cells, thus resulting in differences in the pharmacokinetics of chemotherapeutic agents and leading to differences in drug distribution.[16] Phase I clinical trials demonstrate a wide variation in pharmacokinetic parameters between patients, which could partially explain the significant individual differences in therapeutic responses and / or toxicities seen with individual drugs. This is particularly pertinent with antineoplastic drugs because of their `narrow therapeutic window'. The effects of differences in age, sex and bodyweight have long been known to impact factors such as absorption, distribution, >

metabolism and excretion of drugs. The impact of genetic variability is a more recent focus.[16] One theory, for example, is that tumors can alter the systemic distribution of antitumor drugs by releasing soluble factors with high binding affinity.[16] Differences in drug distribution between the primary tumor and metastases may be the consequence of several factors. One of the most likely possibilities is differences in perfusion – larger tumor masses are often more poorly perfused causing necrotic regions, compared with small tumors or metastases that tend to have more regular vascularization.[16] Differences in blood supply may also explain – particularly for drugs that depend on blood circulation for their delivery – intratumor variation in drug distribution. Differential movement across cell membranes, sequestration and the extent to which drugs are bound intracellularly, may also have an impact.[16] Furthermore, heterogeneity in drug distribution is important because resistant cancer cell phenotypes may develop in regions with poor or restricted drug penetration.[16]

Implications for precision medicine

Unquestionably, patient selection strategies and our ability of setting apart different subgroups of patients each requiring specific therapeutic strategies represents the most important and revolutionary advance in cancer care in the last two decades. However, and paradoxically, tumor heterogeneity is perhaps one of the greatest barriers to personalized or precision medicine, where treatment aims to address specific molecular abnormalities or differences from one individual to another.[17] In view of tumor heterogeneity, cancer cells can be seen as dynamic moving targets.[18]

Importantly, the response to a targeted therapy is determined among many other factors by the heterogeneity in a driver-gene alteration – be it intratumoral, intermetastatic or intrametastatic. A clone is likely to grow if it does not have the driver-gene mutation being targeted by a particular treatment, exemplifying the possibility of outgrowing a pre-existent resistant clone.

In different tumor types, genomic testing can variably inform optimal therapy by identifying driver alterations. In certain tumor types such as NSCLC, the identification of different driver abnormalities has led to dramatic changes in the treatment of patient subgroups.[19] In other cases, agnostic markers such as microsatellite instability have resulted in revolutionary outcomes for patients treated with immunotherapies.[20]

In breast cancer, genomic changes in genes such as ERBB2, PIK3CA, AKT1, ESR1 and NTRK can indicate targeted strategies.[8] For example, chemotherapy combined with HER2-targeted agents is the best treatment for 20–25% of patients with breast cancer who have tumor cells that overexpress HER2 or have amplification of ERBB2.[8] Heterogeneity of receptor expression has been identified as a resistance mechanism and has been associated with worse prognosis.[8]

The recent development of antibody-drug conjugates with the ability of releasing a highly toxic payload and impacting neighbouring cells through the so called “bystander effect” represents a revolution in our treatment approach to breast cancer patients and suggests an alternative to address heterogeneous populations of cells, some of which may not express high levels of a specific target-antigen.[21]

It is important to note that recent research suggests that cells in the tumor microenvironment may be significant for predicting response to treatment. For example, HER2+ carcinomas and triple negative breast cancers (TNBCs) have the highest levels of immune cell infiltrate and are sometimes considered immune-enriched –referred to as ‘hot’ tumors; in contrast to luminal carcinomas with few immune cells – so-called ‘cold’ tumors. Although it remains unclear the exact threshold to call a highly infiltrated tumor, TILs are nevertheless thought to be a good prognostic indicator and are beginning to be included in clinical diagnosis algorithms. Comparatively, immunoregulatory cells such as Tregs and tumour-associated macrophages have been associated with poor prognosis.[22] Characterization of not only the presence of an immune infiltrate, but also the nature of the immune cells, represents an area of important and intense research.

Implications for detection, prognosis and assessment

Heterogeneity in breast cancer and the increased ability of tumors to adapt to constantly changing constraints poses a significant challenge to diagnosis.[5]

A single tumor biopsy provides only a snapshot of the complete anatomy of a tumor and of its history in time.[10] It does not necessarily give a complete picture of its clonal composition. A biopsy of the primary tumor does not necessarily indicate the characteristics of metastases as they too can differ in a variety of respects from the primary.[12]

Biopsy of metastases is valuable in relation to molecular evaluation and obviously has an impact in precision medicine. However, it may not be practical when the metastases are not easily accessible or when a patient has multiple organs with metastases.[23] The recent development of technologies that allow the detection of circulating tumor DNA (ctDNA) and circulating tumor cells (CTC), does provide a more accurate assessment of the temporal and spatial heterogeneity of cancer.[10] The need for continual monitoring to keep track of the evolution of tumors will no doubt require more non-invasive diagnostic techniques to make the process

practically feasible. Liquid biopsy is a promising development destined to revolutionize our classification of patients. For example, an early disease breast cancer patient following primary surgery will be either ctDNA positive or negative, and this could have prognostic and therapeutic implications. The same reasoning can be applied to a patient with metastatic disease who is responding to treatment.[24]

Some evidence shows that intratumoral heterogeneity may be a useful clinical prognostic indicator. Yu and colleagues analysed data from 21 studies involving 9804 patients with solid tumors, including breast cancer.[25] They found that overall survival time was shorter [hazard ratio (HR) 1.65; 95% CI 1.42–1.91] in cases with high intratumoral heterogeneity. A similar relationship exists for progression-free survival [HR 1.89; 95% CI 1.41–2.54] and disease-specific survival [HR 1.87; 95% CI 1.15–3.04].[25]

Among some of the other useful techniques that can help us deal with heterogeneity, is positron emission tomography. It relies on the uptake of radiotracers and has been validated in the assessment of estrogen receptor expression in breast cancer tumors.[26] This information can be invaluable in decision making regarding endocrine therapy with potential impact on diagnosis, treatment selection, response evaluation and follow up. It is also thought that it could be used to analyse intratumoral and interlesional heterogeneity in patients with cancer in general using different biomarkers.[27] Indeed the field of radiomics in which high quality images from mammography, ultrasound, magnetic resonance imaging and PET are collated and analysed to produce a three-dimensional representation of an area of interest is an active field of investigation. The imaging information can then be combined with clinical and genomic data, which can subsequently be interrogated using artificial intelligence, machine learning techniques or statistical analysis to help improve diagnostic accuracy of breast imaging, for example.[28]

Conclusion

Cancer is heterogenous by its very nature. This poses a challenge to treatment because the evolving nature of the disease and the variation in the characteristics of the tumors themselves means it is difficult to envision a one-size fits all treatment. Looking back to our previous successes with chemotherapy, treatment regimens were generally associated with combination strategies where we used agents with different mechanisms of action, an initial recognition of the complex and heterogeneous nature of cancer. At our current stage of development, and moving forward, greater understanding and even more sophisticated diagnostic and monitoring tools are needed to allow clinicians to optimize management strategies tailored to this challenging evolving biology.

References

1 Schmelz K et al. Spatial and temporal intratumour heterogeneity has potential consequences for single biopsy-based neuroblastoma treatment decisions. Nat Commun 2021;12:6804.
2 Munnik C et al. Drosophila melanogaster: A platform for anticancer drug discovery and personalized therapies. Front Genet 2022;13:949241.
3 Zhang A et al. Tumor heterogeneity reshapes the tumor microenvironment to influence drug resistance. Int J Biol Sci 2022;18(7):3019–33.
4 Yang J et al. Guidelines and definitions for research on epithelial–mesenchymal transition. Nat Rev Mol Cell Biol 2020;21:341–352.
5 Lüönd F, Tiede S, Christofori G. Breast cancer as an example of tumour heterogeneity and tumour cell plasticity during malignant progression. Br J Cancer 2021;125(2):164–75.
6 Fares J et al. Molecular principles of metastases: a hallmark of cancer revisited. Sign Tranduc Targeted Ther 2020;5:28
7 Janku F. Tumor heterogeneity in the clinic: is it a real problem? Ther Adv Med Oncol 2014;6(2):43–51.
8 Baliu-Piqué M, Pandiella A, Ocana A. Breast Cancer Heterogeneity and Response to Novel Therapeutics. Cancers (Basel). 2020 Nov 5;12(11):3271.
9 Dzobo K et al. Not Everyone Fits the Mold: Intratumor and Intertumor Heterogeneity and Innovative Cancer Drug Design and Development. OMICS 2018;22(1):17–34.
10 Fumagalli C, Barberis M. Breast Cancer Heterogeneity. Diagnostics (Basel) 2021;11(9):1555.
11 Thompson AM et al. Prospective comparison of switches in biomarker status between primary and recurrent breast cancer: the Breast Recurrence In Tissues Study (BRITS). Breast Cancer Res 2010;12:R92.
12 Ramón Y Cajal S et al. Clinical implications of intratumor heterogeneity: challenges and opportunities. J Mol Med (Berl) 2020;98(2):161–77.
13 Salgado R et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol 2015;26:259-71.
14 Mantovani A et al. Macrophages as tools and targets in cancer therapy. Nat Rev 2022;21.
15 Caswell-Jin JL, Lorenz C, Curtis C. Molecular heterogeneity and the evolution of breast cancer. Annu Rev Cancer Biol 2021;5:79–94.
16 Garattini S, Fuso Nerini I, D'Incalci M. Not only tumor but also therapy heterogeneity. Ann Oncol 2018;29(1):13–19.
17 De Lartigue J. Tumor heterogeneity: a central foe in the war on cancer. J Community Support Oncol 2018;16(3):e167–e174.
18 Jacquemin V et al. Dynamic Cancer Cell Heterogeneity: Diagnostic and Therapeutic Implications. Cancers (Basel) 2022;14(2):280.
19 Li T et al. Genotyping and Genomic Profiling of Non-Small-Cell Lung Cancer: Implications for Current and Future Therapies. J Clin Oncol 2013;31:1039-49.
20 Weis L et al. Tissue agnostic drug approvals: how does this apply to patients with breast cancer? npj Breast Cancer 2021;7:120.
21 Ferraro E et al. Implementing antibody-drug conjugates (ADCs) in HER2-positive breast cancer: state of the art and future directions. Breast Cancer Research 2021;23:84.
22 Perrone M et al. Impact of Immune Cell Heterogeneity on HER2+ Breast Cancer Prognosis and Response to Therapy. Cancers (Basel) 2021;13(24):6352.
23 Kutasovic JR et al. Morphologic and Genomic Heterogeneity in the Evolution and Progression of Breast Cancer. Cancers (Basel) 2020;12(4):848.
24 Sanz Garcia E et al. Monitoring and adapting cancer treatment using circulating tumor DNA kinetics: Current research, opportunities, and challenges. Sci Adv 2022;8:1-14.
25 Yu T et al. Intratumor Heterogeneity as a Prognostic Factor in Solid Tumors: A Systematic Review and Meta-Analysis. Front Oncol 2021;11:744064.
26 Edmonds C et al. Novel applications of molecular imaging to guide breast cancer therapy. Cancer Imaging 2022;22:31.
27 Bailly C et al. Exploring Tumor Heterogeneity Using PET Imaging: The Big Picture. Cancers (Basel) 2019;11(9):1282.
28 Guo L et al Breast cancer heterogeneity and its implication in personalized precision therapy. Exp Hematol Oncol 2023;12(1):3.

Conclusion

Adam Brufsky MD PhD
Professor of Medicine and Co-Director, Comprehensive Breast Cancer Center, UPMC Hillman Cancer Center, University of Pittsburgh, Pittsburgh, PA, USA

In this handbook, our distinguished panel of authors has reviewed the concept of heterogeneity and described how it manifests in different types of cancer. They have discussed the emerging picture of the way the complex and wide variation of some of the characteristics of tumor cells manifests in breast cancer, in particular, and some of the factors that influence these phenomena. The impact of heterogeneity on diagnosis and management and the implications for future management have also been discussed.

Cancer is intrinsically heterogenous and diagnostic tests to detect heterogeneity and therefore inform treatment decisions are crucial in the management of a number of cancers in the era of personalized medicine.

For example, three biomarkers are used to categorize breast cancer based on its histology and immunohistochemical (IHC) expression: the estrogen receptor (ER); progesterone receptor (PR); and human epidermal growth factor receptor 2 (HER2). These subtypes not only have different prognoses but also require distinct treatment strategies.

However, the expression of these markers can change during the natural history of cancer, for example, between initial diagnosis and metastatic relapse or successive progression – a phenomenon known as phenotypic discordance. The detection of any discordance in the expression of markers is crucial for managing patients with metastatic breast cancer because the efficacy of personalized treatments relies heavily on the dynamic changes of these markers over time. That is why the latest recommendations strongly advocate for performing a biopsy at presentation or first recurrence of metastatic lesions.

Successive biopsies during metastatic disease are also recommended in case of abnormal progression, such as primary resistance to treatment or acceleration of tumor growth, to detect potential discordance and adapt treatment accordingly.

However, repeat biopsies have some limitations. They are invasive, costly, and can be painful for the patient. In addition, repeat biopsies are not risk-free, as they may cause serious adverse events. And it is as yet unclear what the impact of phenotypic discordance is on patient survival. The loss of hormone receptors has been identified as a poor prognostic factor associated with poorer overall survival. Loss of ER or PR alone has also been linked to worse survival. By contrast, the gain of hormone receptors did not appear to affect survival. Similarly, no difference in overall survival was observed in patients with discordant HER2 status (loss or gain). However, it should be noted that these findings may not consider the impact of therapeutic management changes resulting from the change in cancer status.

So the picture is complex and our understanding of heterogeneity is at an early stage. As a result our understanding of a patient's cancer, oftentimes based on a biopsy or excision, equates to a mere glimpse into a portion of one lesion at one time point. These variables translate to many things we do not know about the full picture of a patient's cancer. Blood-based 'liquid' biopsies can help some but do not eliminate this problem. Ultimately, temporal and spatial heterogeneity add to the complexity of the disease.

While localized forms of cancer can be effectively managed by available local therapies, the dissemination of cancer cells and the development of drug resistance are the main reasons for treatment failure and the main barriers compromising our ability to cure disseminated forms of the disease.

The challenge for researchers will be to develop a deeper understanding of heterogeneity, the factors that drive it and its impact on prognosis to inform the development of new treatments and strategies that clinicians can employ to continue improving patient outcomes.

Content Director, Secondary Care
Andrea Porter

Design/artwork support
Steve Powell

Director Secondary Care
David Ling

Executive Director
Edward Burkle

 Published in the United Kingdom by Cogora Limited, 1 Giltspur Street, London EC1A 9DD, UK.