Chapter 6
Breast Cancer Heterogeneity in Primary and Metastatic Disease

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Abstract Breast cancer encompasses a heterogeneous collection of neoplasms with diverse morphologies, molecular phenotypes, responses to therapy, probabilities of relapse and overall survival. Traditional histopathological classification aims to categorise tumours into subgroups to inform clinical management decisions, but the diversity within these subgroups remains considerable. Application of massively parallel sequencing technologies in breast cancer research has revealed the true depth of variability in terms of the genetic, phenotypic, cellular and microenvironmental constitution of individual tumours, with the realisation that each tumour is exquisitely unique. This poses great challenges in predicting the development of drug resistance, and treating metastatic disease. Central to achieving fully personalised clinical management is translating new insights on breast cancer heterogeneity into the clinical setting, to evolve the taxonomy of breast cancer and improve risk stratification.

Keywords Breast cancer classification · Breast cancer molecular subtypes · Clonal evolution · Heterogeneity · Metastasis · Pathology

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6.1 Introduction

Complexity pervades breast tumours at every level – from (epi)genomic, transcriptomic and proteomic landscapes, through to cellular composition and clinical behaviour. This lack of compositional uniformity is referred to as heterogeneity. In breast cancer, this has been historically categorised as intertumoural heterogeneity (diversity between separate tumours) and intratumoural heterogeneity (diversity within a tumour). However, the distinction is becoming increasingly blurred as we understand more about the pathobiology of breast cancer progression. While a single cell acquires the somatic mutations sufficient to launch oncogenic transformation, the cells that eventually comprise clinically detectable deposits arise from clonal selection and expansion as a consequence of a range of different selection pressures, and this has important implications for diagnosis, treatment and drug resistance.

Molecular confirmation of breast cancer heterogeneity has been driven by unparalleled expansion of next generation sequencing technologies over the last decade, with advances in tumour profiling also evolving the traditional taxonomy. Categorising breast tumours into diagnostic and prognostic groups has always been the basis for clinical management, but the *fully* personalised model we are striving for will feature an unprecedented level of precision, matching each patient with the best possible treatments according to specific molecular alterations underpinning their disease. Navigating and rationalising the exponentially growing wealth of new molecular information remains a major challenge to clinical translation.

This chapter will consider the traditional histopathologic classification of breast cancer, broadly examine the molecular basis of genetic, cellular and microenvironment heterogeneity and the ways in which new knowledge is being integrated to complement the existing taxonomy. Finally, we examine the impact of breast cancer heterogeneity on clinical management and translation of emerging research.

6.2 Current Histopathologic Classification of Breast Cancer

6.2.1 Histological Subtypes

Breast carcinoma encompasses a large group of tumours with different morphological, phenotypic and molecular characteristics, and the current classification includes a spectrum of in situ (pre-invasive) to invasive disease. This chapter focuses on invasive disease, where tumour cells breach the basement membrane and invade surrounding tissue, although there is increasing recognition of heterogeneity within in situ carcinoma [1]. The World Health Organisation (WHO) maintains a diagnostic framework that provides practical information to guide tumour diagnosis and patient management (Table 6.1) [2, 3]. Invasive cancers are initially stratified according to

Table 6.1 WHO classification of breast carcinoma (2012)

	Histological type	Frequency
1	Invasive carcinoma of no special type (IC-NST) *Includes: pleomorphic carcinoma, carcinoma with osteoclast-like stromal giant cells, carcinoma with choriocarcinomatous features, carcinoma with melanotic features*	40–75%
2	Invasive lobular carcinoma (ILC) *Includes: classic, solid, alveolar, pleomorphic, tubulolobular, mixed subtypes*	5–15%
3	Tubular carcinoma	2%
4	Cribriform carcinoma	0.3–0.8% (up to 4%)
5	Mucinous carcinoma	2%
6	Carcinoma with medullary features *Includes: medullary carcinoma, atypical medullary carcinoma, IC-NST with medullary features*	<1%
7	Carcinoma with apocrine differentiation	4%
8	Carcinoma with signet ring cell differentiation	<1%
9	Invasive micropapillary carcinoma	0.9–2%
10	Metaplastic carcinoma of no special type *Includes: low-grade adenosquamous carcinoma, fibromatosis-like metasplastic carcinoma, squamous cell carcinoma, spindle cell carcinoma, metasplastic carcinoma with mesenchymal differentiation (chondroid, osseuous, other types), mixed metaplastic carcinoma, myoepithelial carcinoma*	0.2–5%
11	Carcinoma with neuroendocrine features *Includes: well differentiated neuroendocrine tumour, poorly differentiated neuroendocrine tumour (small cell carcinoma), carcinoma with neuroendocrine differentiation*	<1%
12	Secretory carcinoma	<0.15%
13	Invasive papillary carcinoma	<1%
14	Acinic cell carcinoma	<1%
15	Mucoepidermoid carinoma	0.3%
16	Polymorphous carcinoma	<1%
17	Oncocytic carcinoma	<1%
18	Lipid-rich carcinoma	<1–1.6%
19	Glycogen rich clear cell carcinoma	1–3%
20	Sebaceous carcinoma	<1%
21	Adenomyoepithelioma with carcinoma	<1%
22	Adenoid cystic carcinoma	<0.1%
23	Encapsulated papillary carcinoma	<2%
24	Invasive solid papillary carcinoma	<1%

cellular and architectural growth patterns, into histological ‘special types’ with distinct morphology (25–30% of cases, including 5–15% lobular carcinomas; for examples see Fig. 6.1a, b). As a diagnosis of exclusion, tumours without discriminating morphological features are classified as invasive carcinoma of no special type (IC-NST; 40–75% of cases) [3]. Whilst this distinction appears straightforward,

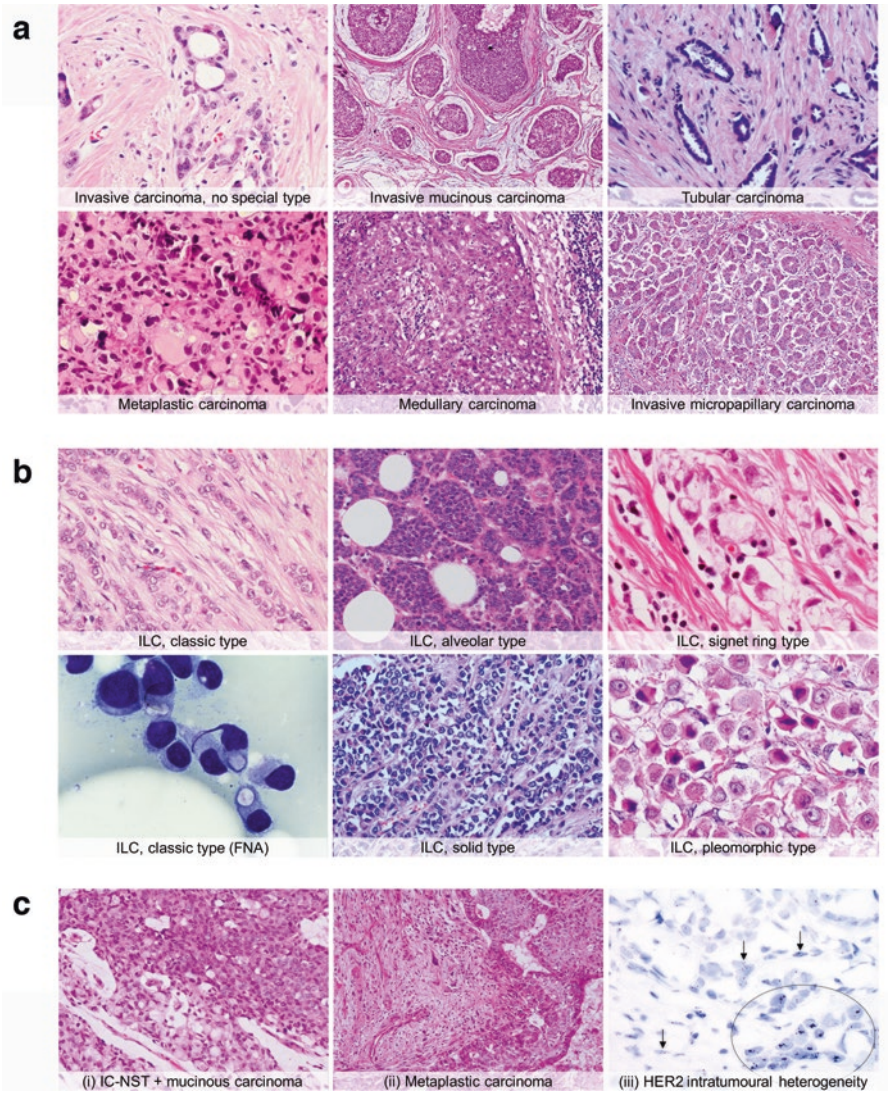

Fig. 6.1 (**a**) Breast cancer heterogeneity exemplified by histological subtypes. Haematoxylin and eosin-stained breast cancer tissues visualised by light microscopy. (**b**) Morphological variation within one histological type, invasive lobular carcinoma (ILC). *FNA, fine needle aspiration cytology.* (**c**) Mixed histological subtypes: (i) Invasive carcinoma (no special type) mixed with invasive mucinous carcinoma; (ii) metaplastic carcinoma exhibiting marked variability in both epithelial and stromal compartments; (iii) intratumoural heterogeneity for HER2 shown by silver *in situ* hybridisation (SISH) – a nest of cells exhibits *ERBB2* gene amplification (circled), while others remain diploid (arrows)

many tumours comprise mixed histology (Fig. 6.1c) – thresholds are used to definitively categorise individual cases, though these are somewhat arbitrary. For example, a pure 'special type' diagnosis is applied if >90% of the tumour area comprises the special morphology, but in 'mixed' cases, separate areas within the same tumour exhibit both 'non-special' and 'special' morphology (10–49% [2]).

In some instances, tumours with distinct morphological features share underlying genetic mutations [4]. Secretory carcinomas are associated with a t(12;15)(p13;q25) translocation and the resulting *ETV6-NTRK3* fusion gene [5]; and, like their counterpart in the salivary gland, adenoid cystic breast carcinomas consistently harbour the t(6;9)(q22–23;p23–24) translocation, leading to *MYB-NFIB* gene fusion and over-expression of the *MYB* oncogene [6]. Genotype-phenotype correlation is epitomised by E-cadherin, which is genomically 'lost' or dysregulated in lobular carcinomas, and tends to occur concomitantly with specific mutations in *PTEN*, *TBX3* and *FOXA1* [7, 8]. However, even subtypes with shared morphological features and mutations exhibit substantial inter-tumoural diversity. For example, within lobular carcinoma, the largest group of special types, genomic and transcriptomic analysis highlighted the existence of distinct prognostic subtypes [8]. Thus overall, histological subtyping alone provides imperfect prognostic information – its value comes from integration with other histopathologic information, namely grade, stage and biomarker status.

6.2.2 Prognostic and Predictive Subgroups

Histopathologic assessment routinely involves quantification of prognostic factors, which predict the natural history of disease irrespective of therapy, and predictive factors, which indicate the likely response to a specific treatment. The disease stage, histological grade and tumour expression of receptors for oestrogen, progesterone and human epidermal growth factor (ER, PR and HER2) are the cornerstones of current prognostic and predictive algorithms. The American Joint Committee on Cancer TNM (tumour/node/metastasis) staging system stratifies broadly based on the burden of the disease by measuring the tumour size, the number of lymph nodes involved, and the extent of distant metastatic disease; stage IV is the most advanced disease, while stage I is the least advanced.

Histological grade is a powerful prognostic indicator [9, 10] and correlates with morphology and molecular features [11]. It is calculated from the degree of nuclear pleomorphism, 'tubule' formation (resemblance to normal ducto-lobular gland structure) and the number of mitoses per ten high power microscope fields [12]. Grading reflects a collective morphological assessment of the biological characteristics of a tumour and therefore encompasses intra-tumoural heterogeneity. It is highly reproducible, and remains a component of widely used prognostic algorithms (e.g. Nottingham and Kalmar Prognostic Indices [13–15]), as well as predictive algorithms used to guide the prescription of chemotherapy [16, 17]. Pathologists

have been describing heterogeneity for decades, but given that clinical behaviour is still diverse within these three broad categories, so there is much scope for grading to be complemented by molecular information.

Breast cancers are routinely analysed for ER, PR and HER2 using IHC-based assessment of protein expression levels and frequency. This information is both prognostic and predictive, reflecting critical growth factor signalling dependencies that can be targeted for therapeutic benefit. PR is induced by oestrogen signalling (thus is a surrogate for ER activity), and adds value to the power of ER for predicting response to therapy [18, 19]. ER/PR-positive tumours tend to be lower grade and associated with better outcomes than ER/PR-negative cases, and are candidates for endocrine therapy (*e.g.* tamoxifen, fulvestrant, aromatase inhibitors). The gene encoding HER2 (*ERBB2*) is amplified and/or over-expressed in 15–20% of invasive breast cancers, and correlates with poor prognosis but is also a marker of sensitivity to HER2-targeted therapy (standardly trastuzumab and pertuzumab with chemotherapy) [20–24]. Tumours that are negative for ER/PR and HER2 are currently classified as triple negative breast cancers (TNBC), where there are intensive research efforts ongoing to substratify molecularly distinct subgroups that could be suitable for new therapeutic approaches targeting antitumour host immunity, DNA repair and/or specific signalling pathways [25] (see Sect. 6.4.2).

Importantly, the expression of ER/PR and HER2 is not always uniform, implying that not all tumour cells are dependent on their growth factor ligands. ER/PR-positivity is currently defined by a diagnostic threshold of only 1% [26]. Testing to define HER2 status is based on either protein over-expression as demonstrated by IHC and/or testing for gene amplification. Criteria for gene amplification are based on *ERBB2* copy number or *ERBB2:CEP17* ratio, determined using *in situ* hybridisation (ISH). If the results are equivocal, orthogonal testing is recommended [27]. Whilst conservative cut-offs ensure patients are eligible for treatments that may confer marginal benefit, heterogeneity undoubtedly impacts the clinical response. For example, HER2 heterogeneity is related to low levels of gene amplification, which is more common in ER/PR-positive tumours and associated with shorter disease-free survival [28–30] (Fig. 6.1c).

6.3 Molecular Basis for Heterogeneity in Breast Cancer

The molecular basis for heterogeneity can be divided into tumour cell-intrinsic factors, such as genomic alterations, and their impact on pre-existing differentiation programs in the cell-of-origin; as well as extrinsic factors in the tumour microenvironment. However this division is purely for academic purposes – in reality the tumour and nontumour components are admixed and constantly engaged in feedback signalling [31, 32].

6.3.1 Genetic Heterogeneity

Tumourigenesis occurs by inappropriate expansion of genetically altered clones (groups of isogenic tumour cells derived from a common ancestor; Fig. 6.2, inset) via branching evolution, where the acquisition of a new genetic alteration in a multipotent cell capable of self-renewal represents an evolutionary branching point, and the initiation of a new 'subclone' (Fig. 6.2). Mutations that confer a selective

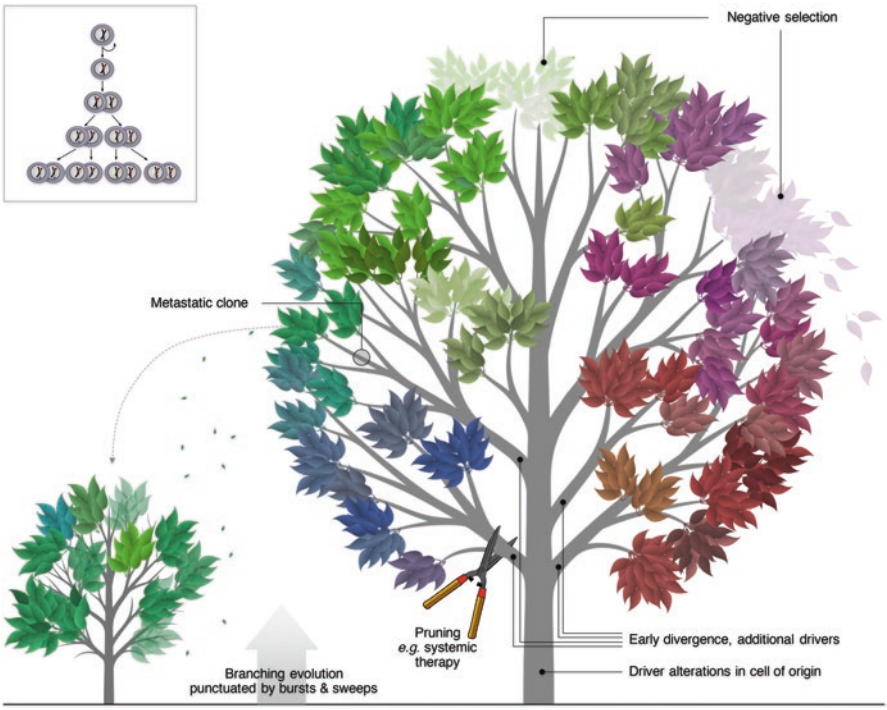

Fig. 6.2 The branching phylogenetic tree analogy of clonal progression, where major branchpoints represent the acquisition of each new driver, and distance from the ground is proportional to divergence from the original founding clone. Coloured bunches of leaves represent major subclones with the same driver combinations, with individual leaves as minor subclones harbouring additional genomic alterations and phenotypic differences (see Fig. 6.3). Evidence suggests that clonal evolution occurs intermittently, with essential fitness advantages acquired at the earliest stages of tumourigenesis in short mutational bursts (often copy-number alterations), followed by stable clonal expansions that form the bulk of the tumour. Selective clonal 'sweeps' may also occur in response to new extrinsic selection pressure (e.g. systemic agents that prune particular subclones). Circulating tumour cell seeds are shed into the blood and lymphatics – those with the requisite capabilities may colonise suitable soil in distant organs, where the branching evolution process continues. **(inset)** Classic clonal expansion, where genetic diversity arises through iterative rounds of somatic mutation (coloured dots) and cell division (arrows), and heritable alterations are passed to daughter cells. *Curved arrow = self-renewal of a cancer stem cell at the top of the clonal hierarchy*

advantage to a clone in its particular microenvironment are referred to as ‘drivers’, while those that do not immediately confer a selective advantage are ‘passengers’. They can be distinguished using algorithms that calculate the rates of non-synonymous *vs* synonymous mutation in each gene, non-random clustering of mutations and/or gene amplification combined with over-expression, which imply positive evolutionary selection [33–35].

Historically, heterogeneity has been considered a byproduct of classical Darwinian evolution, where *de novo* mutations conferring a fitness advantage result in rapid expansion and positive selection of the new clone at the expense of others, resulting in its mutation profile dominating a whole region of the tumour (a so-called ‘clonal sweep’) [36]. This model implies that tumour cells sustain mutations at a fairly constant tempo, but recent studies suggest that tumourigenesis can be driven by just a few major expansion events followed by long periods of relative evolutionary stasis, challenging gradualistic clonal expansion dogma [37–42]. By sequencing multiple single cells from a tumour, several groups have now found evidence for so-called ‘big-bang’ dynamics in breast cancer, where critical copy-number alterations are thought to occur as early ‘bursts’, superimposed with cumulative point mutations that contribute to the genetic diversity observed in tumour biopsies [41, 43]. These detailed studies have given weight to the more progressive ‘punctuated’ model of evolution – a hybrid of the big-bang and classic clonal expansion paradigms where evolutionary tempos are sporadic.

Large international consortia (The Cancer Genome Atlas (TCGA [44]) and the International Cancer Genome Consortium (ICGC [45, 46])) have made significant inroads characterising the genomic diversity of breast cancer using next-generation sequencing of RNA and DNA from human clinical samples (Table 6.2). An initial survey of 100 tumours identified at least 40 different genes harbouring driver mutations, but these were present in over 70 different combinations [46], with each occurring in less than 10% of tumours [47, 48]. Due to the low overall frequencies of driver mutations, larger cohorts have been required to confirm recurrent alterations. More recently, a landmark whole genome sequencing study using the largest cohort to date (n = 560), identified 93 protein-coding genes as probable drivers, including five with no previously described link to breast cancer (*MED23*, *FOXP1*, *MLLT4*, *XBP1*, *ZFP36L1*) [49]. Whilst almost all the tumours harboured at least one driver, no two tumours out of 560 shared the same combination. Some of the more frequent changes identified include *ERRB2, CCND1, AKT1* (amplified and over-expressed), *PIK3CA*, *GATA3* (amplified and overexpressed and/or activating mutation), *TP53*, *PTEN* and *CDH1* (copy-number loss or inactivating mutation leading to functional insufficiency) [44, 46, 48, 50, 51]. It is thought that breast cancer driver mutations occurring with a frequency of greater than 2% are now known, but it is expected that additional, low frequency drivers are still likely to be found in minor subgroups (e.g. male breast cancer, histological special types) [52]. The particular combination of driver alterations, together with thousands of passenger mutations and structural rearrangements make each breast cancer unique [49, 53, 54]. In general, high levels of genomic heterogeneity tend to be associated with worse clinical outcomes [55].

Table 6.2 Landmark next generation sequencing studies in breast cancer

Study	Year	Cohort size	Approach	Key findings
Shah [156]	2009	1	WGS, RNASeq	First to apply WGS to a matched pair of primary and metastasis 5/32 somatic non-synonymous coding mtuations identified in the metastasis were detected in the primary lobular breast tumour diagnosed 9 years earlier, 6/32 were at low frequency in the primary
Curtis, METABRIC [50]	2012	1992	CNA, GEX	Integration of CNA and GEX data derived 10 molecular subgroups called 'integrative clusters' with distinct clinical outcomes Groups include a high-risk, ER-positive 11q13/14 subgroup and a favourable prognosis subgroup devoid of CNAs Identified *PPP2R2A*, *MTAP* and *MAP2K4* as putative cancer genes
Shah [51]	2012	104	aCGH, WES, WGS, RNASeq,	Basal-like tumours show greater variation in mutations than non-basal TNBC *TP53*, *PIK3CA* and *PTEN* somatic mutations are clonally fominant but in some cases are inconsistent with founder status Mutations in cell shape, cytoskeleton and motitilty genes tend to occur later in tumour progression
TCGA [44]	2012	466	CNA, GEX, methylation, microRNAseq, RPPA, WES	Only *TP53*, *PIK3CA* and *PTEN* somatic mutations occurred in >10% of samples Enrichment of *GATA3*, *PIK3CA* and *MAP3K1* mtuations in luminal tumours Identified two novel protein expression defined subgroups related to microenvironment Molecular commonalities between basal-like tumours and serous ovarian cancers
Stephens [46]	2012	100	CNA, WES	Correlations between number of mutations, age of cancer diagnosis and histological grade Somatic driver point mutations and/or copy number changes were identified in over 40 cancer genes in 73 combinations Maximum of 6 mutated driver genes in a single tumour; 28 tumours showed only 1 driver mutation *TP53, PIK3CA, ERRB2, MYC, FGFR1/ ZNF703, GATA3* and *CCND1* were mutated in >10% of cancers (58% of the driver mutations); remaining 33 mutated cancer genes contributed to the other 42% of driving genetic events 9 new candidate driver genes: *AKT2, ARIDIB, CASP8, CDKN1B, MAP3K1, MAP3K13, NCOR1, SMARCD1*, and *TBX3*.

(continued)

Table 6.2 (continued)

Study	Year	Cohort size	Approach	Key findings
Banerji [48]	2012	103 + 22	WES (103), WGS (22)	Confirmed recurrent mutations *PIK3CA, TP53, AKT1, GATA3*, and *MAP3K1* driver mutations Novel mutation *in CBFB* and deletions in *RUNX1* Recurrent *MAGI3-AKT3* fusion enriched in TNBC; leads to activation of AKT kinase
Ellis [172]	2012	77	WES (31), WGS (46)	Biopsies of ER+ tumours from two neo-adjuvant aromatase inhibitor trials were assessed to elucidate biomarkers of response Distinct phenotypes in ER-positive tumours are driven by specific patterns of somatic alteration: *GATA3* – luminal A, low grade, low prolif.; *TP53* – non-luminal A, high grade, high prolif *GATA3* mutations correlated with a treatment-related suppression of proliferation
Wang [41]	2014	2 cases	CNA, nucSeq, WGS, WES	Developed nucSeq approach for single cell sequencing Population and single nuclei sequencing approach in an ER positive tumour and a TNBC No 2 single tumour cells are genetically identical Large numbers of subclonal and de novo mutations
Yates [36]	2015	303 tumours, 50 patients	Targeted sequencing WGS	12 treatment-naive tumours with spatially heterogeneous subclones; all tumours showed at least one clonal driver 4 multi-focal cancers with 2–5 foci; individual foci clonally related but had private mutations suggestive of clonal sweeps Created 'index of heterogeneity', which correlated with age at diagnosis and larger tumour size; no correlation with histology, grade, ER status, intra-tumoral lymphocytes or Ki67 No specific temporal pattern observed – mutations in common breast cancer genes (*PIK3CA, TP53, PTEN, BRACA2* and *MYC*) occurred early in some tumours and late in others
Ciriello [8]	2015	817	CNA, methylation, RPPA, WES	E-cadherin loss and mutations in *PTEN, TBX3* and *FOXA1* in ILC; conversely luminal A IC-NST had mutations in *GATA3* Identified 3 mRNA derived prognostic subgroups of ILC: immune-related, proliferation and reactive-like

Table 6.2 (continued)

Study	Year	Cohort size	Approach	Key findings
Nik-Zainal [49]	2016	560	WGS; mutational signature analysis	Defined 93 protein-coding genes with probable driver mutations (31 dominant, 60 recessive, 2 uncertain) 5 new cancer genes described (*MED23*, *FOXP1*, *MLLT4*, *XBP1*, *ZFP36L1)* At least 1 driver mutation in >95% of cancers 10 most frequently mutated (62% of drivers): *TP53, PIK3CA, MYC, CCND1, PTEN, ERBB2, ZNF703/FGFR1, GATA3, RB1, MAP3KI* Characterised mutational signatures: 12 base substitutions and 6 rearrangement signatures Specific mutational signatures associated with *BRCA1/2* alterations
Smid [60]	2016	266	RNASeq, meta-analysis of WGS	In luminal tumours, mutation burden correlates directly with adverse outcome Signatures 3 and 13 associated with immune response, increased TILs and better outcomes Specific substitutions more effective in eliciting an immune response than sheer number
Periera [55]	2016	2433	CNA, GEX, targeted sequencing, WES	Assessed intra-tumorual heterogeneity using mutant-allel fractions 40 mutation-driver genes (6/40 oncogenes; 8/40 tumour suppressor genes); most common *PIK3CA* (40.1%) and *TP53* (35.4%) Five genes with coding mutations in >10% samples: *MUC16* (16%), *AHNAK2* (16.2%), *SYNE1* (12%), *KMT2C* (11.4%), *GATA3* (11.1%) *PIK3CA* in lower grade ER+ tumours (associated with reduced survival in 3 subgroups); *TP53* in higher grade tumours, and only associated with worse outcome in ER+ tumours Difference in mutation frequency based on HER status: *TP53* ER-/HER2+ (67.5%) vs ER+/HER2+ (42.6%) 42.5% of tumours had a mutation in the Akt pathway (*PIKC3A, AKT1, PIK3R1, PTEN, FOXO3*) Mutations associated with longer (ER+ *MAP3K1*, *GATA3*) vs shorter (*SMAD4, USP9X*) survival

aCGH array comparative genomic hybridisation, *CNA* copy number aberration, usually SNP-based, *GEX* gene expression profiling, array-based, *METABRIC* Molecular Taxonomy of Breast Cancer International Consortium, *RNASeq* RNA sequencing, *RPPA* reverse phase protein assays, *SNP* single nucleotide polymorphism, *TCGA* The Cancer Genome Atlas, *WES* whole exome sequencing, *WGS* whole genome sequencing

Focusing on driver mutations has helped to understand the hallmark processes underpinning breast cancer development and define possible drug targets, but there is also increasing interest in passenger mutations – not only in terms of their influence on progression in the context of exposure to extrinsic selection pressures, but as a genomic record of the mutational processes that occurred throughout the development of each tumour. Mutational process signatures are dynamic, varying spatially and temporally depending on both exogenous and endogenous factors (e.g. carcinogen exposure, age-related change or DNA repair defects) [53]. Complex mathematical analysis has identified 21 substitution signatures with different clinicopathologic associations and underlying aetiologies. Like individual mutations, signatures are clonal and coexist at variable frequencies within cancer deposits of each patient. Some are common to different cancers (e.g. age-related), while others are tumour type-specific (e.g. C·G ➔ T·A transitions are a feature of signature 7, associated with UV-induced DNA damage in cutaneous cancers) [56, 57].

Breast cancer genomes are characterised by 12 substitution signatures, with six consistently detected in at least 20% of cases [49, 53, 57, 58]. Amongst these, signatures 1 and 5 (which are similar and often classified together as 1B) are associated with age, while signatures 2 and 13 are associated with APOBEC cytidine deaminases, which are normally involved in antiviral immunity and RNA editing, but can also act on long stretches of single-stranded DNA thought to arise during abnormal DNA replication [49]. Signatures 3 and 8 are associated with *BRCA1/BRCA2* deficiency, defective homologous recombination repair, and short (<10 kb) deletions/tandem duplications [49]. Of the rarer signatures (<20% cases), 6, 20, and 26 are associated with mismatch repair deficiency, while 17, 18, and 30 are of unknown aetiology. The potential implications and clinical applications of mutational signature composition are currently under investigation [58]. For example, they may be useful for characterising cancers with unknown primary origin at diagnosis [59]. Also, signatures 3 and 13 are associated with increased lymphocytic infiltrate and better clinical outcomes, raising the possibility that free DNA and/or mutant peptides associated with this pattern are more immunogenic compared with other mutational processes [60] (see Sect. 6.3.3). Finally, there are possibilities for developing signature-based predictive models, such the 'HRDetect' algorithm that quantifies somatic *BRCA1/2* deficiency, a candidate biomarker of response to polyADP-ribose polymerase (PARP) inhibitors [61, 62].

Sequencing the genomes and transcriptomes of single breast tumour cells is now offering additional insights into heterogeneity. For example, single-cell genome analysis has been applied to understand the dynamics of clonal selection across cohorts of patient-derived tumour xenografts [63]. Also, in an elegant and clinically relevant application of RNA-sequencing, Lee and colleagues compared breast cancer cell subpopulations exhibiting resistance to the microtubule poison paclitaxel in vitro [64]. Residual cells that persisted after treatment expressed variants involved a variety of cellular processes logically connected to drug resistance, including microtubule stabilization and stress. But critically, individual cells expressed different combinations of variant transcripts, suggesting that transcriptional heterogeneity

can ultimately generate equivalent phenotypes. The expression profiles of individual cells were not apparent in a pooled analysis of the bulk population, or even as few as five cells. Thus single-cell sequencing has the potential to illuminate aspects of plasticity and clonal evolution that would not be apparent from analysis of tissue homogenates.

6.3.2 Cellular Heterogeneity

Gene expression studies comparing breast tumours with normal breast tissue identified groups of tumours exhibiting transcriptomic similarity to particular mammary epithelial compartments. For example, 'luminal-like' tumours are most similar to the specialised luminal epithelia that line ducts and lobules of the breast (Sect. 6.4.1), while the expression profile of 'basal-like' tumours resembles luminal progenitor cells (Sect. 6.4.2) [65]. Functional evidence supporting the idea that global tumour gene expression profiles could reflect the cell type of origin came from transgenic mouse experiments, where oncogenic mutations were introduced into specific compartments of the mouse mammary gland, resulting in formation of tumours that phenocopied metaplastic or *BRCA1*-mutant breast cancer [66, 67]. Thus, heterogeneity reflects the consequences of superimposing the mutational landscape over pre-programmed phenotypic determinants. The cells comprising a tumour exhibit restricted versions of the normal mammary epithelial lineage hierarchy, depending on which cell type sustained the founding oncogenic hits, and how the unique spectrum of alterations impacted lineage differentiation programming in its daughters (for example, de-differentiation and phenotypic plasticity). Diversification is also achieved through phenotypic drift (stochastic heterogeneity [31, 68]; Fig. 6.3a). The significance of this is highlighted by the association between stem-like phenotypes and poor outcomes in breast and other cancers [69–71], though it is worth considering that primitive, stem-like cells may be associated with metastasis and treatment resistance simply because they have more potential for generating clonal complexity (*i.e.* better substrates for natural selection) [31], not necessarily because they possess equivalent normal stem cell functions like efficient drug efflux and slow cell cycling.

6.3.3 Microenvironment Heterogeneity

Non-tumour elements contributing to breast cancer heterogeneity include soluble and extracellular matrix proteins, fibroblasts, endothelia, adipocytes, macrophages and other leukocytes [72] (Fig. 6.3b). A large effort has been directed at investigating clinical implications of the breast cancer microenvironment [73–75]. One area in which there have been key recent developments is in understanding how vascular perfusion dynamics impacts tumour progression and treatment

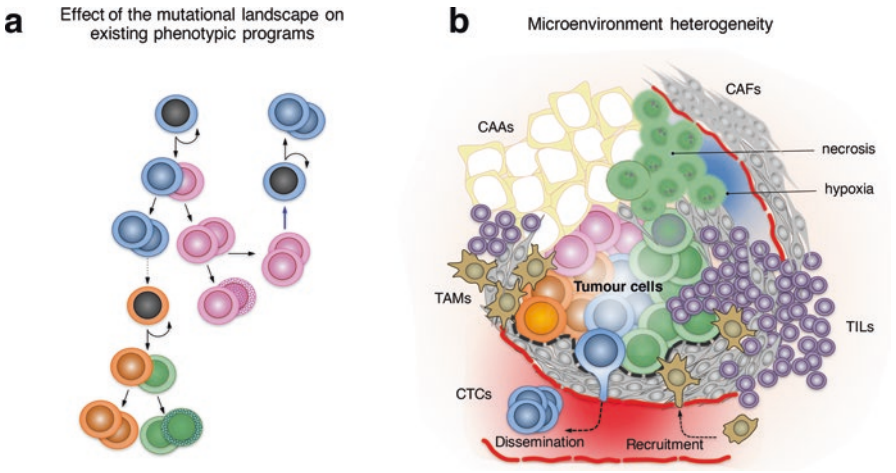

Fig. 6.3 Heterogeneity represents the collective consequences of superimposing the mutational landscape over pre-existing phenotypic programs and interaction with the microenvironment. (**a**) Breast tumour cells exhibit restricted versions of the normal mammary epithelial lineage hierarchy, depending on which cell type sustained the founding oncogenic hits, and how the unique spectrum of alterations impacted lineage differentiation programming in its daughters. The differentiation states (different colours) of stem-like (black nuclei), committed progenitor and daughter cells contribute to phenotypic diversity (deterministic heterogeneity). Phenotypic flux due to cell-specific biochemical processes (patterning in daughter cells) also contributes to phenotypic diversity (stochastic heterogeneity). Daughter cells may acquire stem-cell activity through genetic alteration (dashed arrow) or de-differentiation (blue arrow), acquiring stem cell activity and initiating new clones. (**b**) Stromal elements influence tumour cell phenotypes and clonal selection, and are in turn altered by their interactions with tumour cells, contributing to intratumoural heterogeneity. The figure shows various stromal cell types: cancer-associated adipocytes and fibroblasts (CAAs/CAFs), tumour-infiltrating lymphocytes (TILs), tumour-associated macrophages (TAMs) and circulating tumour cells (CTCs) liberated into surrounding blood vessels. Ongoing tumour cell proliferation fuels pro-tumourigenic cycles of local hypoxia and neoangiogenesis, resulting in chaotic microvascular networks that cannot adequately deliver systemic therapy

efficacy. Ongoing proliferation in solid tumours fuels cycles of hypoxia and neoangiogenesis, and this in turn creates a chaotic, dysfunctional microvascular bed, with (paradoxically) areas of sluggish blood flow in an otherwise hypervascular environment [76–78]. It is thought that inefficient perfusion directly reduce the delivery of systemic therapeutics, but that drug efficacy is also reduced indirectly in poorly oxygenated tissues. This is because radiotherapy and some chemotherapeutics act by damaging tumour DNA, but breaks are more readily repairable in hypoxic conditions, allowing cells to escape fatal chromosome aberrations, and instead, erroneously repair DNA to increase genetic diversity [79]. Hypoxia is also associated with mesenchymal/stem-cell phenotypes, inflammation, fibrosis, poor drug uptake and immune suppression [80]. This knowledge has driven attempts to improve efficacy and reduce the likelihood of relapse using combination therapies that increase oxygenation by ‘normalising’ the vascular bed.

Ironically, this strategy uses agents that target vascular endothelial growth factor and its receptor (VEGF/VEGFR), originally intended to starve tumours of nutrients and oxygen (e.g. bevacizumab).

Tumour-infiltrating lymphocytes (TILs) have also been intensively studied in the last 5 years, with evidence rapidly accumulating to support a role in clinical management. A fundamental function of host immunity is to detect and eradicate abnormalities arising from neoplastic transformation (immune-surveillance). Considering that breast tumours are diagnosed once they are detectable by mammography and/or palpation, they are already successfully evading elimination at diagnosis, but chemotherapy and radiotherapy can produce neoantigens that effectively kick-start the immune response, and new therapies that reactivate anticancer immune responses are currently being assessed in clinical trials (e.g. immune-checkpoint inhibitors, personalised cancer vaccines and adoptive T-cell therapy [81, 82]). TILs are most frequent in HER2+ and TN disease, where the overall degree of infiltrate is associated with better outcome, even amongst TNBC patients with residual disease following neoadjuvant chemotherapy, an otherwise poor prognostic group [83].

The breast cancer immune microenvironment is a complex mixture of different functional subsets – mostly T-cells, with smaller proportions of B-cells, dendritic cells, neutrophils, macrophages and natural killer (NK)-cells; with different effects on tumour progression. For example, NK and CD4+ Th1-cells are generally associated with favourable outcomes, whilst myeloid-derived suppressor cells (MDSC) and gamma-delta regulatory T-cells ($\gamma\delta$-T_{reg}) suppress anti-tumour immunity and are associated with poor response to chemotherapy [84–87]. Ultimately, the particular constitution of lymphocytic infiltrate (ratios of different TIL subsets and effectors/modifiers they produce) reaches equilibrium with the tumour compartment, and shapes the microenvironment along a spectrum from an immuno-stimulatory, anti-tumour milieu, to a pro-tumourigenic environment geared toward wound-healing. Despite this complexity, TILs are routinely enumerated *en masse* by examination of haematoxylin and eosin (H&E)-stained tissue sections. Special IHC stains are used occasionally, but at this stage this is purely to help discriminate intratumoural TILs from tumour cells, rather than identify different functional subpopulations [88]. Apart from the fact that the full clinical implications of functional TILs heterogeneity are still being elucidated, there are challenges with standardisation in the diagnostic laboratory, as TILs reside mostly in the stromal compartment, which varies with tumour architecture, and enumeration on two-dimensional tissue sections is difficult because they are heterogeneously distributed in three dimensions. But even without universal standardisation, and somewhat crude histopathologic assessment of overall infiltrate density, there is already strong evidence from multiple prospective clinical trials supporting the prognostic and predictive significance of TILs in HER2+ and TN disease [81]. Thus, improvements in the precision of TIL-based biomarker development and companion therapies will likely play favourably into personalised clinical management models.

6.4 Molecular Classification of Breast Cancer

Molecular profiling has shifted the ways that breast cancer development and heterogeneity are considered. Transcriptomic studies began more than 15 years ago, with the segregation of 38 invasive breast carcinomas by unsupervised hierarchical clustering of gene expression profiles [89, 90]. These 'intrinsic subtypes' have since been extensively confirmed in the field as robust biological entities with distinct mutation profiles and clinical outcomes [70, 91–93] (Table 6.3). In some instances, underlying expression profiles provide a molecular explanation for well-known clinical or morphological features. For example, the clinical behaviour of ER-positive tumours depends largely on histologic grade, and in the intrinsic subtype taxonomy they segregate into luminal-A and -B groups, distinguished by expression of proliferation gene networks [89, 90, 93–95].

Subsequent technological advances have increased the breadth and resolution of the transcriptomic taxonomy (more tumours, more extensive coverage and more accurate RNA quantification), providing a deeper understanding of the underlying biology and potential clinical implications. For example, a subgroup of ER-negative IC-NST that frequently exhibits medullary and metaplastic features is enriched with a 'claudin-low' gene cluster [71, 96]. Another is the 'molecular apocrine' (mApo) group, which is largely triple-negative, yet paradoxically expresses ER-responsive, luminal genes due to expression of the androgen receptor (AR; Table 6.3) [97, 98]. In smaller cohorts with less statistical power, mApo tumours would be classed as basal-like or HER2-enriched [94, 97, 99]. The Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) took expression profile analysis to a different level by integrating expression with copy-number data to stratify 2000 breast tumours on the basis of cancer driver profiles [50]. The ten 'integrative subgroups' overlap variably with intrinsic subtypes and are associated with distinct survival trends.

6.4.1 Heterogeneity in Luminal/ER+ Breast Cancer

The largest subgroup of breast cancers is defined by expression of ER in at least 1% of tumour cells, a conservative cut-off that qualifies around 70% of breast cancer patients for endocrine therapy; though ER+ disease still exhibits marked clinical variability, particularly with respect to late recurrences [50, 100]. While endocrine therapy significantly increases relapse-free survival overall, almost a quarter of patients still relapse within 10 years of diagnosis, with some evidence that mutations in the gene itself (*ESR1)*, or ER signalling regulators (e.g. *SMRT*) could contribute [101–103]. In terms of predictive molecular features, luminal-B tumours tend to respond less well to endocrine therapy but better to chemotherapy than luminal-A tumours, with the exception of 'atypical' luminal-A cases, which harbour more *TP53* mutations and copy-number aberrations [101, 104]. Deep molecular analyses have also identified unique differences between luminal-A ILC and IC-NST, with ILCs featuring loss of *CDH1*, AKT signalling activation and *FOXA1*

Table 6.3 Molecular breast cancer subtypes

	Histopath features			Phenotypic features	Mutations [44]	Differentiation state
	ER	HER2	Grade			
Intrinsic subtypes						
Luminal A	+	−	1–2	Expression ER-signalling networks and low molecular-weight cytokeratins (CK8/18)	*PIKC3A* (49%), *GATA3* (14%), *MAP3K1* (14%), *TP53* (12%), *CDH1* (9%)	Enriched with the gene expression signature of fully differentiated luminal epithelia
Luminal B	+/−	−/+	2–3	Expression of ER-signalling and proliferation networks	*PIKC3A* (32%), *TP53* (32%), *PTEN* (24%)	Enriched with the gene expression signature of fully differentiated luminal epithelia
Her2-enriched	−/+	+	2–3	HER2 overexpressed/amplified, over-expression of genes at 17q22.24	*TP53* (75%), *PIK3CA* (42%) *PTEN* (19%)	Modest expression of mature luminal and luminal progenitor gene expression signatures
Basal-like	−	−	3	Often triple-negative, high grade, frequent expression of myoepithelial markers (EGRF, CK5/6, CK14), c-kit and FOXC1	*TP53* (84%) *PIK3CA* (7%)	Most similar to luminal epithelial progenitor cells
Normal-like	−/+	−	1	Expression of adipose and myoepithelium genes; low expression of luminal genes; clusters with fibroadenoma and normal breast samples		Enriched with mammary stem cell and stromal gene expression signatures

(continued)

Table 6.3 (continued)

	Histopath features			Phenotypic features	Mutations [44]	Differentiation state
	ER	HER2	Grade			
Additional subtypes						
Claudin-low	–	–	3	Enriched with primitive, $CD44^{+}/CD24^{-}$, mesenchymal-like cells [71, 96]. Low expression of cell-cell junction proteins, high expression of immune response and epithelial-to-mesenchymal signatures. Overall survival worse than Luminal A [70, 71]	*CDH1, CLDN3/4/7, OCLN*	Primitive, enriched with stem cell and stromal signatures Epithelial to mesenchymal transition markers
Molecular apocrine	–	+/–	2–3	Frequent apocrine histological features; gene networks associated with AR, calcium and ErbB signalling, lipid and fatty acid synthesis; HER2 over-expression (not associated with 17q22.24 amplification); early recurrence but good response to neoadjuvant therapy [99]	Genes associated with AR, calcium and Her2 signalling, lipid and fatty acid synthesis [97, 99]	Primitive [99]

mutations, while IC-NST showed intact cellular adhesion and *GATA3* mutations [8]. Analysing ILCs as a separate group stratified them into reactive-like, immune-related and proliferative subgroups [8]. It is also worth highlighting that there are two different ligand-activated oestrogen receptors, encoded by separate genes: ERα (*ESR1*) and ERβ *(ESR2)* [105]. ERα is the dominant isoform and the best predictor of response to hormone therapy, but a possible role for ERβ in regulating the immune microenvironment is also now emerging [106], and may provide further insights into luminal breast cancer heterogeneity in the future.

6.4.2 Heterogeneity in Triple-Negative Breast Cancer

TNBC is arguably the most heterogeneous of the major breast cancer subgroups. It is more frequent in individuals with inherited mutations in *BRCA1* and other DNA repair genes and carries a poor prognosis overall, although some patients have complete, durable responses to treatment. TNBCs are usually highly proliferative and of higher histological grade, though there are low-grade variants with a more protracted natural history (namely, adenoid cystic and secretory carcinomas) [70, 90, 91, 107–115]. A defining characteristic of TNBC is its paradoxically impressive initial response to neoadjuvant chemotherapy, yet poor 5-year survival rate. The discrepancy is due to a chemotherapy-resistant subgroup found to have residual disease at the time of breast surgery, which is associated with brain and liver metastasis [116]. TNBC patients who do not relapse within 3 years have a prognosis comparable to ER+ disease [109].

Despite high overall mutation loads, the most common variants are in *TP53* (around 80% of cases, with more frequent nonsense and frameshift mutations than ER/HER2+ disease) and *PI3K* in around 10% of cases [117]. On the other hand, TNBCs harbour characteristic chromosomal alterations, categorised as ‘simple’, ‘amplifier/firestorm’ or ‘complex/sawtooth’ depending on the complexity of causal rearrangements [118]. Integrating CNA with expression data in basal-like TNBC revealed that alterations tend to converge on two major oncogenic signalling pathways: EGFR-ras-MEK and PI3K-mTOR. Common alterations include loss of *PTEN* and *INPP4B* and amplification of *CDK1*, *MYC* and *AKT3*. Interestingly, this profile confers more similarity to serous ovarian cancer than other breast cancers, providing a genomic link between two malignancies associated with germline *BRCA1* mutation [117, 119]. mTOR/MEK inhibitors are promising as combination agents with neoadjuvant chemotherapy, however they are associated with significant toxicity and dosing schedules are still being optimised [120].

Together, these studies have produced critical information on somatic events underpinning TNBC development, however the tumour microenvironment receives little weight in genome-focused approaches. The percentage of ‘contaminating’ non-tumour cell types is considered a limitation in genomics, but given that immunogenicity is a strong determinant of clinical outcome in TNBC, with TILs at the

forefront of personalised therapy for this patient group, there is a valid argument for stratifying TNBCs in a way that encompasses the complexity in tissue homogenates, rather than filtering it out. Two landmark studies attempted to classify TNBC on the basis of unsupervised clustering of gene expression data from large tumour cohorts [121, 122]. They identified four to six major clusters associated with distinct functional gene networks, genomic alterations and clinical outcomes. Independent analyses incorporating laser capture-micro-dissected tumour samples, patient-derived xenografts and cell lines (which lack human non-tumour elements) subsequently showed that tumour cellularity is a critical determinant of clustering [123, 124], and the field is not yet united on a robust classifier that mathematically accounts for this. But overall, the data suggest that basal-like TNBCs (70–80% of cases) can be further segregated according to the degree of active immune infiltrate and tumour-specific immunity, while non-basal TNBCs (20–30%) can be defined by whether they engage ER-independent hormone signalling or exhibit mesenchymal and stem-like features (previously identified as 'claudin-low' as an extension of the intrinsic subtype classifier) [71, 121, 124]. Interest is also gathering around epigenetic dysregulation, and whether differentially methylated regions of the TNBC genome could underpin some of this biological variability [125].

6.5 Clinical Implications of Breast Cancer Heterogeneity

6.5.1 Molecular Diagnostic Tools

As molecular subtypes have emerged, the clinical corollary has been to develop risk stratification signatures. However, many offer no significant benefit beyond current practice standards. In order to be useful, risk stratification signatures must add value to existing histopathological data, accurately classify individual cases (so-called 'single sample predictor') and be readily implemented in the diagnostic laboratory. From a clinical perspective, the most important contribution of molecular subtyping has been the recognition of the luminal A/B subdivision in ER-positive disease, which has informed the development of MammaPrint® [126–130], Oncotype DX® [131–133], EndoPredict® [134] and Prosigna® [94, 131–133]. These tests quantitatively assign the risk of recurrence in ER-positive, node-negative patients, and have implications for sparing a proportion of low-stage patients from receiving chemotherapy. The 'Nottingham Prognostic Index Plus' is another good example of early transitioning to precision medicine – it attempts to blend traditional parameters with a broader IHC panel to better define molecular subtypes and improve stratification [135]. The topic of molecular signatures in breast cancer prognostication has been recently reviewed [136].

Precision medicine is predicated on employing genomics and gene expression profiling to personally tailor a treatment regimen. Theoretically, this is optimal as it allows targeting of appropriate pathways while minimising treatment with agents

that may have limited or no benefit. Yet, identifying a targetable mutation does not guarantee that the matching treatment will be effective (for example, one study reported that only 36% of somatic variants were actually expressed [51]). With the exception of *ERRB2*, no single genomic alteration is a clinically useful predictor of therapeutic efficacy [137]. This was exemplified recently in the PALOMA-1 trial where *CDK4/6* amplification was not a reliable predictor of response to a CDK4/6 inhibitor [138]. Predictive tools that incorporate multiple alterations or that complement existing algorithms such as the Nottingham Prognostic Index, are the ideal way to move forward.

It is clear that molecular profiling has a role in breast cancer management, but incorporating the technology and knowledge into routine clinical practice is a major challenge. In the public health setting, there are considerable logistic and economic barriers related to standardization, accreditation and reliable service delivery. The requirements will include infrastructure that is cost-effective and can adapt to evolving technologies; dynamic, curated databases of clinically actionable variants/signatures; major changes to pathology and oncology training programs, and possibly an entirely new precision oncology specialization stream that incorporates genetic counselling, as there are important ethical issues around synthesizing and communicating complex diagnostic results to the clinician and patient in a meaningful way. Finally, how should mutations which are of as-yet-unknown clinical significance be dealt with? Sequencing of close to 1000 breast cancers has identified 128 genes with *putative* targetable alterations [139]. For the vast majority, genotype-drug efficacy relationships are still being elucidated in preclinical and clinical studies, and the cost-effectiveness and overall benefit of matching treatments are unknown [140].

A tumour biopsy is a static representation of a small fraction of a larger mass taken at a single point in time. Given that diagnostic tests used today are still relatively low-resolution with conservative thresholds, tumour under-sampling is not currently a major consideration in clinical practice. However given that precision medicine aims to select rational combinations of targeted agents according to an individual tumour's profile, and yet drug resistant, metastatic clones may represent a minor component of the tumour, the risk of under-sampling will be magnified in a precision oncology context [141, 142]. The potential consequences of basing clinical management on small tumour biopsies has been exemplified by spatial and temporal heterogeneity in amplification and over-expression of HER2 during disease progression [30]. In the case of HER2+ breast cancer, trastuzumab therapy does not preclude later development of metastatic disease; in fact distant recurrence is common and can be HER2-negative [70, 143].

6.5.2 Heterogeneity in Metastatic Breast Cancer

Historically, metastasis was viewed as a complication of end-stage disease with the assumption that distant cancer deposits were virtually the same as the primary tumour from which they arose (linear progression) [144]. However, we have known

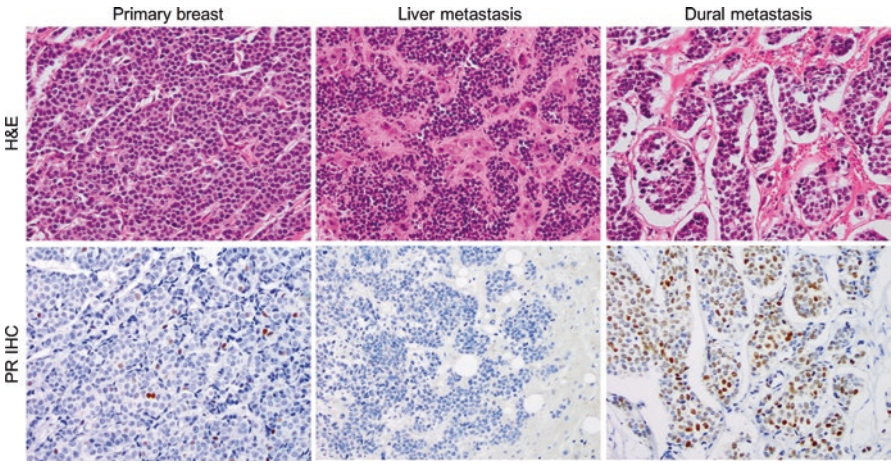

Fig. 6.4 Metastatic breast cancer heterogeneity exemplified by variable expression of progesterone receptor (PR). Approximately 1% of tumour cells in the primary breast tumour are positive for PR, whereas the liver metastasis is negative, and the dural lesion is virually 100% positive. *IHC, immunohistochemistry*

for some time that the expression of clinically relevant biomarkers can differ between primary and metastatic tumours from the same patient (e.g. Fig. 6.4; [145–152]), and American Society of Clinical Oncology (ASCO) practice guidelines now recommend direct biopsy of accessible metastases for repeat HER2/ER/PR testing [153]. More recently, sequencing studies have provided compelling evidence for parallel evolution in regional metastases [154–158]. By applying whole genome sequencing to a large cohort of breast and matching metastatic tumours, Yates and colleagues found that at the time of initial diagnosis, primary tumour genomes are suitable proxies for subclinical metastatic deposits (very encouraging in terms of guiding adjuvant therapy for early breast cancer), but resistant deposits undergo clonal expansion and further diversification, acquiring additional driver alterations before becoming clinically detectable [158] (Fig. 6.2). These findings suggest that early detection of therapeutic resistance will be crucial for optimising therapy, and that re-biopsy will be critical for the success of molecular-targeted therapies in the metastatic setting.

Single-cell genomics can be used to track genomic and transcriptional changes in individual cells, and track clonal evolution over time. There has also been great enthusiasm around applying this technology to circulating tumour cells (CTCs), shed from solid tumours into the circulation or lymphatics and detectable as a source of genetic material [159–161]. CTCs predict survival, disease-related mortality, response to treatment and early disease recurrence [162–164], and it has been argued that the ‘liquid biopsy’ could represent the entire tumour genome, and provide a means for sensitively monitoring response to treatment over time [165].

However, there are major logistic barriers to realising this goal, not least of which are the low and variable concentrations of CTCs in peripheral blood (1–10 CTC per 10 mL [166]). On the other hand, multiple proof-of-principle studies have underscored the utility of circulating tumour (ct)DNA as an early indicator of therapeutic resistance and the presence of residual disease [165, 167, 168], and suggested value as a collective representation of the tumour genome [169], helping to overcome issues related to tissue sampling bias [170]. As ctDNA profiling is incorporated into more clinical trials, significant developments are expected in the forseeable future [171].

6.6 Concluding Remarks

New knowledge about the extent of heterogeneity in breast cancer and how this relates to clonal evolution is changing the way we think about research, diagnosis and treatment. From a clinical standpoint, the most dire complication of breast cancer heterogeneity is therapeutic resistance. To tackle this, the field has taken a three-pronged approach: (1) reduce the likelihood of relapse in the first place by using more effective agents (and likewise, use agents more effectively) for early breast cancer; (2) detect therapeutic resistance early so that treatment regimens can be optimised; and (3) expand the arsenal of second- and subsequent- line agents so that metastatic disease can be stabilised for as long as possible (though a future goal will be to treat metastatic disease with curative intent).

Achieving these expectations will require continued investment in research and development to identify new therapeutic targets; including druggable alterations, synthetic lethal vulnerabilities, and innovative strategies that combine therapies for maximal efficacy and/or to simultaneously target minor subclones that cause resistance. In parallel, we will need to implement high-resolution companion molecular diagnostic assays that are scalable, adaptive and cost-effective in the public health setting. Implicit in this will be algorithms that accurately predict risk profiles and rank suitable therapeutic regimens according to each tumour's molecular profile, potentially highlighting clinical trial suitability. Clinical training programs will need to evolve to meet precision oncology demands, as oncologists of the future will be expected to synthesise complex diagnostic information, assess optimal therapeutic strategies and deliver complicated diagnostic results and recommendations to their patients. Finally, we need public investment and innovation in clinical trials, including measures to drastically increase access to patients outside major metropolitan centres. Apart from improving equity, conducting trials on a broader scale will be necessary to determine dosing regimens, timing and drug interactions, and to achieve adequate recruitment as the numbers of new targets and agents coming online increases.

References

1. Pinder SE (2010) Ductal carcinoma in situ (DCIS): pathological features, differential diagnosis, prognostic factors and specimen evaluation. Modern Pathol: Off J US Can Acad Pathol 23(Suppl 2):S8–S13
2. Lakhani SR et al (2012) In: Bosman FT (ed) WHO classification of tumours of the breast, World Health Organisation Classification of Tumours. IARC, Lyon
3. Ellis IO et al (2003) Invasive breast carcinomas, WHO Classification of Tumours. Pathology and Genetics of Tumours of the Breast and Female Genital Organs. International Agency for Research on Cancer (IARC), Lyon, France
4. Weigelt B et al (2008) Refinement of breast cancer classification by molecular characterization of histological special types. J Pathol 216(2):141–150
5. Tognon C et al (2002) Expression of the ETV6-NTRK3 gene fusion as a primary event in human secretory breast carcinoma. Cancer Cell 2(5):367–376
6. Persson M et al (2009) Recurrent fusion of MYB and NFIB transcription factor genes in carcinomas of the breast and head and neck. Proc Natl Acad Sci U S A 106(44):18740–18744
7. Rakha EA, Ellis IO (2010) Lobular breast carcinoma and its variants. Semin Diagn Pathol 27(1):49–61
8. Ciriello G et al (2015) Comprehensive molecular portraits of invasive lobular breast cancer. Cell 163(2):506–519
9. Rakha EA et al (2008) Prognostic significance of Nottingham histologic grade in invasive breast carcinoma. J Clin Oncol Off J Am Soc Clin Oncol 26(19):3153–3158
10. Rakha EA et al (2010) Breast cancer prognostic classification in the molecular era: the role of histological grade. Breast Cancer Res: BCR 12(4):207
11. Sotiriou C et al (2006) Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. J Natl Cancer Inst 98(4):262–272
12. Elston CW, Ellis IO (1991) Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 19(5):403–410
13. Dalton LW, Page DL, Dupont WD (1994) Histologic grading of breast carcinoma. A reproducibility study. Cancer 73(11):2765–2770
14. Galea MH et al (1992) The Nottingham Prognostic Index in primary breast cancer. Breast Cancer Res Treat 22(3):207–219
15. Sundquist M et al (1999) Applying the Nottingham Prognostic Index to a Swedish breast cancer population. South East Swedish Breast Cancer Study Group. Breast Cancer Res Treat 53(1):1–8
16. Mook S et al (2009) Calibration and discriminatory accuracy of prognosis calculation for breast cancer with the online Adjuvant! program: a hospital-based retrospective cohort study. Lancet Oncol 10(11):1070–1076
17. Goldhirsch A et al (2009) Thresholds for therapies: highlights of the St Gallen International Expert Consensus on the primary therapy of early breast cancer 2009. Ann Oncol: Off J Eur Soc Med Oncol/ESMO 20(8):1319–1329
18. Early Breast Cancer Trialists' Collaborative Group (1998) Tamoxifen for early breast cancer: an overview of the randomised trials. Lancet 351(9114):1451–1467
19. Ravdin PM et al (1992) Prognostic significance of progesterone receptor levels in estrogen receptor-positive patients with metastatic breast cancer treated with tamoxifen: results of a prospective Southwest Oncology Group study. J Clin Oncol Off J Am Soc Clin Oncol 10(8):1284–1291
20. Wolff AC et al (2007) American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med 131(1):18–43
21. Slamon DJ et al (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235(4785):177–182

22. Tandon AK et al (1989) HER-2/neu oncogene protein and prognosis in breast cancer. J Clin Oncol Off J Am Soc Clin Oncol 7(8):1120–1128
23. Chia S et al (2008) Human epidermal growth factor receptor 2 overexpression as a prognostic factor in a large tissue microarray series of node-negative breast cancers. J Clin Oncol Off J Am Soc Clin Oncol 26(35):5697–5704
24. Madarnas Y et al (2008) Adjuvant/neoadjuvant trastuzumab therapy in women with HER-2/neu-overexpressing breast cancer: a systematic review. Cancer Treat Rev 34(6):539–557
25. Dent R et al (2007) Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res 13(15 Pt 1):4429–4434
26. Hammond ME et al (2010) American society of clinical oncology/college of american pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Oncolo Prac/Am Soc Clin Oncol 6(4):195–197
27. Wolff AC et al (2013) Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol 31(31):3997–4013
28. Lee HJ et al (2014) Two histopathologically different diseases: hormone receptor-positive and hormone receptor-negative tumors in HER2-positive breast cancer. Breast Cancer Res Treat 145(3):615–623
29. Lee HJ et al (2015) Clinicopathologic significance of the intratumoral heterogeneity of HER2 gene amplification in HER2-positive breast cancer patients treated with adjuvant trastuzumab. Am J Clin Pathol 144(4):570–578
30. Seol H et al (2012) Intratumoral heterogeneity of HER2 gene amplification in breast cancer: its clinicopathological significance. Mod Pathol: Off J US Can Acad Pathol 25:938
31. Marusyk A, Almendro V, Polyak K (2012) Intra-tumour heterogeneity: a looking glass for cancer? Nat Rev Cancer 12(5):323–334
32. Marusyk A, Polyak K (2010) Tumor heterogeneity: causes and consequences. Biochim Biophys Acta 1805(1):105–117
33. Carter H et al (2009) Cancer-specific high-throughput annotation of somatic mutations: computational prediction of driver missense mutations. Cancer Res 69(16):6660–6667
34. Wood LD et al (2007) The genomic landscapes of human breast and colorectal cancers. Science 318(5853):108–113
35. Torkamani A, Schork NJ (2008) Prediction of cancer driver mutations in protein kinases. Cancer Res 68(6):1675–1682
36. Yates LR et al (2015) Subclonal diversification of primary breast cancer revealed by multiregion sequencing. Nat Med 21(7):751–759
37. Greaves M, Maley CC (2012) Clonal evolution in cancer. Nature 481(7381):306–313
38. Nowell PC (1976) The clonal evolution of tumor cell populations. Science 194(4260):23–28
39. Navin N et al (2011) Tumour evolution inferred by single-cell sequencing. Nature 472(7341):90–94
40. Stephens PJ et al (2011) Massive genomic rearrangement acquired in a single catastrophic event during cancer development. Cell 144(1):27–40
41. Wang Y et al (2014) Clonal evolution in breast cancer revealed by single nucleus genome sequencing. Nature 512(7513):155–160
42. Gao Y et al (2017) Single-cell sequencing deciphers a convergent evolution of copy number alterations from primary to circulating tumour cells. Genome Res 27:1312
43. Gao R et al (2016) Punctuated copy number evolution and clonal stasis in triple-negative breast cancer. Nat Genet 48(10):1119–1130
44. Cancer Genome Atlas, N (2012) Comprehensive molecular portraits of human breast tumours. Nature 490(7418):61–70
45. Zhang J et al (2011) International Cancer Genome Consortium Data Portal – a one-stop shop for cancer genomics data. Database (Oxford) 2011:bar026
46. Stephens PJ et al (2012) The landscape of cancer genes and mutational processes in breast cancer. Nature 486(7403):400
47. Polyak K, Metzger Filho O (2012) SnapShot: breast cancer. Cancer Cell 22(4):562–562 e1

48. Banerji S et al (2012) Sequence analysis of mutations and translocations across breast cancer subtypes. Nature 486(7403):405–409
49. Nik-Zainal S et al (2016) Landscape of somatic mutations in 560 breast cancer whole-genome sequences. Nature 534(7605):47–54
50. Curtis C et al (2012) The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature 486:346
51. Shah SP et al (2012) The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature 486(7403):395–399
52. Yates LR, Desmedt C (2017) Translational genomics: practical applications of the genomic revolution in breast cancer. Clin Cancer Res 23(11):2630–2639
53. Nik-Zainal S et al (2012) Mutational processes molding the genomes of 21 breast cancers. Cell 149(5):979–993
54. Nik-Zainal S et al (2012) The life history of 21 breast cancers. Cell 149(5):994–1007
55. Pereira B et al (2016) The somatic mutation profiles of 2,433 breast cancers refines their genomic and transcriptomic landscapes. Nat Commun 7:11479
56. Alexandrov LB, Stratton MR (2014) Mutational signatures: the patterns of somatic mutations hidden in cancer genomes. Curr Opin Genet Dev 24:52–60
57. Alexandrov LB et al (2013) Signatures of mutational processes in human cancer. Nature 500(7463):415–421
58. Nik-Zainal S, Morganella S (2017) Mutational signatures in breast cancer: the problem at the DNA level. Clin Cancer Res 23(11):2617–2629
59. Saunus JM et al (2015) Integrated genomic and transcriptomic analysis of human brain metastases identifies alterations of potential clinical significance. J Pathol 237(3):363–378
60. Smid M et al (2016) Breast cancer genome and transcriptome integration implicates specific mutational signatures with immune cell infiltration. Nat Commun 7:12910
61. Davies H et al (2017) HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. Nat Med 23(4):517–525
62. Lord CJ, Ashworth A (2016) BRCAness revisited. Nat Rev Cancer 16(2):110–120
63. Eirew P et al (2015) Dynamics of genomic clones in breast cancer patient xenografts at single-cell resolution. Nature 518(7539):422–426
64. Lee MC et al (2014) Single-cell analyses of transcriptional heterogeneity during drug tolerance transition in cancer cells by RNA sequencing. Proc Natl Acad Sci U S A 111(44):E4726–E4735
65. Lim E et al (2009) Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. Nat Med 15(8):907–913
66. Keller PJ et al (2012) Defining the cellular precursors to human breast cancer. Proc Natl Acad Sci U S A 109(8):2772–2777
67. Molyneux G et al (2010) BRCA1 basal-like breast cancers originate from luminal epithelial progenitors and not from basal stem cells. Cell Stem Cell 7(3):403–417
68. Chaffer CL et al (2011) Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. Proc Natl Acad Sci U S A 108(19):7950–7955
69. Clevers H (2011) The cancer stem cell: premises, promises and challenges. Nat Med 17(3):313–319
70. Harrell JC et al (2011) Genomic analysis identifies unique signatures predictive of brain, lung, and liver relapse. Breast Cancer Res Treat 132:523
71. Prat A et al (2010) Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Res: BCR 12(5):R68
72. Korkaya H, Liu S, Wicha MS (2011) Breast cancer stem cells, cytokine networks, and the tumor microenvironment. J Clin Invest 121(10):3804–3809
73. Finak G et al (2008) Stromal gene expression predicts clinical outcome in breast cancer. Nat Med 14(5):518–527
74. Allinen M et al (2004) Molecular characterization of the tumor microenvironment in breast cancer. Cancer Cell 6(1):17–32

75. Ma XJ et al (2009) Gene expression profiling of the tumor microenvironment during breast cancer progression. Breast Cancer Res: BCR 11(1):R7
76. Kienast Y et al (2010) Real-time imaging reveals the single steps of brain metastasis formation. Nat Med 16(1):116–122
77. Carmeliet P, Jain RK (2011) Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. Nat Rev Drug Discov 10(6):417–427
78. Monsky WL et al (2002) Role of host microenvironment in angiogenesis and microvascular functions in human breast cancer xenografts: mammary fat pad versus cranial tumors. Clin Cancer Res 8(4):1008–1013
79. Moeller BJ, Richardson RA, Dewhirst MW (2007) Hypoxia and radiotherapy: opportunities for improved outcomes in cancer treatment. Cancer Metastasis Rev 26(2):241–248
80. Jain RK (2014) Antiangiogenesis strategies revisited: from starving tumors to alleviating hypoxia. Cancer Cell 26(5):605–622
81. Savas P et al (2016) Clinical relevance of host immunity in breast cancer: from TILs to the clinic. Nat Rev Clin Oncol 13(4):228–241
82. US National Institutes of Health (n.d.). www.clinicaltrials.gov. 7th Aug 2017
83. Dieci MV et al (2014) Prognostic value of tumor-infiltrating lymphocytes on residual disease after primary chemotherapy for triple-negative breast cancer: a retrospective multicenter study. Ann Oncol 25(3):611–618
84. Mahmoud SM et al (2011) Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol Off J Am Soc Clin Oncol 29(15):1949–1955
85. Rody A et al (2011) A clinically relevant gene signature in triple negative and basal-like breast cancer. Breast Cancer Res: BCR 13(5):R97
86. Ye J et al (2013) Specific recruitment of gammadelta regulatory T cells in human breast cancer. Cancer Res 73(20):6137–6148
87. Ladoire S et al (2008) Pathologic complete response to neoadjuvant chemotherapy of breast carcinoma is associated with the disappearance of tumor-infiltrating foxp3+ regulatory T cells. Clin Cancer Res 14(8):2413–2420
88. Salgado R et al (2015) The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an international TILs working group 2014. Ann Oncol 26(2): 259–271
89. Perou CM et al (2000) Molecular portraits of human breast tumours. Nature 406(6797): 747–752
90. Sorlie T et al (2001) Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 98(19):10869–10874
91. Kennecke H et al (2010) Metastatic behavior of breast cancer subtypes. J Clin Oncol Off J Am Soc Clin Oncol 28(20):3271–3277
92. Korde LA et al (2010) Gene expression pathway analysis to predict response to neoadjuvant docetaxel and capecitabine for breast cancer. Breast Cancer Res Treat 119(3):685–699
93. Rouzier R et al (2005) Breast cancer molecular subtypes respond differently to preoperative chemotherapy. Clin Cancer Res 11(16):5678–5685
94. Parker JS et al (2009) Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 27(8):1160–1167
95. Sorlie T et al (2003) Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A 100(14):8418–8423
96. Hennessy BT et al (2009) Characterization of a naturally occurring breast cancer subset enriched in epithelial-to-mesenchymal transition and stem cell characteristics. Cancer Res 69(10):4116–4124
97. Farmer P et al (2005) Identification of molecular apocrine breast tumours by microarray analysis. Oncogene 24(29):4660–4671
98. Doane AS et al (2006) An estrogen receptor-negative breast cancer subset characterized by a hormonally regulated transcriptional program and response to androgen. Oncogene 25(28):3994–4008

99. Guedj M et al (2011) A refined molecular taxonomy of breast cancer. Oncogene 31:1196
100. Haque R et al (2012) Impact of breast cancer subtypes and treatment on survival: an analysis spanning two decades. Cancer Epidemiol Biomark Prev 21(10):1848–1855
101. Ciriello G et al (2013) The molecular diversity of Luminal A breast tumors. Breast Cancer Res Treat 141(3):409–420
102. (EBCTCG), E.B.C.T.C.G (2005) Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 365(9472):1687–1717
103. Jeselsohn R et al (2015) ESR1 mutations-a mechanism for acquired endocrine resistance in breast cancer. Nat Rev Clin Oncol 12(10):573–583
104. Creighton CJ (2012) The molecular profile of luminal B breast cancer. Biologics 6:289–297
105. Huang B, Warner M, Gustafsson JA (2015) Estrogen receptors in breast carcinogenesis and endocrine therapy. Mol Cell Endocrinol 418(Pt 3):240–244
106. Huang B et al (2014) Differential expression of estrogen receptor alpha, beta1, and beta2 in lobular and ductal breast cancer. Proc Natl Acad Sci U S A 111(5):1933–1938
107. Badve S et al (2011) Basal-like and triple-negative breast cancers: a critical review with an emphasis on the implications for pathologists and oncologists. Mod Pathol: Off J US Can Acade Pathol 24(2):157–167
108. Turner NC et al (2007) BRCA1 dysfunction in sporadic basal-like breast cancer. Oncogene 26(14):2126–2132
109. Lehmann BD, Pietenpol JA (2015) Clinical implications of molecular heterogeneity in triple negative breast cancer. Breast 24(Suppl 2):S36–S40
110. Fulford LG et al (2006) Specific morphological features predictive for the basal phenotype in grade 3 invasive ductal carcinoma of breast. Histopathology 49(1):22–34
111. Livasy CA et al (2006) Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. Mod Pathol 19(2):264–271
112. Banerjee S et al (2006) Basal-like breast carcinomas: clinical outcome and response to chemotherapy. J Clin Pathol 59(7):729–735
113. Bergamaschi A et al (2006) Distinct patterns of DNA copy number alteration are associated with different clinicopathological features and gene-expression subtypes of breast cancer. Genes Chromosomes Cancer 45(11):1033–1040
114. Chin K et al (2006) Genomic and transcriptional aberrations linked to breast cancer pathophysiologies. Cancer Cell 10(6):529–541
115. Hu Z et al (2006) The molecular portraits of breast tumors are conserved across microarray platforms. BMC Genomics 7:96
116. Fulford LG et al (2007) Basal-like grade III invasive ductal carcinoma of the breast: patterns of metastasis and long-term survival. Breast Cancer Res 9(1):R4
117. TCGA, Cancer Genome Atlas Network (2012) Comprehensive molecular portraits of human breast tumours. Nature 490(7418):61–70
118. Kwei KA et al (2010) Genomic instability in breast cancer: pathogenesis and clinical implications. Mol Oncol 4(3):255–266
119. Encinas G et al (2015) Somatic mutations in breast and serous ovarian cancer young patients: a systematic review and meta-analysis. Rev Assoc Med Bras (1992) 61(5):474–483
120. Jokinen E, Koivunen JP (2015) MEK and PI3K inhibition in solid tumors: rationale and evidence to date. Ther Adv Med Oncol 7(3):170–180
121. Burstein MD et al (2015) Comprehensive genomic analysis identifies novel subtypes and targets of triple-negative breast cancer. Clin Cancer Res 21(7):1688–1698
122. Lehmann BD et al (2011) Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 121(7):2750–2767
123. Lehmann BD et al (2016) Refinement of triple-negative breast cancer molecular subtypes: implications for neoadjuvant chemotherapy selection. PLoS One 11(6):e0157368
124. Prat A et al (2013) Molecular characterization of basal-like and non-basal-like triple-negative breast cancer. Oncologist 18(2):123–133

125. Stirzaker C et al (2015) Methylome sequencing in triple-negative breast cancer reveals distinct methylation clusters with prognostic value. Nat Commun 6:5899
126. van 't Veer LJ et al (2002) Gene expression profiling predicts clinical outcome of breast cancer. Nature 415(6871):530–536
127. van de Vijver MJ et al (2002) A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 347(25):1999–2009
128. Buyse M et al (2006) Validation and clinical utility of a 70-gene prognostic signature for women with node-negative breast cancer. J Natl Cancer Inst 98(17):1183–1192
129. Bueno-de-Mesquita JM et al (2009) Validation of 70-gene prognosis signature in node-negative breast cancer. Breast Cancer Res Treat 117(3):483–495
130. Cardoso F et al (2008) Clinical application of the 70-gene profile: the MINDACT trial. J Clin Oncol Off J Am Soc Clin Oncol 26(5):729–735
131. Goldstein LJ et al (2008) Prognostic utility of the 21-gene assay in hormone receptor-positive operable breast cancer compared with classical clinicopathologic features. J Clin Oncol Off J Am Soc Clin Oncol 26(25):4063–4071
132. Paik S (2007) Development and clinical utility of a 21-gene recurrence score prognostic assay in patients with early breast cancer treated with tamoxifen. Oncologist 12(6):631–635
133. Paik S et al (2004) A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 351(27):2817–2826
134. Filipits M et al (2011) A new molecular predictor of distant recurrence in ER-positive, HER2-negative breast cancer adds independent information to conventional clinical risk factors. Clin Cancer Res 17(18):6012–6020
135. Rakha EA et al (2014) Nottingham Prognostic Index Plus (NPI+): a modern clinical decision making tool in breast cancer. Br J Cancer 110(7):1688–1697
136. Lal S et al (2017) Molecular signatures in breast cancer. Methods 131:135
137. Santarpia L et al (2016) Deciphering and targeting oncogenic mutations and pathways in breast cancer. Oncologist 21(9):1063–1078
138. Finn RS et al (2015) The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. Lancet Oncol 16(1):25–35
139. Van Allen EM et al (2014) Whole-exome sequencing and clinical interpretation of formalin-fixed, paraffin-embedded tumor samples to guide precision cancer medicine. Nat Med 20(6):682–688
140. Kalita-de Croft P et al (2016) Omics approaches in breast cancer research and clinical practice. Adv Anat Pathol 23(6):356–367
141. Ding L et al (2012) Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. Nature 481(7382):506–510
142. Gerlinger M et al (2012) Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 366(10):883–892
143. Wu JM, Halushka MK, Argani P (2010) Intratumoral heterogeneity of HER-2 gene amplification and protein overexpression in breast cancer. Hum Pathol 41(6):914–917
144. Weigelt B et al (2003) Gene expression profiles of primary breast tumors maintained in distant metastases. Proc Natl Acad Sci U S A 100(26):15901–15905
145. Da Silva L et al (2010) HER3 and downstream pathways are involved in colonization of brain metastases from breast cancer. Breast Cancer Res 12(4):R46
146. Wu JM et al (2008) Heterogeneity of breast cancer metastases: comparison of therapeutic target expression and promoter methylation between primary tumors and their multifocal metastases. Clin Cancer Res: Off J Am Assoc Cancer Res 14(7):1938–1946
147. Arslan C et al (2011) Variation in hormone receptor and HER-2 status between primary and metastatic breast cancer: review of the literature. Expert Opin Ther Targets 15(1):21–30
148. St Romain P et al (2012) Organotropism and prognostic marker discordance in distant metastases of breast carcinoma: fact or fiction? A clinicopathologic analysis. Hum Pathol 43(3):398–404

149. Houssami N et al (2011) HER2 discordance between primary breast cancer and its paired metastasis: tumor biology or test artefact? Insights through meta-analysis. Breast Cancer Res Treat 129(3):659–674
150. Cummings MC et al (2014) Metastatic progression of breast cancer: insights from 50 years of autopsies. J Pathol 232(1):23–31
151. Cejalvo JM et al (2017) Intrinsic subtypes and gene expression profiles in primary and metastatic breast cancer. Cancer Res 77(9):2213–2221
152. Amir E et al (2012) Prospective study evaluating the impact of tissue confirmation of metastatic disease in patients with breast cancer. J Clin Oncol Off J Am Soc Clin Oncol 30(6):587–592
153. Van Poznak C et al (2015) Use of biomarkers to guide decisions on systemic therapy for women with metastatic breast cancer: American Society of Clinical Oncology Clinical Practice Guideline. J Clin Oncol 33(24):2695–U174
154. Almendro V et al (2014) Genetic and phenotypic diversity in breast tumor metastases. Cancer Res 74(5):1338–1348
155. Ding L et al (2010) Genome remodelling in a basal-like breast cancer metastasis and xenograft. Nature 464(7291):999–1005
156. Shah SP et al (2009) Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution. Nature 461(7265):809–813
157. Klein CA (2009) Parallel progression of primary tumours and metastases. Nat Rev Cancer 9(4):302–312
158. Yates LR et al (2017) Genomic evolution of breast cancer metastasis and relapse. Cancer Cell 32(2):169–184 e7
159. Shaw JA et al (2017) Mutation analysis of cell-free DNA and single circulating tumor cells in metastatic breast cancer patients with high circulating tumor cell counts. Clin Cancer Res 23(1):88–96
160. Sieuwerts AM et al (2011) mRNA and microRNA expression profiles in circulating tumor cells and primary tumors of metastatic breast cancer patients. Clin Cancer Res 17(11):3600–3618
161. Babayan A et al (2013) Heterogeneity of estrogen receptor expression in circulating tumor cells from metastatic breast cancer patients. PLoS One 8(9):e75038
162. Smerage JB et al (2014) Circulating tumor cells and response to chemotherapy in metastatic breast cancer: SWOG S0500. J Clin Oncol 32(31):3483–3489
163. Janni WJ et al (2016) Pooled analysis of the prognostic relevance of circulating tumor cells in primary breast cancer. Clin Cancer Res 22(10):2583–2593
164. Lv Q et al (2016) Prognostic value of circulating tumor cells in metastatic breast cancer: a systemic review and meta-analysis. Clin Transl Oncol 18(3):322–330
165. Dawson SJ et al (2013) Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 368(13):1199–1209
166. Alix-Panabieres C, Pantel K (2014) Challenges in circulating tumour cell research. Nat Rev Cancer 14(9):623–631
167. Murtaza M et al (2013) Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. Nature 497(7447):108–112
168. Garcia-Murillas I et al (2015) Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. Sci Transl Med 7(302):302ra133
169. Murtaza M et al (2015) Multifocal clonal evolution characterized using circulating tumour DNA in a case of metastatic breast cancer. Nat Commun 6:8760
170. Chan KC et al (2013) Cancer genome scanning in plasma: detection of tumor-associated copy number aberrations, single-nucleotide variants, and tumoral heterogeneity by massively parallel sequencing. Clin Chem 59(1):211–224
171. Openshaw MR et al (2016) The role of ctDNA detection and the potential of the liquid biopsy for breast cancer monitoring. Expert Rev Mol Diagn 16(7):751–755
172. Ellis MJ et al (2012) Whole-genome analysis informs breast cancer response to aromatase inhibition. Nature 486(7403):353–360