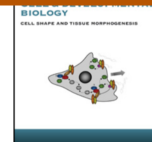
G Model
YSCDB-2126; No. of Pages 8
ARTICLE IN PRESS

Seminars in Cell & Developmental Biology xxx (2016) xxx–xxx

View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb



Review

Molecular heterogeneity in breast cancer: State of the science and implications for patient care

Rachel E. Ellsworth[a], Heather L. Blackburn[b], Craig D. Shriver[c], Patrick Soon-Shiong[d], Darrell L. Ellsworth[b,*]

[a] Murtha Cancer Center, Windber, PA, USA
[b] Chan Soon-Shiong Institute of Molecular Medicine at Windber, Windber, PA, USA
[c] Murtha Cancer Center, Walter Reed National Military Medical Center, Bethesda, MD, USA
[d] NantWorks, Culver City, CA, USA

ARTICLE INFO

Article history:
Received 12 August 2016
Accepted 24 August 2016
Available online xxx

Keywords:
Intratumor heterogeneity
Breast cancer
Metastasis
Circulating tumor cells
Cell-free DNA

ABSTRACT

The identification of extensive genetic heterogeneity in human breast carcinomas poses a significant challenge for designing effective treatment regimens. Significant genomic evolution often occurs during breast cancer progression, creating variability within primary tumors as well as between the primary carcinoma and metastases. Current risk allocations and treatment recommendations for breast cancer patients are based largely on characteristics of the primary tumor; however, genetic differences between disseminated tumor cells and the primary carcinoma may negatively impact treatment efficacy and survival. In this review we (1) present current information about genomic variability within primary breast carcinomas, between primary tumors and regional/distant metastases, among circulating tumor cells (CTCs) and disseminated tumor cells (DTCs), and in cell-free nucleic acids in circulation, and (2) describe how this heterogeneity affects clinical care and outcomes such as recurrence and therapeutic resistance. Understanding the evolution and functional significance of the composite breast cancer genome within each patient is critical for developing effective therapies that can overcome obstacles presented by molecular heterogeneity.

© 2016 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Contents

1. Introduction 00
2. Historical perspective 00
3. Cancer stem cells 00
4. Evaluation of breast tumor heterogeneity by single-cell analysis 00
   4.1. Breast cancer cell lines 00
   4.2. Primary breast tumors 00
5. Patient derived xenografts 00
6. Heterogeneity between primary breast carcinomas and metastases 00
   6.1. Regional and distant metastases 00
   6.2. Circulating and disseminated tumor cells 00
7. Heterogeneity among CTCs 00
   7.1. Genomic heterogeneity among CTCs 00
   7.2. Epithelial-mesenchymal transition in CTCs 00
8. Cell-free DNA 00
9. Clinical implications 00
10. Conclusions 00
Conflicts if interest 00
Acknowledgements 00
References 00

* Corresponding author at: Chan Soon–Shiong Institute of Molecular Medicine at Windber, 620 Seventh Street, Windber, PA 15963, USA.
E-mail address: d.ellsworth@wriwindber.org (D.L. Ellsworth).

http://dx.doi.org/10.1016/j.semcdb.2016.08.025
1084-9521/© 2016 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Please cite this article in press as: R.E. Ellsworth, et al., Molecular heterogeneity in breast cancer: State of the science and implications for patient care, Semin Cell Dev Biol (2016), http://dx.doi.org/10.1016/j.semcdb.2016.08.025

1. Introduction

Decades of observation and research have shown that malignant carcinomas are heterogeneous. Three main types of heterogeneity have been described: (1) population heterogeneity or differences among tumors from different patients, (2) intratumor or spatial heterogeneity within a single tumor mass, and (3) temporal heterogeneity reflecting variability over time during tumor growth and development or in response to treatment [1]. In this review, we focus on intratumor heterogeneity in breast cancer, with particular emphasis on molecular and genomic variability within carcinomas and among disseminated cells and cell-free nucleic acids, as well as the effect of heterogeneity on treatment response.

2. Historical perspective

Early observational studies of heterogeneity within a single carcinoma were conducted in animals. Histological studies of mouse mammary tumors revealed extensive variability in morphology of cells derived from a single tumor [2]. Further research detected the presence of distinct cell types within mammary tumors that differed markedly in growth properties, antigen expression, karyotype, and sensitivity/response to therapeutic agents [3,4]. These studies provided some of the first evidence that mammary carcinomas contain a number of distinct tumorigenic cell types that differ at the molecular level.

In humans, early studies observed that human breast tumors are also composed of multiple subpopulations of cells that differ in their histologic and biochemical properties. Cytogenetic studies detected heterogeneity in chromosomal alterations within archival breast cancer specimens [5] and found that human breast carcinomas exhibit intratumor differences in the expression of clinical biomarkers, such as estrogen (ER) and progesterone (PR) receptors, and the extent of human epidermal growth factor receptor 2 (HER2) gene amplification [6–8]. More recent studies have detected substantial spatial heterogeneity at the genomic level in primary breast carcinomas using comparative genomic hybridization [9] and genomic fingerprinting [10], with different patterns of chromosomal changes observed in different regions of the primary tumor.

3. Cancer stem cells

The idea that cancer develops from morphologically normal cells through the accumulation of genetic changes that activate oncogenic pathways and inactivate tumor suppressor genes is well-established [11]; however, the cells in which these mutations originate have not been well-characterized. Normal adult stem cells are relatively rare, largely quiescent cells that survive in an undifferentiated state for extended periods of time and have the capacity for unlimited self-renewal and the ability to generate morphologically diverse progeny cells [12]. Over time (years to decades), stem cells are hypothesized to acquire specific carcinogenesis-initiating mutations and become cancer stem cells. Further mutations in pathways that alter genome stability, proliferative potential, growth inhibition, normal cellular differentiation, and resistance to apoptosis may occur in more differentiated cells, leading to substantial genetic diversification among clonal populations of cells within the primary tumor [13,14]. The mutational history of stem cells from which carcinomas arise may influence heterogeneity and metastatic potential. Accordingly, tumors derived from early stem cells may have a greater capacity for genomic heterogeneity compared to carcinomas that arise from later stem-like cells [15].

In breast cancer, substantial intratumor heterogeneity for genetic alterations and activated signaling pathways has been observed among populations of putative breast cancer stem cells [16]. The Notch, Sonic hedgehog, and Wnt signaling pathways control cell growth, differentiation, and apoptosis in the regulation of normal breast development, and increasing evidence indicates that inherited or acquired mutations in one or more of these pathways may occur in cancer stem cells [17]. This genetic heterogeneity within a developing carcinoma may lead to phenotypic heterogeneity, which influences important clinical outcomes such as metastatic potential and therapeutic resistance [18].

4. Evaluation of breast tumor heterogeneity by single-cell analysis

Technological advancements for genomic analysis now permit the assessment of copy number changes and sequence variants at the single cell level. Next-generation sequencing of individual cells is sufficient to examine more than 90% of the genome with ~10× average exome coverage depth per cell [19]. Because rare *de novo* mutations and/or transcriptional changes in individual cells or small populations of cells cannot be detected when analyzing larger sections of tumors [20], "single-cell genomics" is increasingly being used to study individual cells from primary tumors, metastases, cancer stem cells, and circulating (CTCs) or disseminated (DTCs) tumor cells to guide diagnosis, prognosis, and treatment.

4.1. Breast cancer cell lines

Studies focusing on breast cancer cell lines have identified extensive cell to cell heterogeneity within a single cell line. One study focusing on single-cell-derived sub-clones from the HCC38 breast cancer cell line found extensive copy number heterogeneity among individual cells that could be attributed to novel DNA changes during cell division in genomically unstable cancer cells [21]. Sequencing of single cells from an ER-/HER2+ cell line revealed additional mutations not detected in bulk genomic DNA from the same cell line and showed that no two cells had identical mutation profiles [19]. Investigating the effects of heterogeneity on paclitaxel (Taxol) resistance using single-cell RNA-sequencing (RNAseq), Lee and colleagues [22] found significant molecular heterogeneity among individual cells that could not be detected in pooled cell populations of the MDA-MB-231 metastatic breast cancer cell line. Cell-specific RNA variants led to transcriptional heterogeneity and survival of cells capable of developing a drug-resistant phenotype.

4.2. Primary breast tumors

One of the earliest studies performing copy number evaluation on single cells from triple negative breast carcinomas identified varying numbers of distinct clonal subpopulations of cells, suggesting a punctuated clonal evolution of tumor growth, rather than gradual tumor progression [23]. A novel whole-genome and whole-exome single-cell sequencing method called Nuc-Seq showed that no two single cells from either luminal A or triple negative breast carcinomas exhibited identical genomic profiles [24]. The single-cell sequencing data suggest that the earliest steps of tumor development involve copy number changes that occur in punctuated bursts, but point mutations evolve gradually, generating extensive sequence diversity that may play an important role in the development of drug resistance (Fig. 1) [25]. Knowledge gained from single-cell studies may improve early detection and disease monitoring in breast cancer patients and promote the development of precise and personalized cancer therapy [26].

Please cite this article in press as: R.E. Ellsworth, et al., Molecular heterogeneity in breast cancer: State of the science and implications for patient care, Semin Cell Dev Biol (2016), http://dx.doi.org/10.1016/j.semcdb.2016.08.025

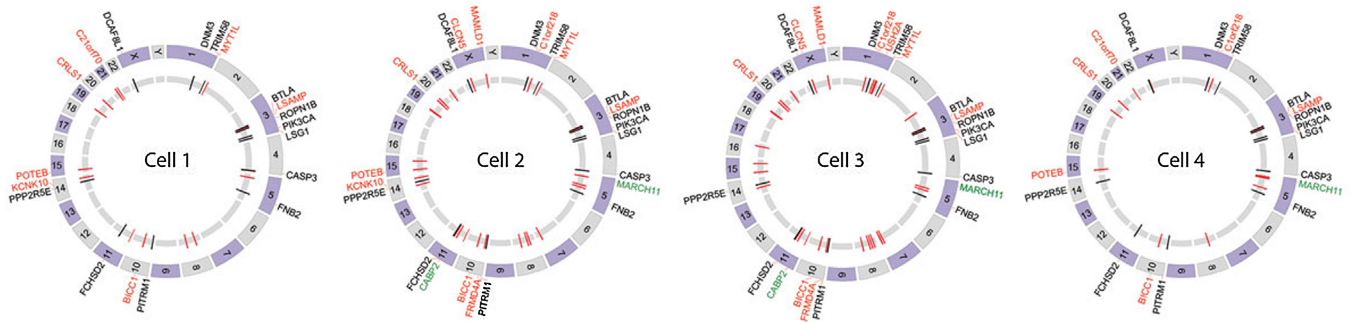


Fig. 1. Whole-genome sequencing of four individual tumor cells from an ER+ breast cancer patient showing extensive genetic heterogeneity at the single-cell level. From Ref. [25]. Reprinted with permission from AAAS.

5. Patient derived xenografts

To improve our understanding of tumor responses to various pharmacologic agents, patient-derived breast tumor xenografts have been established for several breast tumor subtypes. Xenografts are created by engrafting human breast tumor fragments derived from surgical specimens into immunodeficient mice. Xenografts are believed to faithfully recapitulate the histological complexity and molecular heterogeneity of the primary carcinoma, and thus, may serve as effective models for preclinical evaluation of potential chemotherapeutics. By monitoring tumor responses to various cancer therapies, the optimum treatment regimen for the patient can be selected [27].

Although xenograft models exhibit histopathological characteristics similar to the primary carcinoma, next-generation sequencing of a primary tumor, a xenograft derived from the primary tumor, and a brain metastasis from a triple negative breast cancer patient detected a wide range of allele frequencies for various mutations, enrichment of some *de novo* sequence mutations, and copy number variation in the metastasis and xenograft compared to the primary tumor. These data suggest that considerable genetic heterogeneity exists within the primary tumor and formation of metastases and xenografts selects for specific cells carrying a distinct subset of mutations present in the primary tumor [28].

Human breast cancer xenografts are generally assumed to be reasonable models of the primary tumors from which they were established; however, molecular heterogeneity within primary carcinomas can influence xenograft clonal dynamics and clonal selection. Whole genome single-cell sequencing has shown that in a minority of cases, clonal lineages observed in xenografts are similar to those in the primary tumor. In the majority of patients, molecular heterogeneity among xenografts is evident, suggesting that preexisting rare clones from the primary tumor often expand to dominate the xenografts [29].

6. Heterogeneity between primary breast carcinomas and metastases

6.1. Regional and distant metastases

Metastasis represents an important step in the progression of malignant breast cancer from a localized stage to an aggressive systemic disease. Despite the fact that distant metastases are responsible for the majority of cancer-related mortality, current risk allocations and treatment recommendations targeting breast cancer metastases are based largely on histological and molecular characteristics of the primary tumor [30]. However, genetic differences between metastases and the primary tumor may affect treatment efficacy.

Research on genetic differences between primary breast carcinomas and paired locoregional metastases have shown tremendous variation in the degree of genetic differentiation between primary tumors and metastases within patients [31]. Phylogenetic analyses have shown that multiple genetically divergent lineages of metastatic cells independently colonize the axillary lymph nodes [32]. Using large-scale DNA sequencing [28,33] and immunoFISH [34], researchers have observed marked single nucleotide and copy number differences between primary breast carcinomas and metastases.

These studies indicate that although metastases descend from the primary tumor, substantial genetic differences attributable to significant genomic evolution that often occurs with disease progression may be evident between the primary carcinoma and metastases. Genomic heterogeneity characteristic of metastatic breast cancer explains why biomarkers measured exclusively from the primary tumor may not be sufficiently informative for predicting responsiveness to therapy. To illustrate this point, the progressive Intensive Trial of Omics in Cancer (ITOMIC) was designed to prospectively enroll patients with triple negative breast cancer to appropriate therapies based on the cumulative molecular profile of the cancer over space (primary vs metastases) and time (progression) [35]. Comprehensive genomic, transcriptomic, and proteomic profiling were performed on multiple biopsies taken at study outset for each ITOMIC patient, followed by multiple biopsies at each progression and finally at autopsy. Spatial and temporal heterogeneity in single nucleotide variants, copy number variants, chromosomal rearrangements, and insertion/deletion polymorphisms were observed in primary carcinomas and between metastases (Fig. 2) [36]. These molecular differences within primary disease and between metastases may serve as the basis for outgrowth of sub-clonal disease in response to each administered therapy and suggest that the optimal strategy for treatment must include in-depth genomic and proteomic profiles of multiple biopsies across space and time for each individual patient.

6.2. Circulating and disseminated tumor cells

Research on the role of CTCs found in the bloodstream and DTCs in bone marrow of cancer patients has grown exponentially in recent years because the dissemination of cells from a primary carcinoma through the circulatory system is believed to be a critical step in the process of disease progression and metastasis. CTCs are viable cancer cells that are shed from a primary carcinoma and circulate throughout the vasculature, carrying genetic alterations found in the primary tumor [37]. Because CTCs are believed to initiate growth of metastatic deposits in distant organs, the ability to detect and quantify CTCs in peripheral blood of breast cancer patients may provide an easily accessible marker with high prognostic and predictive value. The ability to quantify CTCs through

Please cite this article in press as: R.E. Ellsworth, et al., Molecular heterogeneity in breast cancer: State of the science and implications for patient care, Semin Cell Dev Biol (2016), http://dx.doi.org/10.1016/j.semcdb.2016.08.025

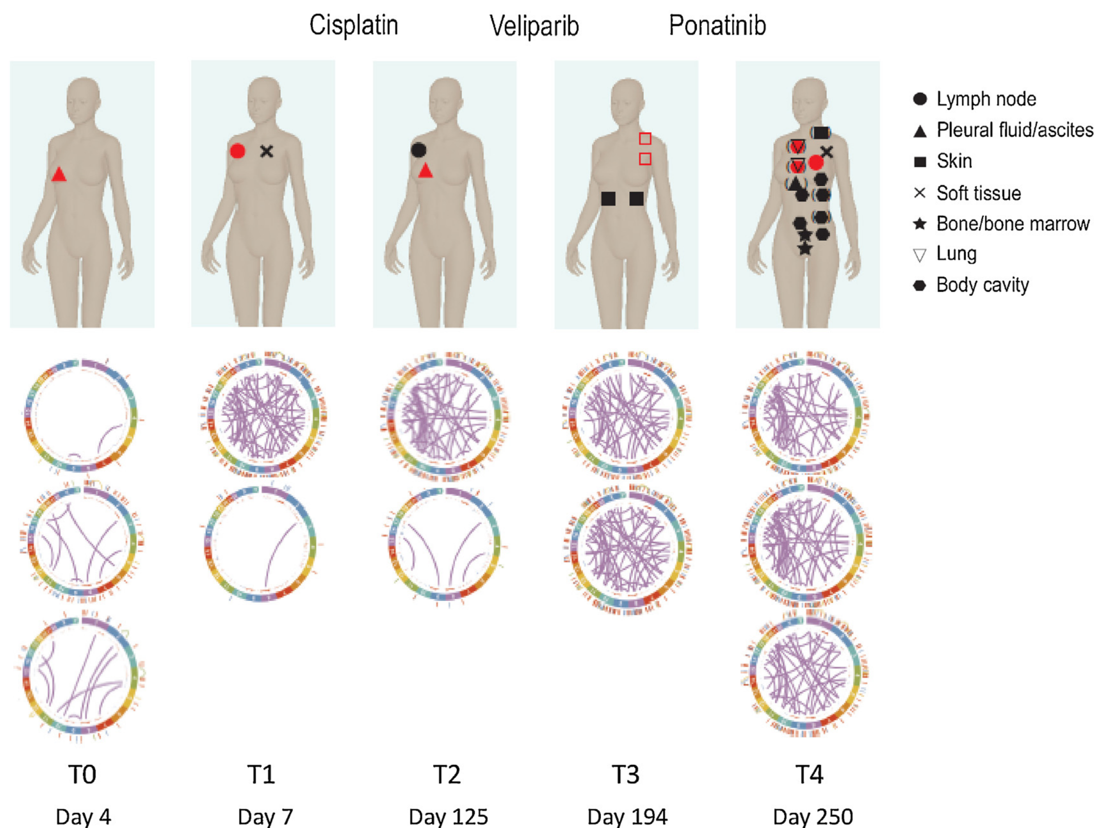


Fig. 2. Whole genome analysis of multiple biopsies from a 45-year-old woman diagnosed with triple-negative breast cancer demonstrates extensive spatial and temporal heterogeneity during treatment and reveals the evolution of molecular signatures that may lead to treatment failure.

minimally-invasive blood collection during treatment has led to the concept of using CTC assays as a "liquid biopsy". Pioneering research evaluated the prevalence of CTCs in the bloodstream of patients with metastatic disease versus healthy individuals to determine clinical utility. Evaluation of 422 metastatic breast cancer patients showed that CTC count varied dramatically among patients (84 ± 885 CTCs per 7.5 ml of whole blood) and 26% of patients had increased CTC counts (≥5 CTCs/7.5 ml whole blood) [38]. Further studies have established the presence and/or abundance of CTCs in whole blood as a reliable independent predictor of poor overall survival, disease-related mortality, unfavorable response to treatment, and early recurrence in numerous cancer types including breast cancer [39–43].

Although presence of CTCs is strongly prognostic in patients with breast cancer, enumeration alone is not sufficient to predict potential benefits from therapy. The SWOG S0500 randomized trial found that switching to an alternate cytotoxic therapy in metastatic breast cancer patients whose CTC counts remained high after 21 days of first-line chemotherapy did not improve overall survival [41]. Consequently, the American Society of Clinical Oncology (ASCO) guidelines on use of tumor markers in breast cancer recently concluded: (1) there is no evidence at this time that changing therapy solely on the basis of circulating biomarkers improves health outcomes, quality of life, or cost effectiveness; and (2) current data are insufficient to recommend use of CTC counts alone for monitoring response to treatment [44].

At present, ER, PR, and HER2 status are assessed in primary breast carcinomas as part of standard clinical practice; however, whole-genome sequence data suggest that DTCs arise from sub-clonal populations of cells in the primary tumor and undergo further sequence evolution after dissemination [45]. Cancer biomarkers and genetic variation therefore may change during disease progression, and significant molecular discordance with important therapeutic implications may develop between the primary tumor and corresponding CTCs [46,47].

In a substantial percentage of patients with early stage breast cancer, CTCs/DTCs exhibit amplification of the HER2 gene and expression of the HER2 gene product independent of HER2 status in the primary carcinoma [48]. Presence of CTCs expressing HER2 has been associated with increased mortality risk and is predictive of poor disease-free and overall survival. In women with early stage HER2-negative breast cancer, traditional adjuvant chemotherapy or tamoxifen could not completely eliminate HER2+ CTCs, and secondary adjuvant therapy with trastuzumab was required to eliminate chemotherapy-resistant HER2+ CTCs and improve disease-free survival [49]. Similarly, lapatinib administered to metastatic breast cancer patients with therapy-resistant HER2+ CTCs significantly decreased the number of HER2+ CTCs per patient but did not eliminate all HER2+ CTCs or significantly stop disease progression. Over the course of treatment, a population of lapatinib-resistant HER2+ and HER2- CTCs emerged that may be associated with disease recurrence [50].

Although the efficacy of changing treatment regimens based on conflicting biomarker status between CTCs and the primary tumor is currently inconclusive, clinical trials are ongoing to better characterize CTCs and evaluate their suitability to predict treatment response. The DETECT trials are evaluating therapies based on HER2 status of CTCs in patients with metastatic breast cancer and the safety of dual HER2-targeted therapy plus traditional endocrine or chemotherapy [51]. The TREAT-CTC trial is examining the usefulness of trastuzumab in patients with early HER2- primary breast carcinomas who have detectable HER2+ CTCs after chemotherapy [52].

7. Heterogeneity among CTCs

7.1. Genomic heterogeneity among CTCs

One reason that CTC counts have not been clinically useful for predicting potential benefits from therapy is the considerable molecular and functional heterogeneity observed among CTCs [53]. Genomic heterogeneity, including loss of heterozygosity and DNA variants in the phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA) gene, was detected among single CTCs within individual metastatic breast cancer patients [54]. Single cell analyses have detected differences in mutation patterns in the PIK3CA gene among CTCs from blood and disseminated tumor cells in tissues, and have observed changes in the mutational status in CTCs with disease progression [55,56]. Further studies using next-generation sequencing of 50 cancer-related genes in multiple CTCs from ER+/HER2- and triple negative breast tumors found significant genomic heterogeneity between CTCs and the matched primary tumor, as well as extensive heterogeneity among CTCs from the same patient [57]. These data suggest that assessing the genomic signatures of many circulating cancer cells via liquid biopsy in all cancer patients may be more informative than traditional biopsies of the primary tumor for designing targeted therapies and monitoring therapeutic response.

Studies examining gene and protein expression in single or pooled CTCs have found marked heterogeneity in the expression of known tumor biomarkers, even when comparing CTCs from a single venipuncture [58]. Considerable intra-patient heterogeneity in ER status was detected among CTCs in metastatic breast cancer patients with ER-positive primary tumors [59]. Gene expression profiling has detected heterogeneity in the expression of numerous genes among individual CTCs [60]. *In situ* hybridization and immunofluorescence analyses have detected a heterogeneous pattern of expression for miR-10b, a microRNA known to be upregulated in many cancers, within individual CTCs from breast cancer patients [61]. These studies suggest that overall tumor burden may be more thoroughly quantified by examining multiple CTCs/DTCs in each patient due to the vast heterogeneity among individual cells. As heterogeneity among circulating and disseminated tumor cells may be one mechanism by which patients become refractory to endocrine therapy, analysis of many circulating and disseminated tumor cells may provide important clinical information for improving treatment decisions. However, at present, determining which CTCs are clinically important and the number of CTCs that should be examined to provide clinically actionable information is largely unknown.

Beyond the value of CTC counts for predicting survival and recurrence, functional characteristics of CTCs are now being identified to tailor therapy and monitor disease progression. To better predict development of resistance to therapy in metastatic breast cancer patients, Paoletti and colleagues are evaluating combining CTC counts with protein expression assays of the ER, HER2, B-cell lymphoma 2 (BCL-2), and Ki-67 in individual CTCs. The resulting CTC-Endocrine Therapy Index is designed to provide a more comprehensive view of tumor heterogeneity within individual patients and may accommodate molecular heterogeneity that evolves during treatment [62].

Most studies of CTCs in peripheral blood and/or DTCs in bone marrow have focused on the prognostic significance of CTC/DTC burden, but recent reports show that molecular characterization of these cells is pivotal to optimizing therapeutic regimens. Overall genomic complexity and specific driver mutations present in disseminated cells may be an important factor in treatment failure; therefore, assessing the genomic signatures of many circulating cancer cells in all patients may be more informative than traditional biopsies of the primary tumor for designing targeted therapies and monitoring therapeutic response.

7.2. Epithelial-mesenchymal transition in CTCs

The epithelial-mesenchymal transition (EMT) is a process by which epithelial cells undergo biochemical changes causing them to lose their cellular polarity and cell-cell adhesiveness, hence acquiring properties commonly observed in mesenchymal cells, such as motility, invasiveness, and resistance to apoptosis. EMT is important in the progression of epithelial cancers such as breast cancer and may be induced by a diverse array of cytokines and growth factors. Cells that disseminate from the primary carcinoma undergo aberrant activation of EMT, thereby losing their epithelial characteristics and acquiring more mesenchymal-like phenotypes [63]. CTCs isolated from breast cancer patients show extensive variability in the expression of epithelial cell adhesion molecule (EpCAM) and other epithelial markers, and patients with metastatic breast cancer may carry several populations of CTCs defined by the presence of epithelial markers, mesenchymal markers, or co-expression of epithelial and mesenchymal markers. Analysis of CTCs captured from whole blood of breast cancer patients defined five categories of cells with exclusively epithelial, intermediate, and exclusively mesenchymal characteristics [64]. The fraction of CTCs co-expressing epithelial and mesenchymal markers has been significantly associated with poorer progression-free and overall survival [65].

EMT in CTCs and DTCs adds to the already complex heterogeneity of these disseminated cells and may complicate their use for outcome prediction [66]. CTCs that are in the process of EMT or have transitioned to a mesenchymal cell phenotype may provide important information regarding tumor progression and metastasis, but current CTC-enrichment technologies that specifically target epithelial markers to differentiate CTCs from other cell types may underestimate the number of CTCs and potentially miss critical subpopulations of cells that are undergoing or have completed EMT [67]. As current methods do not completely detect the heterogeneous population of CTCs, new technologies are needed to improve the detection and isolation of CTCs with mesenchymal characteristics. The cell-surface protein vimentin has shown promise for detecting epithelial-mesenchymal transitioned CTCs in the blood of patients with epithelial cancers [68]. Similarly, the melanoma cell adhesion molecule (MCAM), which when combined with traditional EpCAM enrichment, significantly improved detection of CTCs in breast cancer patients undergoing neoadjuvant chemotherapy compared to EpCAM alone [69]. These studies demonstrate the potential importance of detecting cells with mesenchymal features, which likely represent clinically relevant subpopulations of CTCs, as part of a comprehensive liquid biopsy.

8. Cell-free DNA

Cell-free DNA (cfDNA) is composed of nucleic acid fragments released from cells during apoptosis, necrosis, and macrophage phagocytosis that are found in the blood stream and other bodily fluids. Although cfDNA can be identified in healthy individuals, cancer patients tend to exhibit higher levels of cfDNA, with significantly higher cfDNA concentrations observed in serum of patients with metastatic disease (209 ± 39 ng/ml) compared to non-metastatic patients (100 ± 30 ng/ml, $p < 0.02$) [70]. A portion of the cfDNA, termed circulating tumor DNA (ctDNA), is far more abundant in the bloodstream than CTCs and is rapidly cleared from the circulation within hours. In addition, ctDNA profiles in metastatic breast cancer patients accurately reflect the mutational composition of individual CTCs [71].

Please cite this article in press as: R.E. Ellsworth, et al., Molecular heterogeneity in breast cancer: State of the science and implications for patient care, Semin Cell Dev Biol (2016), http://dx.doi.org/10.1016/j.semcdb.2016.08.025

Recent years have seen growing enthusiasm for developing clinical applications for ctDNA. Methods have been developed to quantify levels of ctDNA in the circulation and conduct genomic analysis of tumor-specific mutations in circulating DNA. Primary breast carcinomas usually contain a mixture of clonal populations of cells, which in part, account for the intratumor genetic heterogeneity discussed above. During disease progression, tumor DNA may be released from these genetically-unique subpopulations of cells, contributing to sequence heterogeneity in ctDNA. Studies have shown concordance between the mutation status of PIK3CA gene mutations in matched primary tumor and plasma-derived circulating DNA from breast cancer patients [72,73]; however, recent studies using next-generation sequencing of circulating ctDNA have identified genomic heterogeneity with potentially important clinical consequences between primary carcinomas and ctDNA [74].

Investigating ctDNA by monthly sampling of plasma in patients with various cancer types who had completed two courses of targeted therapy identified variability at baseline among patients in the pattern of mutations in ctDNA, with TP53, PIK3CA, and KRAS being the most mutated genes [75]. Continued monitoring of ctDNA suggested different clinical responses in these patients may be attributable to novel genetic variation that arises among clonal populations of tumor cells during therapy. Similarly, targeted amplicon sequencing of an ER+/HER2+ primary breast carcinoma, multiple metastatic deposits, and plasma samples collected during ~3 years of targeted therapy in a 42-year-old woman revealed mutational heterogeneity that correlated with different treatment responses across metastases [76]. In a patient with ER+/HER2- breast cancer that had metastasized to the liver, patterns of mutations in archival primary tumor DNA, synchronous liver metastasis DNA, and ctDNA collected from plasma at various time points were evaluated using massively parallel sequencing of 300 cancer-related genes known to harbor actionable mutations [77]. Sixteen somatic non-synonymous mutations were identified in the liver metastasis, of which, only nine were detected in the primary tumor. However, all mutations present in the primary tumor and/or liver metastasis were captured in the ctDNA. Mutant allele frequencies in ctDNA varied over the course of treatment and mirrored response to targeted therapy.

Overall ctDNA levels in plasma correlate well with changes in tumor burden, and thus, may prove to be a valuable tool for monitoring breast cancer progression [78] and for real-time, noninvasive sampling of treatment response in women with metastatic breast cancer [79]. Sequencing of serial plasma samples has been used successfully to track genomic evolution of metastatic breast cancer in response to therapy [80]. Over the course of treatment, increased frequencies of mutations in important genes, such as PIK3CA, mediator complex subunit 1 (MED1), and growth arrest-specific 6 (GAS6), which are associated with emergence of therapy resistance, have been identified in cfDNA.

These studies suggest that ctDNA may provide a more complete picture of the mutational landscape of metastatic disease and early molecular response to treatment than CTCs or invasive biopsies. Next-generation targeted sequencing of plasma-derived ctDNA constitutes a potentially important tool for detecting *de novo* driver mutations that occur during cancer progression, monitoring changes in the frequency of genetic alterations during the course of targeted therapy, and identifying mutations associated with acquired drug resistance in patients with advanced disease [81]. Other potential applications include detecting residual disease following chemotherapy, noninvasive tumor genotyping, and early detection of cancer recurrence. At present, use of ctDNA has not been validated for routine clinical use in breast cancer patients and additional research is ongoing to identify diagnostic and prognostic uses of ctDNA that overcome the complexities posed by intra-tumor genetic heterogeneity.

9. Clinical implications

In clinical practice, a single tumor biopsy is likely to contain only a minority of genetic aberrations present in the entire carcinoma, which leads to an underestimation of the mutational burden of heterogeneous tumors [10] and inaccurate prediction of appropriate therapy [82]. Currently, breast cancer patients rarely receive a metastatic site biopsy; however, biopsies of metastatic deposits may become a clinical necessity for all patients to ensure therapies effectively target genomic heterogeneity between the primary carcinoma and metastases [83]. Although discordance in receptor status between primary and metastatic lesions may lead to detrimental outcomes, if clinicians alter patient management based on results of a metastatic biopsy, discordance is no longer associated with detrimental effects on outcome [84].

In addition to metastatic deposits, routine serial monitoring of breast cancer heterogeneity during disease progression or in response to treatment in CTCs, DTCs, and/or ctDNA shows great promise for overcoming sampling bias when only a limited number of biopsies are available from the primary tumor or metastases. However, ctDNA studies have not been established in the clinical environment and many insurance companies do not cover multiple molecular tests on liquid biopsies throughout the course of treatment.

Many traditional cancer therapeutics have been developed to target rapidly proliferating cells that comprise the bulk of the primary tumor [85]. These agents usually produce clinically encouraging results in initial phases of treatment, exemplified by impressive decreases in the size of the primary tumor; however, clinical remission is often temporary as relatively quiescent, and possibly genetically diverse, cancer stem cells may survive and lead to recurrence once therapy is completed. Although the choice of targeted therapy is often based on mutations present in an initial biopsy specimen, these “actionable” mutations may no longer drive disease progression once tumor cells disseminate from the primary carcinoma. Likewise, the predominant clone(s) in the primary tumor may not be prevalent in the metastases or CTCs due to clonal selection that occurs with certain therapies. Thus, it is vitally important to identify which clone(s) within a cancer patient is(are) the most biologically relevant to disease progression or therapeutic resistance [86].

Radically new practices for optimal clinical management of breast cancer patients are urgently needed, but identification and implementation of such strategies will be challenging. Large clinical trials must assess the value of matching patients to specific interventions or targeted therapies based on genetic profiling and determine if comprehensive genomic characterization and serial monitoring meaningfully improve patient care [87]. If multiple clinically actionable variants are present, a consistent framework must be developed for interpreting complex genomic results and prioritizing treatments. Other areas of investigation that are needed to improve patient outcomes include: (1) selecting combinations of agents that appropriately target driver mutations; (2) determining how best to deal with deleterious (passenger) mutations that may not be the primary drivers of carcinogenesis or metastases; and (3) fully exploiting the promise of immunotherapy [88].

10. Conclusions

Extensive intraindividual genetic heterogeneity has been identified in breast cancer patients, from primary carcinomas to metastases to disseminated cancer cells and cell-free DNA. This

Please cite this article in press as: R.E. Ellsworth, et al., Molecular heterogeneity in breast cancer: State of the science and implications for patient care, Semin Cell Dev Biol (2016), http://dx.doi.org/10.1016/j.semcdb.2016.08.025

heterogeneity is dynamic and evolves unpredictably during disease progression, creating significant challenges for modern chemotherapeutics [89]. The extent of heterogeneity in breast cancer will likely necessitate a paradigm shift from standard pathological classifications of breast carcinomas to a more personalized approach in which heterogeneity is thoroughly characterized prior to treatment [90]. Recent advances in DNA sequencing technologies have been useful for identifying genomic heterogeneity among subclonal populations of tumor cells, but the extent of genomic (and transcriptional) heterogeneity at the individual cell level within primary breast tumors and metastases remains largely unknown. Large scale clinical trials encompassing various stages of breast cancer will be needed to determine the clinical value of spatial and temporal variation in the genomic landscape of breast cancer for guiding treatment.

Conflicts if interest

Authors disclose no potential conflicts of interest.

Acknowledgements

Opinions and assertions expressed herein are private views of the authors and do not reflect the official policy of the Department of Army/Navy/Air Force, Department of Defense, or US Government. Identification of specific products or scientific instrumentation does not constitute endorsement by the authors, Department of Defense, or any component agency. Supported in part by the Department of Defense Breast Cancer Research Program, W81XWH-11-2-0135.

References

[1] D.R. Welch, Tumor heterogeneity-a “contemporary concept” founded on historical insights and predictions, Cancer Res. 76 (2016) 4–6.
[2] T.B. Dunn, Morphology of mammary tumors in mice, in: F. Homburger, N.H. Fishman (Eds.), Physiopathology of Cancer, 2nd edn., Paul B. Hoeber, Inc., New York, 1959, pp. 38–84.
[3] D.L. Dexter, H.M. Kowalski, B.A. Blazar, Z. Fligiel, R. Vogel, G.H. Heppner, Heterogeneity of tumor cells from a single mouse mammary tumor, Cancer Res. 38 (1978) 3174–3181.
[4] G.H. Heppner, D.L. Dexter, T. DeNucci, F.R. Miller, P. Calabresi, Heterogeneity in drug sensitivity among tumor cell subpopulations of a single mammary tumor, Cancer Res. 38 (1978) 3758–3763.
[5] M. Fiegl, C. Tueni, T. Schenk, et al., Interphase cytogenetics reveals a high incidence of aneuploidy and intra-tumour heterogeneity in breast cancer, Br. J. Cancer 72 (1995) 51–55.
[6] B.W. Davis, D.T. Zava, G.W. Locher, A. Goldhirsch, W.H. Hartmann, Receptor heterogeneity of human breast cancer as measured by multiple intratumoral assays of estrogen and progesterone receptor, Eur. J. Cancer Clin. Oncol. 20 (1984) 375–382.
[7] L.P. Pertschuk, C.A. Axiotis, J.G. Feldman, Y.D. Kim, S.J. Karavattayhayyil, L. Braithwaite, Marked intratumoral heterogeneity of the proto-oncogene Her-2/neu determined by three different detection systems, Breast J. 5 (1999) 369–374.
[8] A. Nassar, A. Radhakrishnan, I.A. Cabrero, G.A. Cotsonis, C. Cohen, Intratumoral heterogeneity of immunohistochemical marker expression in breast carcinoma: a tissue microarray-based study, Appl. Immunohistochem. Mol. Morphol. 18 (2010) 433–441.
[9] L. Torres, F.R. Ribeiro, N. Pandis, J.A. Andersen, S. Heim, M.R. Teixeira, Intratumor genomic heterogeneity in breast cancer with clonal divergence between primary carcinomas and lymph node metastases, Breast Cancer Res. Treat. 102 (2007) 143–155.
[10] R.E. Ellsworth, A.L. Toro, H.L. Blackburn, et al., Molecular heterogeneity in primary breast carcinomas and axillary lymph node metastases assessed by genomic fingerprinting analysis, Cancer Growth Metastasis 8 (2015) 15–24.
[11] K. Polyak, W.C. Hahn, Roots and stems: stem cells in cancer, Nat. Med. 12 (2006) 296–300.
[12] M. Al-Hajj, M.W. Becker, M. Wicha, I. Weissman, M.F. Clarke, Therapeutic implications of cancer stem cells, Curr. Opin. Genet. Dev. 14 (2004) 43–47.
[13] G. Dontu, M. Al-Hajj, W.M. Abdallah, M.F. Clarke, M.S. Wicha, Stem cells in normal breast development and breast cancer, Cell Prolif. 36 (Suppl. 1) (2003) 59–72.
[14] B.M. Boman, M.S. Wicha, Cancer stem cells: a step toward the cure, J. Clin. Oncol. 26 (2008) 2795–2799.
[15] S.M. Tu, S.H. Lin, C.J. Logothetis, Stem-cell origin of metastasis and heterogeneity in solid tumours, Lancet Oncol. 3 (2002) 508–513.
[16] M. Shipitsin, L.L. Campbell, P. Argani, et al., Molecular definition of breast tumor heterogeneity, Cancer Cell 11 (2007) 259–273.
[17] T. Reya, S.J. Morrison, M.F. Clarke, I.L. Weissman, Stem cells, cancer, and cancer stem cells, Nature 414 (2001) 105–111.
[18] H. Clevers, The cancer stem cell: premises, promises and challenges, Nat. Med. 17 (2011) 313–319.
[19] K.E. Szulwach, P. Chen, X. Wang, et al., Single-cell genetic analysis using automated microfluidics to resolve somatic mosaicism, PLoS One 10 (2015) e0135007.
[20] I.C. Macaulay, T. Voet, Single cell genomics: advances and future perspectives, PLoS Genet. 10 (2014) e1004126.
[21] T. Voet, P. Kumar, P. Van Loo, et al., Single-cell paired-end genome sequencing reveals structural variation per cell cycle, Nucleic Acids Res. 41 (2013) 6119–6138.
[22] M.C. Lee, F.J. Lopez-Diaz, S.Y. Khan, et al., Single-cell analyses of transcriptional heterogeneity during drug tolerance transition in cancer cells by RNA sequencing, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) E4726–E4735.
[23] N. Navin, J. Kendall, J. Troge, et al., Tumour evolution inferred by single-cell sequencing, Nature 472 (2011) 90–94.
[24] Y. Wang, J. Waters, M.L. Leung, et al., Clonal evolution in breast cancer revealed by single nucleus genome sequencing, Nature 512 (2014) 155–160.
[25] N.E. Navin, Delineating cancer evolution with single-cell sequencing, Sci. Transl. Med. 7 (2015) 296fs29.
[26] X. Zhang, S.L. Marjani, Z. Hu, S.M. Weissman, X. Pan, S. Wu, Single-cell sequencing for precise cancer research: progress and prospects, Cancer Res. 76 (2016) 1305–1312.
[27] M.D. Landis, B.D. Lehmann, J.A. Pietenpol, J.C. Chang, Patient-derived breast tumor xenografts facilitating personalized cancer therapy, Breast Cancer Res. 15 (2013) 201.
[28] L. Ding, M.J. Ellis, S. Li, et al., Genome remodelling in a basal-like breast cancer metastasis and xenograft, Nature 464 (2010) 999–1005.
[29] P. Eirew, A. Steif, J. Khattra, et al., Dynamics of genomic clones in breast cancer patient xenografts at single-cell resolution, Nature 518 (2015) 422–426.
[30] National Comprehensive Cancer Network, NCCN clinical practice guidelines in oncology, Breast Cancer (2016), version 2. 2016 (accessed: 19.07.16).
[31] T. Kuukasjärvi, R. Karhu, M. Tanner, et al., Genetic heterogeneity and clonal evolution underlying development of asynchronous metastasis in human breast cancer, Cancer Res. 57 (1997) 1597–1604.
[32] T.E. Becker, R.E. Ellsworth, B. Deyarmin, et al., The genomic heritage of lymph node metastases: implications for clinical management of patients with breast cancer, Ann. Surg. Oncol. 15 (2008) 1056–1063.
[33] S.P. Shah, R.D. Morin, J. Khattra, et al., Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution, Nature 461 (2009) 809–813.
[34] V. Almendro, H.J. Kim, Y.K. Cheng, et al., Genetic and phenotypic diversity in breast tumor metastases, Cancer Res. 74 (2014) 1338–1348.
[35] C.A. Blau, A.B. Ramirez, S. Blau, et al., A distributed network for intensive longitudinal monitoring in metastatic triple-negative breast cancer, J. Natl. Compr. Canc. Netw. 14 (2016) 8–17.
[36] P. Soon-Shiong, S. Rabizadeh, S. Benz, et al., Integrating whole exome sequencing data with RNAseq and quantitative proteomics to better inform clinical treatment decisions in patients with metastatic triple negative breast cancer, Cancer Res. 76 (Suppl. 4) (2016), P6-05-08.
[37] T. Fehm, A. Sagalowsky, E. Clifford, et al., Cytogenetic evidence that circulating epithelial cells in patients with carcinoma are malignant, Clin. Cancer Res. 8 (2002) 2073–2084.
[38] W.J. Allard, J. Matera, M.C. Miller, et al., Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases, Clin. Cancer Res. 10 (2004) 6897–6904.
[39] G. Wiedswang, E. Borgen, R. Kåresen, et al., Detection of isolated tumor cells in bone marrow is an independent prognostic factor in breast cancer, J. Clin. Oncol. 21 (2003) 3469–3478.
[40] N. Xenidis, M. Perraki, M. Kafousi, et al., Predictive and prognostic value of peripheral blood cytokeratin-19 mRNA-positive cells detected by real-time polymerase chain reaction in node-negative breast cancer patients, J. Clin. Oncol. 24 (2006) 3756–3762.
[41] J.B. Smerage, W.E. Barlow, G.N. Hortobagyi, et al., Circulating tumor cells and response to chemotherapy in metastatic breast cancer: SWOG S0500, J. Clin. Oncol. 32 (2014) 3483–3489.
[42] W.J. Janni, B. Rack, L.W. Terstappen, et al., Pooled analysis of the prognostic relevance of circulating tumor cells in primary breast cancer, Clin. Cancer Res. 22 (2016) 2583–2593.
[43] Q. Lv, L. Gong, T. Zhang, et al., Prognostic value of circulating tumor cells in metastatic breast cancer: a systemic review and meta-analysis, Clin. Transl. Oncol. 18 (2016) 322–330.
[44] C. Van Poznak, M.R. Somerfield, R.C. Bast, et al., 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 (2015) 2695–2704.
[45] E.K. Møller, P. Kumar, T. Voet, et al., Next-generation sequencing of disseminated tumor cells, Front. Oncol. 3 (2013) 320.
[46] T. Fehm, V. Müller, B. Aktas, et al., HER2 status of circulating tumor cells in patients with metastatic breast cancer: a prospective, multicenter trial, Breast Cancer Res. Treat. 124 (2010) 403–412.

[47] A.M. Sieuwerts, B. Mostert, J. Bolt-de Vries, et al., mRNA and microRNA expression profiles in circulating tumor cells and primary tumors of metastatic breast cancer patients, Clin. Cancer Res. 17 (2011) 3600–3618.
[48] V. Mikulová, M. Cabiòaková, I. Janatková, O. Mestek, T. Zima, P. Tesaøová, Detection of circulating tumor cells during follow-up of patients with early breast cancer: clinical utility for monitoring of therapy efficacy, Scand. J. Clin. Lab. Invest. 74 (2014) 132–142.
[49] V. Georgoulias, V. Bozionelou, S. Agelaki, et al., Trastuzumab decreases the incidence of clinical relapses in patients with early breast cancer presenting chemotherapy-resistant CK-19mRNA-positive circulating tumor cells: results of a randomized phase II study, Ann. Oncol. 23 (2012) 1744–1750.
[50] S. Agelaki, A. Kalykaki, H. Markomanolaki, et al., Efficacy of lapatinib in therapy-resistant HER2-positive circulating tumor cells in metastatic breast cancer, PLoS One 10 (2015) e0123683.
[51] A. Schramm, T.W. Friedl, F. Schochter, et al., Therapeutic intervention based on circulating tumor cell phenotype in metastatic breast cancer: concept of the DETECTstudy program, Arch. Gynecol. Obstet. 293 (2016) 271–281.
[52] F.C. Bidard, T. Fehm, M. Ignatiadis, et al., Clinical application of circulating tumor cells in breast cancer: overview of the current interventional trials, Cancer Metastasis Rev. 32 (2013) 179–188.
[53] F.C. Bidard, C. Proudhon, J.Y. Pierga, Circulating tumor cells in breast cancer, Mol. Oncol. 10 (2016) 418–430.
[54] M. Pestrin, F. Salvianti, F. Galardi, et al., Heterogeneity of PIK3CA mutational status at the single cell level in circulating tumor cells from metastatic breast cancer patients, Mol. Oncol. 9 (2015) 749–757.
[55] G. Deng, S. Krishnakumar, A.A. Powell, et al., Single cell mutational analysis of PIK3CA in circulating tumor cells and metastases in breast cancer reveals heterogeneity, discordance, and mutation persistence in cultured disseminated tumor cells from bone marrow, BMC Cancer 14 (2014) 456.
[56] A. Markou, S. Farkona, C. Schiza, et al., PIK3CA mutational status in circulating tumor cells can change during disease recurrence or progression in patients with breast cancer, Clin. Cancer Res. 20 (2014) 5823–5834.
[57] F. De Luca, G. Rotunno, F. Salvianti, et al., Mutational analysis of single circulating tumor cells by next generation sequencing in metastatic breast cancer, Oncotarget 7 (2016) 26107–26119.
[58] W. Onstenk, J.W. Gratama, J.A. Foekens, S. Sleijfer, Towards a personalized breast cancer treatment approach guided by circulating tumor cell (CTC) characteristics, Cancer Treat. Rev. 39 (2013) 691–700.
[59] A. Babayan, J. Hannemann, J. Spötter, V. Müller, K. Pantel, S.A. Joosse, Heterogeneity of estrogen receptor expression in circulating tumor cells from metastatic breast cancer patients, PLoS One 8 (2013) e75038.
[60] A.A. Powell, A.H. Talasaz, H. Zhang, et al., Single cell profiling of circulating tumor cells: transcriptional heterogeneity and diversity from breast cancer cell lines, PLoS One 7 (2012) e33788.
[61] C. Gasch, P.N. Plummer, L. Jovanovic, et al., Heterogeneity of miR-10b expression in circulating tumor cells, Sci. Rep. 5 (2015) 15980.
[62] C. Paoletti, M.C. Muñiz, D.G. Thomas, et al., Development of circulating tumor cell-endocrine therapy index in patients with hormone receptor-positive breast cancer, Clin. Cancer Res. 21 (2015) 2487–2498.
[63] A. Bonnomet, A. Brysse, A. Tachsidis, et al., Epithelial-to-mesenchymal transitions and circulating tumor cells, J. Mammary Gland Biol. Neoplasia 15 (2010) 261–273.
[64] M. Yu, A. Bardia, B.S. Wittner, et al., Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition, Science 339 (2013) 580–584.
[65] M. Bulfoni, L. Gerratana, F. Del Ben, et al., In patients with metastatic breast cancer the identification of circulating tumor cells in epithelial-to-mesenchymal transition is associated with a poor prognosis, Breast Cancer Res. 18 (2016) 30.
[66] P.K. Grover, A.G. Cummins, T.J. Price, I.C. Roberts-Thomson, J.E. Hardingham, Circulating tumour cells: the evolving concept and the inadequacy of their enrichment by EpCAM-based methodology for basic and clinical cancer research, Ann. Oncol. 25 (2014) 1506–1516.
[67] K.A. Hyun, K.B. Goo, H. Han, et al., Epithelial-to-mesenchymal transition leads to loss of EpCAM and different physical properties in circulating tumor cells from metastatic breast cancer, Oncotarget 7 (2016) 24677–24687.
[68] A. Satelli, A. Mitra, Z. Brownlee, et al., Epithelial-mesenchymal transitioned circulating tumor cells capture for detecting tumor progression, Clin. Cancer Res. 21 (2015) 899–906.
[69] W. Onstenk, J. Kraan, B. Mostert, et al., Improved circulating tumor cell detection by a combined EpCAM and MCAM CellSearch enrichment approach in patients with breast cancer undergoing neoadjuvant chemotherapy, Mol. Cancer Ther. 14 (2015) 821–827.
[70] S.A. Leon, B. Shapiro, D.M. Sklaroff, M.J. Yaros, Free DNA in the serum of cancer patients and the effect of therapy, Cancer Res. 37 (1977) 646–650.
[71] J.A. Shaw, D.S. Guttery, A. Hills, et al., Mutation analysis of cell-free DNA and single circulating tumor cells in metastatic breast cancer patients with high CTC counts, Clin. Cancer Res. (2016), in press.
[72] R.E. Board, A.M. Wardley, J.M. Dixon, et al., Detection of PIK3CA mutations in circulating free DNA in patients with breast cancer, Breast Cancer Res. Treat. 120 (2010) 461–467.
[73] M.J. Higgins, D. Jelovac, E. Barnathan, et al., Detection of tumor PIK3CA status in metastatic breast cancer using peripheral blood, Clin. Cancer Res. 18 (2012) 3462–3469.
[74] T.M. Butler, K. Johnson-Camacho, M. Peto, et al., Exome sequencing of cell-free DNA from metastatic cancer patients identifies clinically actionable mutations distinct from primary disease, PLoS One 10 (2015) e0136407.
[75] J.S. Frenel, S. Carreira, J. Goodall, et al., Serial next-generation sequencing of circulating cell-free DNA evaluating tumor clone response to molecularly targeted drug administration, Clin. Cancer Res. 21 (2015) 4586–4596.
[76] M. Murtaza, S.J. Dawson, K. Pogrebniak, et al., Multifocal clonal evolution characterized using circulating tumour DNA in a case of metastatic breast cancer, Nat. Commun. 6 (2015) 8760.
[77] L. De Mattos-Arruda, B. Weigelt, J. Cortes, et al., Capturing intra-tumor genetic heterogeneity by de novo mutation profiling of circulating cell-free tumor DNA: a proof-of-principle, Ann. Oncol. 25 (2014) 1729–1735.
[78] J.V. Canzoniero, B.H. Park, Use of cell free DNA in breast oncology, Biochim. Biophys. Acta 1865 (2016) 266–274.
[79] S.J. Dawson, D.W. Tsui, M. Murtaza, et al., Analysis of circulating tumor DNA to monitor metastatic breast cancer, N. Engl. J. Med. 368 (2013) 1199–1209.
[80] M. Murtaza, S.J. Dawson, D.W. Tsui, et al., Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA, Nature 497 (2013) 108–112.
[81] H. Schwarzenbach, D.S. Hoon, K. Pantel, Cell-free nucleic acids as biomarkers in cancer patients, Nat. Rev. Cancer 11 (2011) 426–437.
[82] M. Gerlinger, A.J. Rowan, S. Horswell, et al., Intratumor heterogeneity and branched evolution revealed by multiregion sequencing, N. Engl. J. Med. 366 (2012) 883–892.
[83] T. Foukakis, G. Åström, L. Lindström, T. Hatschek, J. Bergh, When to order a biopsy to characterise a metastatic relapse in breast cancer, Ann. Oncol. 23 (Suppl. 10) (2012) x349–x353.
[84] E. Amir, N. Miller, W. Geddie, et al., Prospective study evaluating the impact of tissue confirmation of metastatic disease in patients with breast cancer, J. Clin. Oncol. 30 (2012) 587–592.
[85] S.J. Miller, R.M. Lavker, T.T. Sun, Interpreting epithelial cancer biology in the context of stem cells: tumor properties and therapeutic implications, Biochim. Biophys. Acta 1756 (2005) 25–52.
[86] S. Aparicio, C. Caldas, The implications of clonal genome evolution for cancer medicine, N. Engl. J. Med. 368 (2013) 842–851.
[87] P.L. Bedard, A.R. Hansen, M.J. Ratain, L.L. Siu, Tumour heterogeneity in the clinic, Nature 501 (2013) 355–364.
[88] D. Zardavas, A. Irrthum, C. Swanton, M. Piccart, Clinical management of breast cancer heterogeneity, Nat. Rev. Clin. Oncol. 12 (2015) 381–394.
[89] A. Marusyk, V. Almendro, K. Polyak, Intra-tumour heterogeneity: a looking glass for cancer, Nat. Rev. Cancer 12 (2012) 323–334.
[90] C. Swanton, Intratumor heterogeneity: evolution through space and time, Cancer Res. 72 (2012) 4875–4882.

Please cite this article in press as: R.E. Ellsworth, et al., Molecular heterogeneity in breast cancer: State of the science and implications for patient care, Semin Cell Dev Biol (2016), http://dx.doi.org/10.1016/j.semcdb.2016.08.025