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Review

Metastatic Breast Cancer: Cytology Diagnosis with Implications for Treatment

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Abstract: Breast cancer is among the most frequent malignancies in women worldwide. While early detection and effective treatment provide many women with a cure and prevent their cancer from spreading, metastases to distant sites still occur in around 20% of women suffering from breast cancer. These relapses occur in many forms and locations and are as varied as the primary breast tumors. Metastatic spread makes a cancer incurable and potentially lethal, but new, targeted treatments can offer control of the cancer cells if the features of new targets are unlocked by advanced diagnostic testing. The article offers an overview of the pathomechanisms of metastatic progression and describes the types of metastases, such as hormone-receptor-positive and -negative breast cancers, and *HER2*-overexpressing or triple-negative types. Once distant metastatic spread occurs, cytology allows a precise diagnosis to confirm the breast origin. Other molecular targets include *ESR1* and *PIK3CA* mutations, *MSI*, *NTRK* fusion, *PD-L1* expression and others, which can be obtained also from cytology material and used to determine eligibility for emerging targeted therapeutic options. We outline the diagnostic features of metastatic breast cancer in cytology samples, together with validated and emergent biomarkers that may provide new, targeted treatment options.

Keywords: breast cancer; metastatic; diagnosis; molecular; cytology

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1. Introduction and Overview

More than 2.3 million patients with breast cancer are newly diagnosed globally, and an estimated 685,000 women died of the disease in 2020 [1,2]. Approximately 3.5 million women in the United States are in treatment or living with a history of breast cancer. While the overall survival rate is favorable with early detection and advanced treatment options, 15–25% of women eventually develop distant metastatic disease, and around 40,000 women die from breast cancer each year in the U.S. [1,2].

These overall numbers of breast cancer incidence, obtained through public databases, include a portion of around 25% that are ductal carcinoma in situ, without demonstrating stromal invasion and (theoretically) with no possibility of metastasis. The term "breast cancer" in this article generally refers to invasive breast cancer [3,4].

With increased awareness and breast cancer screening programs in place for decades, only approximately 5% of western women present with breast cancer at an advanced stage—that is, with distant metastatic disease (or stage IV cancer) [5]. Regional lymph nodes are usually the first site of cancer spread outside the breast; these lymph node metastases, typically in the axillary lymph nodes, are considered a local disease for staging and treatment purposes. In this article, emphasis is placed on distant metastatic spread to other organs away from the breast.

Globally, there are significant regional differences in morbidity and mortality. While the rate among women in low-resource countries might be lower than in the western world, their likelihood of dying from this disease is also higher [2]. Estimates prognosticate a steep increase in both frequency and risk of death in the next 20 years. With this worrisome perspective of a higher disease burden on a global scale, effective methods for diagnosis in every layer of the medical resource spectrum are necessary [1,2,6].

In most clinical studies of primary breast cancer treatment, distant metastatic disease represents an endpoint and treatment failure. Although much progress has been made with the early detection of breast cancer, advanced hormonal therapy, chemotherapy regimens and targeted treatments to improve the prognosis of breast cancer patients, only modest improvements are found once the cancer shows a distant metastatic relapse. These patients are excluded from primary treatment schemes, and their psychological, social and economic needs are often not realized [6]. New treatment options are needed to improve the outlook for patients with metastatic breast cancer [7,8].

With the majority of breast cancers being hormone-receptor-positive, hormonal treatment has played an important role for many years [9,10]. As it became better known that hormonally responsive breast cancers may show a pattern of late relapses beyond the first five years after the primary cancer diagnosis and initial treatment, new anti-hormonal agents were discovered and treatment regimens refined [11,12]. These may include agents such as aromatase inhibitors, which subsequently may cause resistance through mutations, or selective estrogen receptor degraders, with which treatment can be continued and also escalated in the metastatic setting [13–15].

The discovery of human epidermal growth factor receptor 2 *(HER2/c-erbB2*) gene amplification and protein overexpression as a predictive factor and treatment target for humanized monoclonal antibodies was a breakthrough in breast cancer treatment. Currently, several treatment regimens target *HER2*-positive tumors in the neoadjuvant and adjuvant setting and have greatly improved the survival of patients with this type of breast cancer [16,17].

In addition to established predictive and prognostic markers for primary breast cancer, new molecular targets are being identified, adding new treatment options and improving the diagnosis for breast cancer patients with metastatic disease [7,10,18]. One such example is immune checkpoint or cyclin dependent kinase (*CDK 4/6*) inhibitors, which may allow further treatment options with agents such as pembrolizumab; primarily, this applies to the triple-negative subset of breast cancers that are found to express programmed death ligand (*PD-L1*) on immune cells in the tumor [19,20]. A new and promising agent to expand the treatment options for triple-negative breast cancers (TNBC), especially in *BRCA 1/2* gene mutation carriers, is poly-ADP ribose or *PARP* inhibitors [21].

A large study that compared genomic profiles between primary and metastatic breast cancers showed more frequent estrogen receptor (*ESR1*), phosphatase and tensin homolog (*PTEN*), cadherin-1 gene (*CDH1*) and retinoblastoma (*RB1*) mutations; mouse double minute 4 (*MDM4*) and myelocytomatosis (*MYC*) gene amplifications; and AT-rich interaction domain 1A (*ARID1A*) deletions in metastatic breast cancers [22].

There are validated biomarkers with actionable treatment options including phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic unit alpha (*PI3KCA)*, estrogen receptor mutations (*ESR1)*, microsatellite instability (*MSI*) and neurotrophic tyrosine receptor kinase (*NTRK)* fusion. Emergent biomarkers include Erb-B2 Receptor Tyrosine Kinase 2 (*ERBB2*), protein kinase B (*AKT*), phosphatase and tensin homolog (*PTEN*), homologous recombination repair (*HRR*), CD274 amplification, retinoblastoma (*RB1*) and neurofibromin 1 (*NF1*) mutations [18,22].

Genomic testing of metastatic lesions is necessary to identify actionable targets, and tissue sampling is at the core of this diagnostic process to determine the presence of therapeutic targets [23]. Cytology is often the method of choice to sample metastatic lesions because of the location, accessibility and choice of a minimally invasive sampling technique [24–26]. The role of liquid-based biopsy from the peripheral blood or other body fluids is considered later in this article.

2. How Does Metastatic Spread Occur?

Most of the diagnostic parameters and initial treatments in early breast cancer focus on the primary tumor. Features that are associated with spread outside of the breast are lymphatic vessel invasion and lymph node metastases. Involvement of axillary lymph

nodes, which provide the first site of lymphatic drainage to the breast, is still indicative of local disease but indicates a higher risk of distant relapse [5]. Regional lymph node involvement is unfavorably correlated with diagnosis as a staging parameter.

Pathogenetically, several models help to explain how tumor cells disseminate into other sites of the body: tumor cells may gain the capacity to metastasize within the primary tumor in a linear progression model to later spread through the genetic evolution of metastasis-capable founder cells. Another possibility assumes the early dissemination of tumor cells with the acquisition of new mutations at the new site that would allow this. In a historical hypothesis formulated by Stephen Paget in 1889, cancer cells ("seed") may settle preferentially in the selected microenvironment ("soil").

Following this model, tumor cells encounter a pre-metastatic niche, which they colonize; after various lengths of dormancy, and mediated by tumor-initiated soluble factors and by possibly creating an immune-suppressed field, these tumor cells eventually cause a metastatic outgrowth at the new site. In the reality of metastatic spread, various pathways might be used by traveling tumor cells to remain dormant or establish new growth sites [27]. Eventually, a metastatic cascade gives rise to innumerable foci of tumor cells that become resistant to treatment and lead to the patient's demise. The genetic underpinnings of why, when and where tumor cells precisely cause distant spread are the subject of ongoing investigation [28].

As to the preferred sites of breast cancer metastases, the molecular subgroups based on hormone receptors and *HER2* expression are associated with certain metastatic patterns and tropisms. Estrogen-receptor-positive types (luminal A/B type carcinomas following the intrinsic subtype model) more frequently cause bone metastases. As a morphological subtype, lobular carcinomas, which are mostly ER-positive, have different metastatic patterns to breast cancers of a ductal/no special type, with more frequent visceral, serosal and gynecological metastases (40). Triple-negative (basal-like) and *HER2*-positive carcinomas were shown to cause brain metastases more frequently, posing particular challenges to treatment [22,29,30].

3. Diagnosis of Breast Cancer Metastases

Different types of breast cancer based on morphology, histologic grade, hormone receptor and *HER2* expression show different patterns in relapse and metastatic behavior. While some changes can occur in morphology and immunohistochemical profile, most metastatic lesions resemble the primary tumor, and key morphologic features in metastases often lead to a further diagnostic investigation [31,32]. For the diagnosis of primary breast cancers and their respective metastases, the current classification for tumor diagnosis is followed [33].

Morphological comparison with the primary may be most helpful when metastasis from a known breast primary is suspected. In conjunction with clinical correlation, a selected immunohistochemical profile of the metastatic focus will help to narrow down the differential diagnosis. A panel of immunohistochemical markers, including estrogen and progesterone receptors, cytokeratins and *GATA3*, may allow us to confirm the primary site in the breast. Other successfully employed markers are mammaglobin and GCDFP-15, but now often the nuclear marker *GATA3* is used. *GATA3* is not specific to the breast but, in the appropriate clinical context, has proven very useful as a diagnostic marker for metastatic breast cancer [34]. In combination with *SOX10* (SRY-box transcription factor 10), it has been shown to be helpful to diagnose triple-negative breast cancer [35]. More recently, the novel marker *TRPS-1* (trichorhinophalangeal syndrome type 1) was used to confirm the breast origin [36]. The hormone receptor and *HER2* profile of a known breast primary can be utilized to confirm the metastatic site. It is important to note at this point is that most metastatic foci resemble the primary tumor immunomorphologically, but exceptions and "switches" from hormone-receptor- or *HER2*-positive to *HER2*-negative, and vice versa, may occur in a considerable proportion of cases [22,26].

The breast ©tself is not a frequent site of metastasis. Nonetheless, some tumors can metastasize to the breast, such as disseminated lymphomas, melanomas and small-cell lung cancers. However, this topic is beyond the scope of this discussion [31].

Estrogen-receptor-positive breast cancers can recur late (after 5, 10 or even 20 years), often causing bone metastases. Other visceral sites, such as multiple liver or lung metastasis, are seen as part of the metastatic cascade.

Triple-negative breast cancers tend to be of high histologic grade and typically recur in the first three to five years after the primary diagnosis. Both triple-negative and *HER2*-positive breast cancers appear to cause brain metastases more frequently than hormone-receptor-positive tumors [29].

With the various organs involved, cytology is often the most accessible means of tissue sampling for breast cancer diagnosis. Depending on the practice patterns, predictive markers such as hormone receptors and *HER2* studies may be determined on the metastatic lesions as a standard of care and following international practice guidelines [37,38]. In addition, molecular profiles are often requested to determine patients eligible for novel targeted treatments such as checkpoint inhibitors, *PIK3CA* inhibitors or immunotherapy [22,39]. Individual protocols vary and depend on departmental preferences and practice patterns: some departments routinely apply molecular tests on liquid cytology remnants, while other use cell block material or send samples for molecular testing.

The subsequent paragraphs illustrate cytological samples of breast cancer subtypes from metastatic lesions, including examples of breast cancer of ductal/no special type, special types such as lobular carcinoma or others and the groups based on hormone receptor and *HER2* expression.

3.1. Metastatic Ductal Breast Cancer (No Special Type)

Invasive ductal carcinomas/carcinoma of no special type constitute approximately 80% of breast carcinomas (Figure 1) and range in histologic grade from well (grade 1) to moderately (grade 2) to poorly differentiated (grade 3). Usually, metastatic lesions are not independently graded. Nuclear pleomorphism, necrosis or other features can of course be used to describe, for example, a poorly differentiated tumor on cytology.

3.2. Metastatic Lobular Carcinoma

Lobular carcinomas are typically low-grade (grade 2 or 1) and represent approximately 15% of breast carcinomas (Figure 2). Metastatic lobular carcinoma shows more frequent involvement of visceral sites, the intestinal or gynecological tract or pleural membranes [40,41]. Characteristically, in effusions, there are medium-sized dyscohesive tumor cells against a background of reactive mesothelial cells.

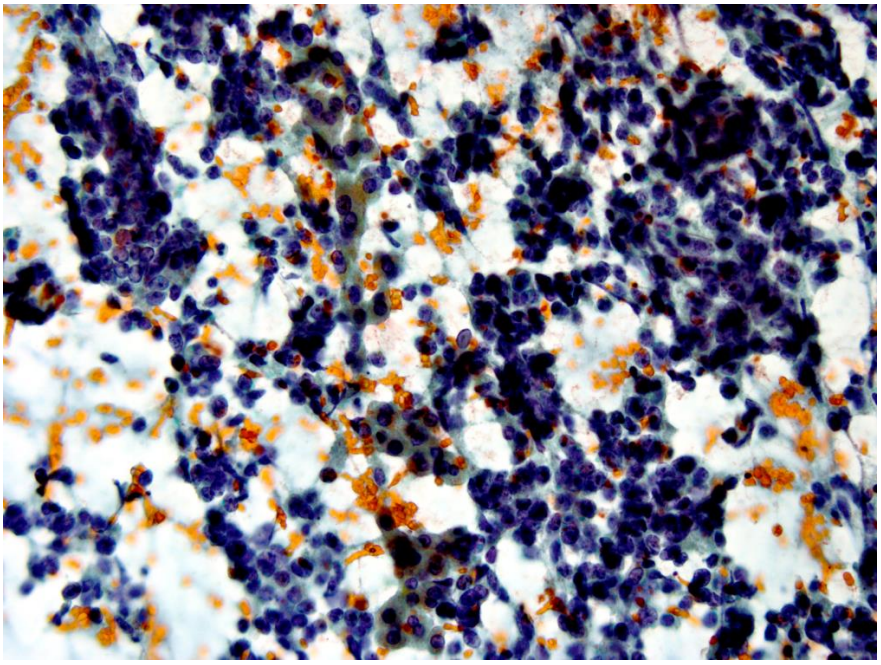
3.3. Metastatic Breast Cancer, Other Special Types

Invasive breast cancers occur in several other special types which can be distinguished by morphological features; only a few can be shown here as examples (33). Some of these special types are associated with a more favorable prognosis, for example mucinous carcinomas of the breast (Figure 3), medullary carcinomas or adenoid cystic carcinomas. On the other hand, matrix producing breast cancers are typically high-grade breast cancers with an aggressive behavior. Micropapillary carcinomas are characterized by a high rate of lymph node metastasis and poor prognosis.

3.4. ER-Positive Metastatic Breast Cancer

Most breast carcinomas are positive for estrogen receptors (Figure 4). Hormone receptors are reported in a semi-quantitative or quantitative way following international guidelines [37]. While it is feasible to perform immunocytochemical stains for nuclear receptors on cytology preparations (direct smears as well as liquid-based preparations), staining protocols need to be separately validated for cytology. Cell block preparations can

be prepared following several techniques and are feasible for immunohistochemical as well as for molecular testing [24,25,42].



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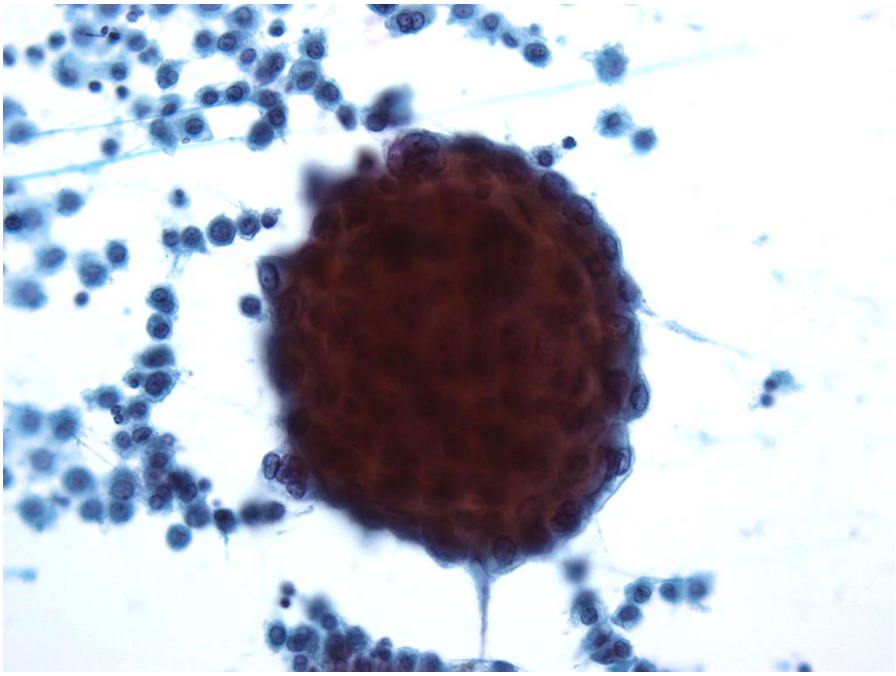

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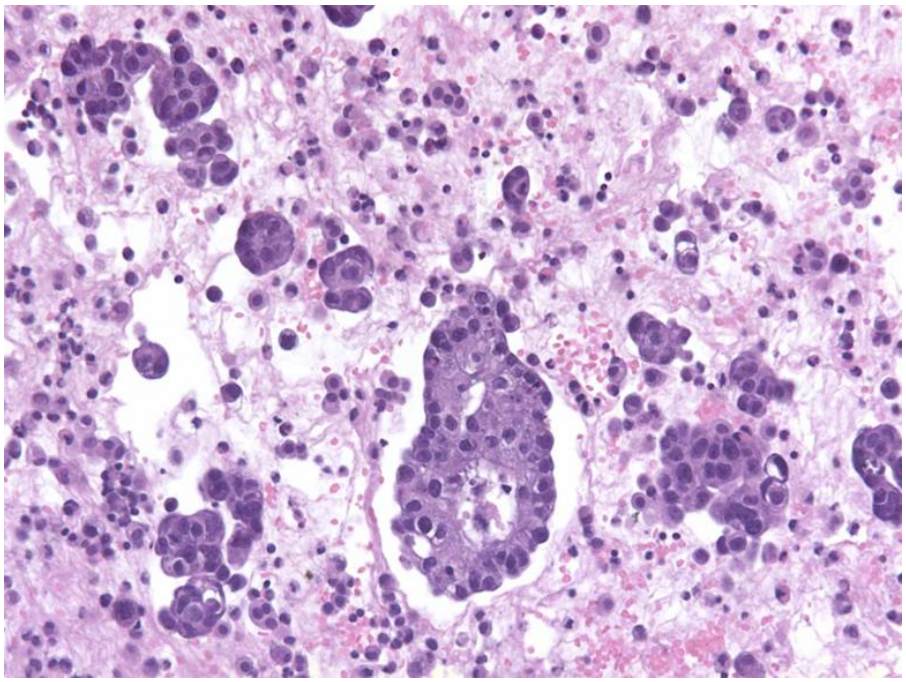

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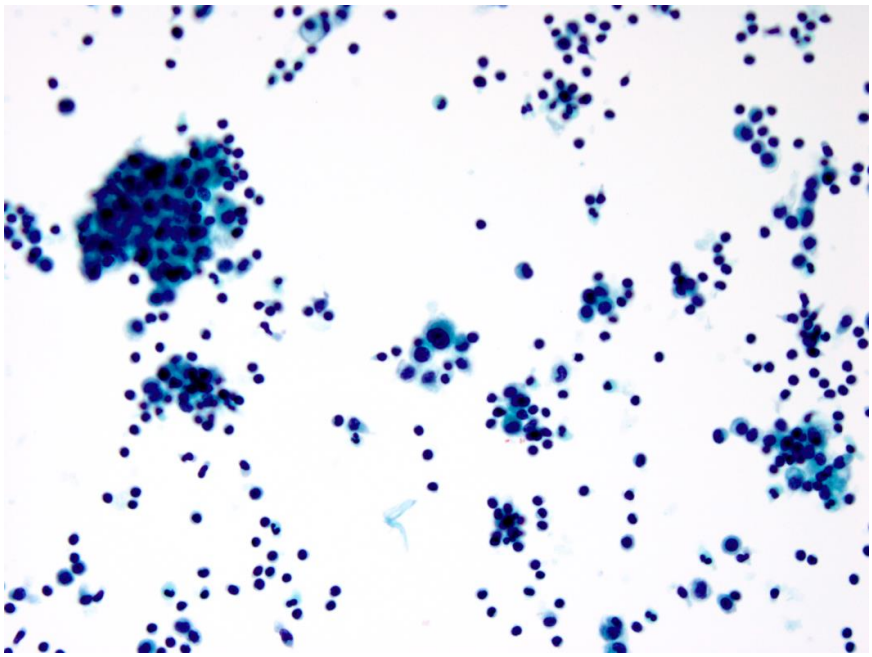
Figure 1. (**a**) Cytology smear of a liver fine-needle aspiration shows metastatic ductal carcinoma (Papanicolaou stain). The smear is very cellular, with three-dimensional clusters and sheets of tumor cells. (**b**) Cytology ThinPrep of a pleural effusion with metastatic breast cancer of ductal type. Characteristic are large, three-dimensional groups of tumor cells, so-called "cannonballs" in an effusion specimen. (Papanicolaou stain). (**c**) Cytology cell block of a pleural effusion with metastatic breast cancer of ductal type. Groups of tumor cells against a background of reactive mesothelial cells, histiocytes and inflammation (hematoxylin and eosin stain). Of note is that the cell block material here is markedly cellular; the cytology sample appears suitable for diagnostic testing as well as advanced molecular testing.



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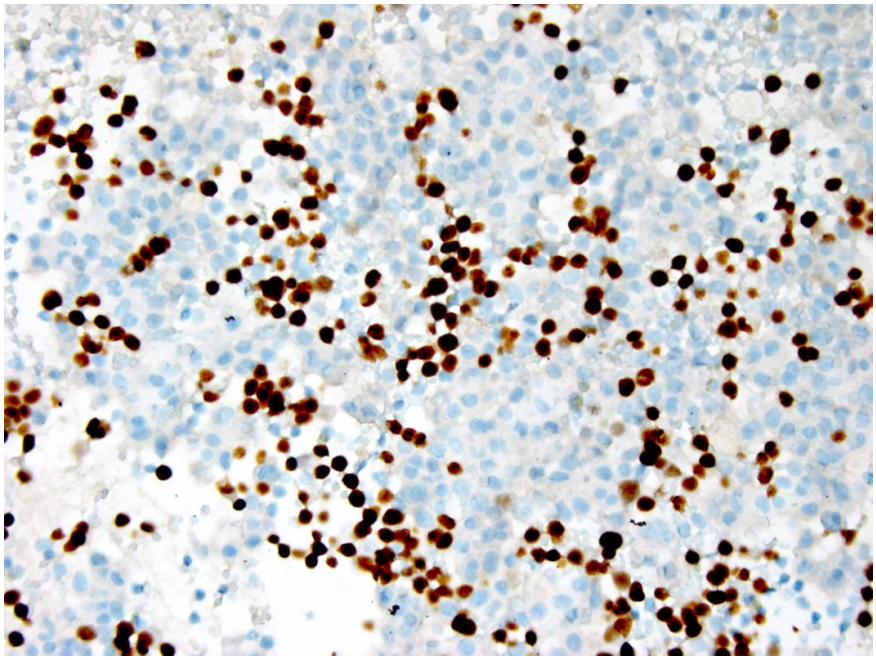

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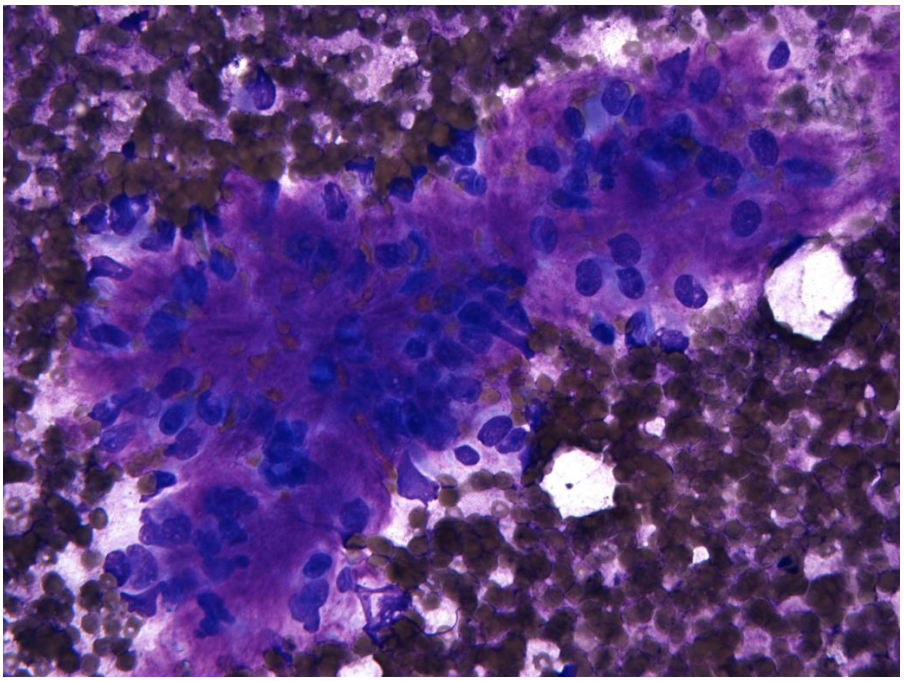
Figure 2. (**a**) Cytology ThinPrep of a pleural effusion aspirate shows metastatic lobular carcinoma (Papanicolaou stain). There are scattered dyscohesive tumor cells that are slightly larger than the surrounding mesothelial cells and lymphocytes. (**b**) Cytology cell block of a pleural effusion aspirate shows metastatic lobular carcinoma that is positive for estrogen receptor protein (immunohistochemical stain).



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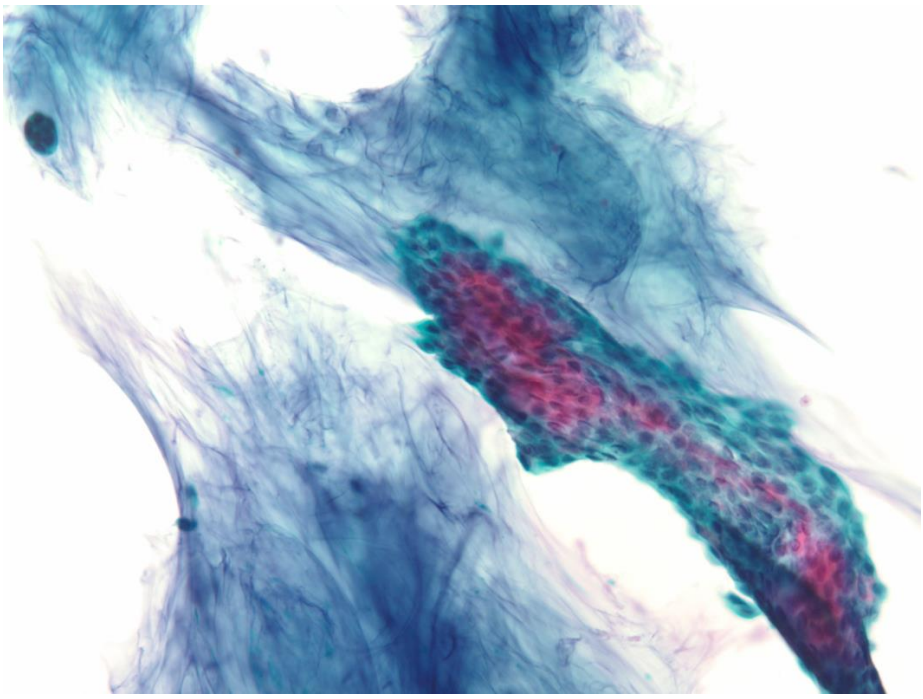

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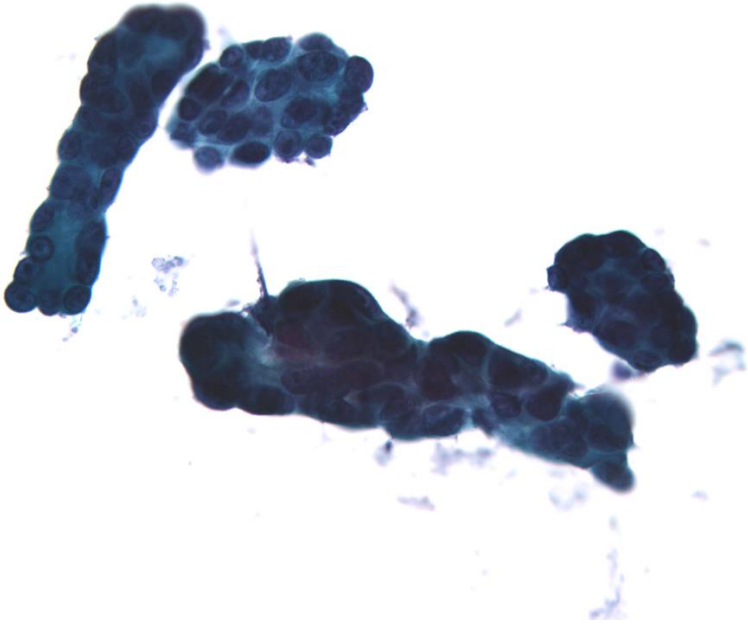

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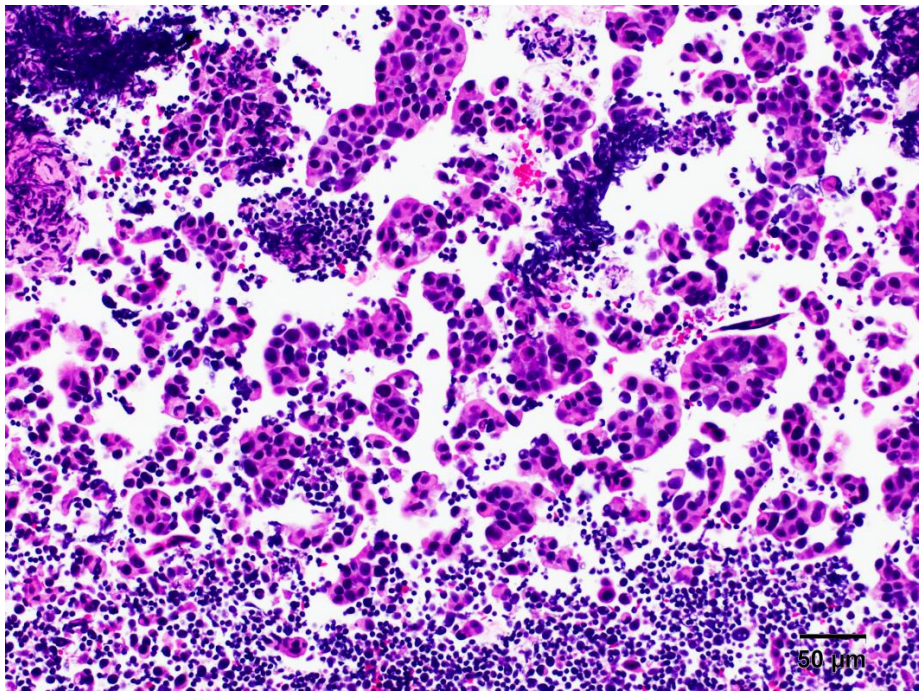
Figure 3. (**a**) Giemsa-stained, air-dried smear of metaplastic carcinoma (matrix-producing carcinoma). (**b**) Papanicolaou-stained, ethanol-fixed direct smear of mucinous breast carcinoma with groups of tumor cells floating in abundant extracellular mucin. This type of breast carcinoma is usually hormone-receptor-positive, with a good prognosis and a relatively low rate of distant metastases. (**c**) Cytology liquid-based SurePath preparation of metastatic micropapillary carcinoma, Papanicolaou-stained. The tumor cells consist of finger-shaped, three-dimensional groups. While cytologically of intermediate grade, this morphological type of breast cancer shows frequent lymphatic vessel invasion and has high metastatic potential.



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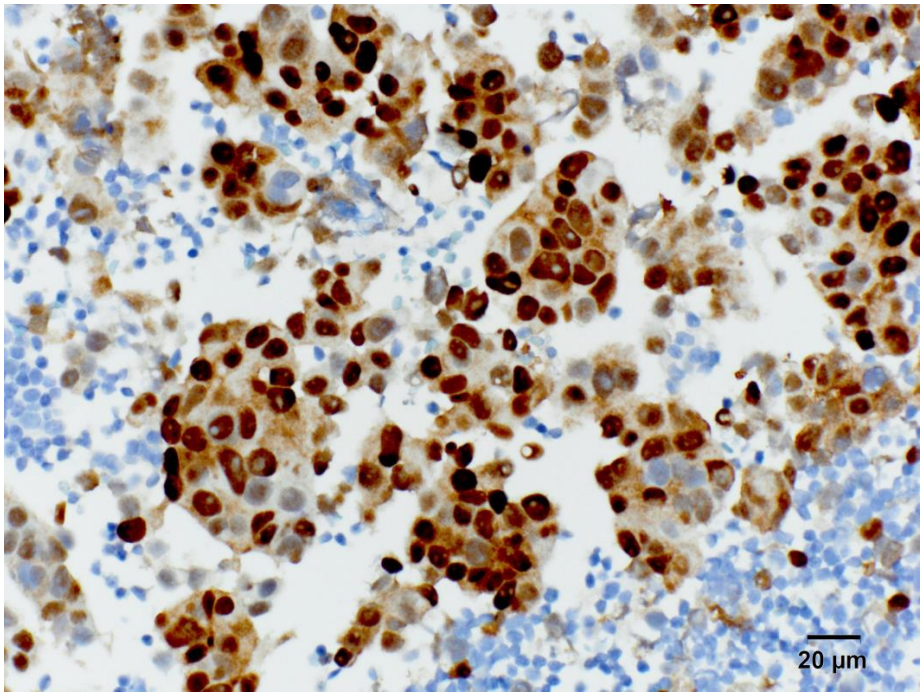

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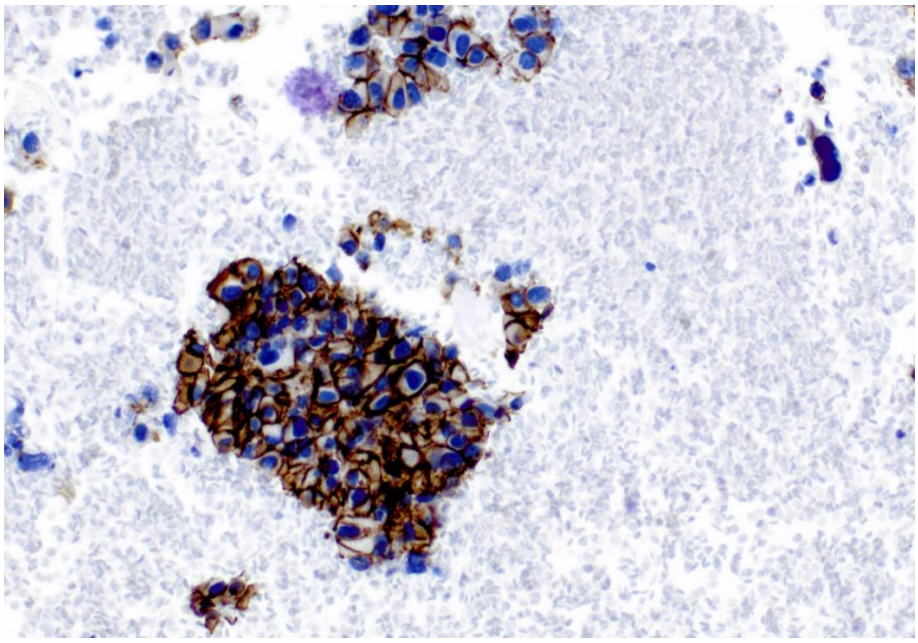
Figure 4. (**a**) Cytology cell block of a supraclavicular lymph node fine-needle aspirate shows metastatic breast carcinoma, present in cords and groups of tumor cells against a background of lymphoid tissue (hematoxylin and eosin stain). (**b**) Cell block of supraclavicular lymph node aspirate with metastatic breast carcinoma (same case as in Figure 4a) shows strong staining for estrogen receptor protein by immunohistochemical stain.

3.5. HER2-Positive Metastatic Breast Cancer

Human epidermal growth factor receptor (*HER2*) positive tumors represent around 10–20% of breast cancers (Figure 5). The scoring of tumor tissue is performed by immunohistochemical and/or in situ hybridization techniques, following international guidelines [38].

Fluorescent in situ hybridization can also be achieved on cytology preparations with adapted protocols. Often preferred, however, is *HER2* testing on formalin-fixed, paraffin-embedded cell block and core biopsy specimens, as routine protocols for both immunohistochemistry and in situ hybridization procedures can be followed, as per guidelines.



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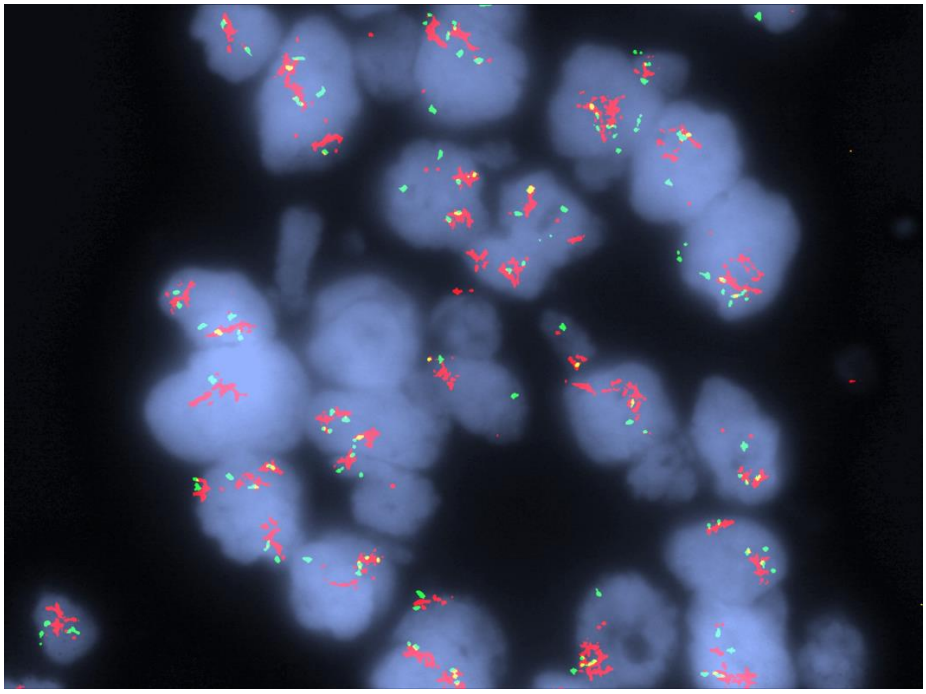

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Figure 5. (**a**) Cell block shows metastatic breast carcinoma with strong circumferential overexpression (3+) of *HER2* (immunohistochemical stain). (**b**) Cell block with *HER2*-amplified breast carcinoma (dual-color fluorescent in situ hybridization, with centromere probe in green (CEP17) and *HER2* gene in orange). Usual protocols for *HER2* testing apply to cell block and core biopsy samples.

3.6. Triple-Negative Metastatic Breast Cancer

Triple-negative breast cancers comprise around 10% of overall breast cancers, which are often of ductal type (no special type) and poorly differentiated. Some special-type breast cancers such as matrix-producing breast cancers, spindle cell carcinomas or adenoid cystic carcinoma (33). As therapeutic targets such as hormonal treatment or *HER2* overexpression do not apply, *PD-L1* can be used for possible immune checkpoint inhibitor treatment. *PD-L1* testing is performed on primary tumors and is feasible also on cytology samples, to test for immunotherapy eligibility [26].

3.7. Molecular Profiles in Cytology Samples and Liquid Biopsies

Circulating tumor cells or cell-free DNA (ctDNA) are in various stages of development to follow treatment responses and for clinical applications [43]. For example, estrogen receptor mutations (ESR1) following hormonal treatment with aromatase inhibitors or *PIK3CA* mutations have been tested by blood samples to follow treatment [10,14,44,45]. Another active area of investigation is liquid biopsies, which are proposed to be used for staging and molecular characterization in blood and in certain body compartments, such as cerebrospinal fluid disease (although, for many places, these methods are still beyond the standard diagnostic repertory) [46–50].

4. Novel Therapeutic Targets in Metastatic Breast Cancer

Multiple novel mutations have been validated and are promising for future personalized, targeted treatment. The phosphatidylinositol 3-kinase gene (PI3KCA) is involved in the cell cycle and cell proliferation, encoding for the class I catalytic isoform p110α. It is mutated in around 40% of hormone-receptor (HR) positive breast cancer cases [51,52]. Microsatellite instability (*MSI*) occurs in less than 1% of cases. Neurotrophin receptor tyrosine kinase (*NTRK*) translocation is found exclusively in secretory carcinoma [53]. The activating mutation of the estrogen receptor α (*ESR1*) gene results in the constitutional activity of the estrogen receptor independently of the ligand; it is commonly found as a result of aromatase inhibitor in *HR+/HER2−* metastatic breast cancer [10,54,55]. Clinical trials already provide levels of evidence, and molecular testing in tissue and liquid biopsies is being established.

Cyclin-dependent kinase 4 and 6 (*CDK4/6*) inhibitors, palbociclib, ribociclib and abemaciclib, are already being used in hormone-receptor-positive metastatic breast cancer [56]. They interrupt cell cycle progression, leading to the inhibition of tumor growth. Adding them to endocrine therapy has shown improved progression-free survival with minimal toxicity. The agent pembrolizumab, also an immune checkpoint inhibitor, is being used for triple-negative breast cancers, for which there are often few treatment options [20]. For metastatic triple-negative breast cancers in *BRCA 1/2* mutation carriers, a patient population with often aggressive tumors and few treatment options, PARP inhibitors are now used, with some success [21].

HER2-positive breast cancers are defined by *HER2* overexpression and/or amplification, which drives the growth of cancer cells. Therapies that target *HER2* include humanized monoclonal antibodies such as trastuzumab, pertuzumab or other conjugates that are used in neoadjuvant and adjuvant treatment settings [16,17]. Adding these agents to the neoadjuvant chemotherapy reduces the tumor size and increases the breast-conserving rate.

Emergent biomarkers include Erb-B2 Receptor Tyrosine Kinase 2 (*ERBB2*), *AKT*, phosphatase and tensin homolog (*PTEN),* homologous recombination repair (*HRR*), CD274 amplification, retinoblastoma protein (*RB1*) and neurofibromin 1 (*NF1*) mutations [18,22].

5. Conclusions and Outlook

This article describes the current practice in sampling metastatic breast cancer lesions and determining their molecular profiles to better apply targeted treatments. This ranges from routine ancillary breast markers such as estrogen receptor proteins to molecular profiles to assess the response and resistance to novel hormonal treatments. *HER2*-targeted

treatments have expanded from a single anti-*HER2* agent to a range of treatment possibilities, also in the metastatic setting. Other actionable treatments include *PIK3CA* mutations and immune checkpoint inhibitors for hormone-receptor-positive and triple-negative breast cancer subtypes. Current oncology guidelines include circulating tumor DNA as a novel technology to determine the mutational profiles of tumor cells in metastatic breast cancer.

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References

1. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **2018**, *68*, 394–424. [CrossRef] [PubMed]
2. Arnold, M.; Morgan, E.; Rumgay, H.; Mafra, A.; Singh, D.; Laversanne, M.; Vignat, J.; Gralow, J.R.; Cardoso, F.; Siesling, S.; et al. Current and future burden of breast cancer: Global statistics for 2020 and 2040. *Breast* **2022**, *66*, 15–23. [CrossRef] [PubMed]
3. Casasent, A.K.; Almekinders, M.M.; Mulder, C.; Bhattacharjee, P.; Collyar, D.; Thompson, A.M.; Jonkers, J.; Lips, E.H.; van Rheenen, J.; Hwang, E.S.; et al. Learning to distinguish progressive and non-progressive ductal carcinoma in situ. *Nat. Rev. Cancer* **2022**, *22*, 663–678. [CrossRef] [PubMed]
4. Giannakeas, V.; Sopik, V.; Narod, S.A. A comparison of two models for breast cancer mortality for women with ductal carcinoma in situ: An SEER-based analysis. *Breast Cancer Res. Treat.* **2018**, *169*, 587–594. [CrossRef]
5. Hortogagyi, G.N.; Connoly, J.L.; D'Orsi, C.J.; Edge, S.B.; Mittendorf, S.A.; Rugo, H.S.; Solin, L.J.; Weaver, D.L.; Winchester, D.J.; Giuliano, A. *Breast-AJCC Cancer Staging Manual*, 8th ed.; American College of Surgeons: Chicago, IL, USA, 2018.
6. Cardoso, F.; Spence, D.; Mertz, S.; Corneliussen-James, D.; Sabelko, K.; Gralow, J.; Cardoso, M.J.; Peccatori, F.; Paonessa, D.; Benares, A.; et al. Global analysis of advanced/metastatic breast cancer: Decade report (2005–2015). *Breast* **2018**, *39*, 131–138. [CrossRef] [PubMed]
7. Loibl, S.; Poortmans, P.; Morrow, M.; Denkert, C.; Curigliano, G. Breast cancer. *Lancet* **2021**, *397*, 1750–1769. [CrossRef]
8. Gennari, A.; Andre, F.; Barrios, C.H.; Cortes, J.; de Azambuja, E.; DeMichele, A.; Dent, R.; Fenlon, D.; Gligorov, J.; Hurvitz, S.A.; et al. ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer. *Ann. Oncol.* **2021**, *32*, 1475–1495. [CrossRef]
9. Ward, H.W. Anti-oestrogen therapy for breast cancer: A trial of tamoxifen at two dose levels. *Br. Med. J.* **1973**, *1*, 13–14. [CrossRef]
10. Burstein, H.J.; Somerfield, M.R.; Barton, D.L.; Dorris, A.; Fallowfield, L.J.; Jain, D.; Johnston, S.R.D.; Korde, L.A.; Litton, J.K.; Macrae, E.R.; et al. Endocrine Treatment and Targeted Therapy for Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Metastatic Breast Cancer: ASCO Guideline Update. *J. Clin. Oncol.* **2021**, *39*, 3959–3977. [CrossRef]
11. Goss, P.E.; Ingle, J.N.; Martino, S.; Robert, N.J.; Muss, H.B.; Piccart, M.J.; Castiglione, M.; Tu, D.; Shepherd, L.E.; Pritchard, K.I.; et al. A randomized trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer. *N. Engl. J. Med.* **2003**, *349*, 1793–1802. [CrossRef]
12. Sgroi, D.C.; Sestak, I.; Cuzick, J.; Zhang, Y.; Schnabel, C.A.; Schroeder, B.; Erlander, M.G.; Dunbier, A.; Sidhu, K.; Lopez-Knowles, E.; et al. Prediction of late distant recurrence in patients with oestrogen-receptor-positive breast cancer: A prospective comparison of the breast-cancer index (BCI) assay, 21-gene recurrence score, and IHC4 in the TransATAC study population. *Lancet Oncol.* **2013**, *14*, 1067–1076. [CrossRef] [PubMed]
13. Bardia, A.; Hovnanian, M.D.; Brachtel, E.F.; Nardi, V. Case 35-2018: A 68-Year-Old Woman with Back Pain and a Remote History of Breast Cancer. *N. Engl. J. Med.* **2018**, *379*, 1946–1953. [CrossRef] [PubMed]
14. Lloyd, M.R.; Wander, S.A.; Hamilton, E.; Razavi, P.; Bardia, A. Next-generation selective estrogen receptor degraders and other novel endocrine therapies for management of metastatic hormone receptor-positive breast cancer: Current and emerging role. *Ther. Adv. Med. Oncol.* **2022**, *14*, 17588359221113694. [CrossRef] [PubMed]
15. Li, Z.; Wu, Y.; Yates, M.E.; Tasdemir, N.; Bahreini, A.; Chen, J.; Levine, K.M.; Priedigkeit, N.M.; Nasrazadani, A.; Ali, S.; et al. Hotspot ESR1 Mutations Are Multimodal and Contextual Modulators of Breast Cancer Metastasis. *Cancer Res.* **2022**, *82*, 1321–1339. [CrossRef] [PubMed]

16. Slamon, D.; Eiermann, W.; Robert, N.; Pienkowski, T.; Martin, M.; Press, M.; Mackey, J.; Glaspy, J.; Chan, A.; Pawlicki, M.; et al. Adjuvant trastuzumab in HER2-positive breast cancer. *N. Engl. J. Med.* **2011**, *365*, 1273–1283. [CrossRef] [PubMed]
17. Spring, L.M.; Clark, S.L.; Li, T.; Goel, S.; Tayob, N.; Viscosi, E.; Abraham, E.; Juric, D.; Isakoff, S.J.; Mayer, E.; et al. Phase 1b clinical trial of ado-trastuzumab emtansine and ribociclib for HER2-positive metastatic breast cancer. *NPJ Breast Cancer* **2021**, *7*, 103. [CrossRef] [PubMed]
18. Verret, B.; Bottosso, M.; Hervais, S.; Pistilli, B. The Molecular Predictive and Prognostic Biomarkers in Metastatic Breast Cancer: The Contribution of Molecular Profiling. *Cancers* **2022**, *14*, 4203. [CrossRef] [PubMed]
19. Hammerl, D.; Martens, J.W.M.; Timmermans, M.; Smid, M.; Trapman-Jansen, A.M.; Foekens, R.; Isaeva, O.I.; Voorwerk, L.; Balcioglu, H.E.; Wijers, R.; et al. Spatial immunophenotypes predict response to anti-PD1 treatment and capture distinct paths of T cell evasion in triple negative breast cancer. *Nat. Commun.* **2021**, *12*, 5668. [CrossRef]
20. Schmid, P.; Salgado, R.; Park, Y.H.; Munoz-Couselo, E.; Kim, S.B.; Sohn, J.; Im, S.A.; Foukakis, T.; Kuemmel, S.; Dent, R.; et al. Pembrolizumab plus chemotherapy as neoadjuvant treatment of high-risk, early-stage triple-negative breast cancer: Results from the phase 1b open-label, multicohort KEYNOTE-173 study. *Ann. Oncol.* **2020**, *31*, 569–581. [CrossRef]
21. Barchiesi, G.; Roberto, M.; Verrico, M.; Vici, P.; Tomao, S.; Tomao, F. Emerging Role of PARP Inhibitors in Metastatic Triple Negative Breast Cancer. Current Scenario and Future Perspectives. *Front. Oncol.* **2021**, *11*, 769280. [CrossRef]
22. Aftimos, P.; Oliveira, M.; Irrthum, A.; Fumagalli, D.; Sotiriou, C.; Gal-Yam, E.N.; Robson, M.E.; Ndozeng, J.; Di Leo, A.; Ciruelos, E.M.; et al. Genomic and Transcriptomic Analyses of Breast Cancer Primaries and Matched Metastases in AURORA, the Breast International Group (BIG) Molecular Screening Initiative. *Cancer Discov.* **2021**, *11*, 2796–2811. [CrossRef] [PubMed]
23. Najjar, S.; Allison, K.H. Updates on breast biomarkers. *Virchows Arch.* **2022**, *480*, 163–176. [CrossRef] [PubMed]
24. Brachtel, E.F.; Operana, T.N.; Sullivan, P.S.; Kerr, S.E.; Cherkis, K.A.; Schroeder, B.E.; Dry, S.M.; Schnabel, C.A. Molecular classification of cancer with the 92-gene assay in cytology and limited tissue samples. *Oncotarget* **2016**, *7*, 27220–27231. [CrossRef] [PubMed]
25. Beca, F.; Schmitt, F.C. Ancillary Tests in Breast Cytology: A Practical Guide. *Acta Cytol.* **2019**, *63*, 302–313. [CrossRef] [PubMed]
26. Souza da Silva, R.; Schmitt, F. Optimal assessment of metastatic breast carcinoma: The value of cytopathology combined with molecular analysis. *J. Mol. Pathol.* **2022**, *3*, 329–338. [CrossRef]
27. Medeiros, B.; Allan, A.L. Molecular Mechanisms of Breast Cancer Metastasis to the Lung: Clinical and Experimental Perspectives. *Int. J. Mol. Sci.* **2019**, *20*, 2272. [CrossRef]
28. Harper, K.L.; Sosa, M.S.; Entenberg, D.; Hosseini, H.; Cheung, J.F.; Nobre, R.; Avivar-Valderas, A.; Nagi, C.; Girnius, N.; Davis, R.J.; et al. Mechanism of early dissemination and metastasis in Her2(+) mammary cancer. *Nature* **2016**, *540*, 588–592. [CrossRef]
29. Laakmann, E.; Witzel, I.; Fasching, P.A.; Rezai, M.; Schem, C.; Solbach, C.; Tesch, H.; Klare, P.; Schneeweiss, A.; Salat, C.; et al. Development of central nervous system metastases as a first site of metastatic disease in breast cancer patients treated in the neoadjuvant trials GeparQuinto and GeparSixto. *Breast Cancer Res.* **2019**, *21*, 60. [CrossRef]
30. Vitos, N.; Gerlee, P. Model-based inference of metastatic seeding rates in de novo metastatic breast cancer reveals the impact of secondary seeding and molecular subtype. *Sci. Rep.* **2022**, *12*, 9455. [CrossRef]
31. Bombonati, A.; Lerwill, M.F. Metastases to and from the Breast. *Surg. Pathol. Clin.* **2012**, *5*, 719–747. [CrossRef]
32. Hui, Y.; Wang, Y.; Nam, G.; Fanion, J.; Sturtevant, A.; Lombardo, K.A.; Resnick, M.B. Differentiating breast carcinoma with signet ring features from gastrointestinal signet ring carcinoma: Assessment of immunohistochemical markers. *Hum. Pathol.* **2018**, *77*, 11–19. [CrossRef] [PubMed]
33. WHO. *Breast Tumours*, 5th ed.; Board of Editors, WHO Classification of Tumours; International Agency for Research on Cancer: Lyon, France, 2019.
34. Ni, Y.B.; Tsang, J.Y.S.; Shao, M.M.; Chan, S.K.; Cheung, S.Y.; Tong, J.; To, K.F.; Tse, G.M. GATA-3 is superior to GCDFP-15 and mammaglobin to identify primary and metastatic breast cancer. *Breast Cancer Res. Treat.* **2018**, *169*, 25–32. [CrossRef] [PubMed]
35. Tozbikian, G.H.; Zynger, D.L. A combination of GATA3 and SOX10 is useful for the diagnosis of metastatic triple-negative breast cancer. *Hum. Pathol.* **2019**, *85*, 221–227. [CrossRef] [PubMed]
36. Abdelwahed, M.; Yurtsever, N.; Savant, D.; Karam, P.; Gimenez, C.; Das, K.; Sheikh-Fayyaz, S.; Khutti, S. Utility of TRPS-1 immunohistochemistry in diagnosis of metastatic breast carcinoma in cytology specimens. *J. Am. Soc. Cytopathol.* **2022**, *11*, 345–351. [CrossRef]
37. Allison, K.H.; Hammond, M.E.H.; Dowsett, M.; McKernin, S.E.; Carey, L.A.; Fitzgibbons, P.L.; Hayes, D.F.; Lakhani, S.R.; Chavez-MacGregor, M.; Perlmutter, J.; et al. Estrogen and Progesterone Receptor Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Guideline Update. *Arch. Pathol. Lab. Med.* **2020**, *144*, 545–563. [CrossRef]
38. Wolff, A.C.; Hammond, M.E.H.; Allison, K.H.; Harvey, B.E.; Mangu, P.B.; Bartlett, J.M.S.; Bilous, M.; Ellis, I.O.; Fitzgibbons, P.; Hanna, W.; et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *J. Clin. Oncol.* **2018**, *36*, 2105–2122. [CrossRef]
39. Verret, B.; Sourisseau, T.; Stefanovska, B.; Mosele, F.; Tran-Dien, A.; Andre, F. The Influence of Cancer Molecular Subtypes and Treatment on the Mutation Spectrum in Metastatic Breast Cancers. *Cancer Res.* **2020**, *80*, 3062–3069. [CrossRef]
40. Borst, M.J.; Ingold, J.A. Metastatic patterns of invasive lobular versus invasive ductal carcinoma of the breast. *Surgery* **1993**, *114*, 637–641, discussion 641–632.

41. Bennett, J.A.; Young, R.H.; Chuang, A.Y.; Lerwill, M.F. Ovarian Metastases of Breast Cancers With Signet Ring Cells: A Report of 17 Cases Including 14 Krukenberg Tumors. *Int. J. Gynecol. Pathol.* **2018**, *37*, 507–515. [CrossRef]
42. Pinto, D.; Schmitt, F.C. Immunohistochemistry Applied to Breast Cytological Material. *Pathobiology* **2022**, *89*, 343–358. [CrossRef]
43. Cheng, J.; Cao, Y.; MacLeay, A.; Lennerz, J.K.; Baig, A.; Frazier, R.P.; Lee, J.; Hu, K.; Pacula, M.; Meneses, E.; et al. Clinical Validation of a Cell-Free DNA Gene Panel. *J. Mol. Diagn.* **2019**, *21*, 632–645. [CrossRef] [PubMed]
44. Fribbens, C.; Garcia Murillas, I.; Beaney, M.; Hrebien, S.; O'Leary, B.; Kilburn, L.; Howarth, K.; Epstein, M.; Green, E.; Rosenfeld, N.; et al. Tracking evolution of aromatase inhibitor resistance with circulating tumour DNA analysis in metastatic breast cancer. *Ann. Oncol.* **2018**, *29*, 145–153. [CrossRef] [PubMed]
45. Galvano, A.; Castellana, L.; Gristina, V.; La Mantia, M.; Insalaco, L.; Barraco, N.; Perez, A.; Cutaia, S.; Calo, V.; Bazan Russo, T.D.; et al. The diagnostic accuracy of PIK3CA mutations by circulating tumor DNA in breast cancer: An individual patient data meta-analysis. *Ther. Adv. Med. Oncol.* **2022**, *14*, 17588359221110162. [CrossRef] [PubMed]
46. Boire, A.; Brandsma, D.; Brastianos, P.K.; Le Rhun, E.; Ahluwalia, M.; Junck, L.; Glantz, M.; Groves, M.D.; Lee, E.Q.; Lin, N.; et al. Liquid biopsy in central nervous system metastases: A RANO review and proposals for clinical applications. *Neuro-Oncology* **2019**, *21*, 571–584. [CrossRef] [PubMed]
47. Cristofanilli, M.; Pierga, J.Y.; Reuben, J.; Rademaker, A.; Davis, A.A.; Peeters, D.J.; Fehm, T.; Nole, F.; Gisbert-Criado, R.; Mavroudis, D.; et al. The clinical use of circulating tumor cells (CTCs) enumeration for staging of metastatic breast cancer (MBC): International expert consensus paper. *Crit. Rev. Oncol. Hematol.* **2019**, *134*, 39–45. [CrossRef] [PubMed]
48. Eigeliene, N.; Saarenheimo, J.; Jekunen, A. Potential of Liquid Biopsies for Breast Cancer Screening, Diagnosis, and Response to Treatment. *Oncology* **2019**, *96*, 115–124. [CrossRef]
49. Fitzpatrick, A.; Iravani, M.; Mills, A.; Childs, L.; Alaguthurai, T.; Clifford, A.; Garcia-Murillas, I.; Van Laere, S.; Dirix, L.; Harries, M.; et al. Assessing CSF ctDNA to Improve Diagnostic Accuracy and Therapeutic Monitoring in Breast Cancer Leptomeningeal Metastasis. *Clin. Cancer Res.* **2022**, *28*, 1180–1191. [CrossRef]
50. Main, S.C.; Cescon, D.W.; Bratman, S.V. Liquid biopsies to predict CDK4/6 inhibitor efficacy and resistance in breast cancer. *Cancer Drug Resist.* **2022**, *5*, 727–748. [CrossRef]
51. Engelman, J.A.; Luo, J.; Cantley, L.C. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat. Rev. Genet.* **2006**, *7*, 606–619. [CrossRef]
52. Andre, F.; Ciruelos, E.; Rubovszky, G.; Campone, M.; Loibl, S.; Rugo, H.S.; Iwata, H.; Conte, P.; Mayer, I.A.; Kaufman, B.; et al. Alpelisib for PIK3CA-Mutated, Hormone Receptor-Positive Advanced Breast Cancer. *N. Engl. J. Med.* **2019**, *380*, 1929–1940. [CrossRef]
53. Tognon, C.; Knezevich, S.R.; Huntsman, D.; Roskelley, C.D.; Melnyk, N.; Mathers, J.A.; Becker, L.; Carneiro, F.; MacPherson, N.; Horsman, D.; et al. Expression of the ETV6-NTRK3 gene fusion as a primary event in human secretory breast carcinoma. *Cancer Cell* **2002**, *2*, 367–376. [CrossRef] [PubMed]
54. Angus, L.; Smid, M.; Wilting, S.M.; van Riet, J.; Van Hoeck, A.; Nguyen, L.; Nik-Zainal, S.; Steenbruggen, T.G.; Tjan-Heijnen, V.C.G.; Labots, M.; et al. The genomic landscape of metastatic breast cancer highlights changes in mutation and signature frequencies. *Nat. Genet.* **2019**, *51*, 1450–1458. [CrossRef] [PubMed]
55. Bertucci, F.; Ng, C.K.Y.; Patsouris, A.; Droin, N.; Piscuoglio, S.; Carbuccia, N.; Soria, J.C.; Dien, A.T.; Adnani, Y.; Kamal, M.; et al. Genomic characterization of metastatic breast cancers. *Nature* **2019**, *569*, 560–564. [CrossRef] [PubMed]
56. Ribnikar, D.; Volovat, S.R.; Cardoso, F. Targeting CDK4/6 pathways and beyond in breast cancer. *Breast* **2019**, *43*, 8–17. [CrossRef] [PubMed]