Targeting the Tumor Microenvironment in Breast Cancer

Subjects: Oncology

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Breast cancer is one of the most prevalent tumors among women. Its prognosis and treatment outcomes depend on factors related to tumor cell biology. Studies have revealed the critical role of the tumor microenvironment (TME) in the development, progression, and treatment response of breast cancer.

breast cancer tumor microenvironment biomarkers targeted therapy

1. Introduction

Breast cancer (BC) is a prevalent and lethal disease, affecting more women than men and exhibiting geographical disparities [1]. It is considered a heterogeneous disease, and its classic prognostic assessment has relied on clinical and histopathological criteria, including lymph node involvement, tumor size, and tumor cell differentiation [2].

Immunohistochemical (IHC) markers such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) have refined subtype differentiation and influenced treatment decisions. Proliferation markers like Ki67 also offer prognostic value [3][4][5]. Advances in molecular biology have allowed platforms like Oncotype to provide prognostic and predictive information for the selection of patients who should undergo adjuvant chemotherapy [6]. Furthermore, in recent years, the targeted sequencing of specific genes, such as phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) or estrogen receptor alpha 1 (ESR1), has been introduced in clinical decision-making as their mutational profiles provide predictive information regarding the response to certain treatments.

While genetic and epigenetic factors significantly contribute to breast carcinoma, the tumor microenvironment (TME) plays a pivotal role in cancer development, progression, and metastasis. The TME comprises diverse cell types (stromal, immune, endothelial, and adipocytes) that interact with cancer cells, fostering tumor growth and functional support. [7]. From a historical perspective, the first evidence of the decisive role of the stroma in tumor development came from studies on tumor neovascularization, in which new blood vessels are formed in response to pro-angiogenic signals secreted from tumor cells. Since then, numerous studies have contributed to the characterization of the TME, further complicating the already challenging task of understanding and treating cancer.

2. Components of the Breast Cancer Microenvironment and Their Value as Prognostic and Predictive Factors

2.1. The Immune System in the Breast Tumor Microenvironment

The notion that the immune system not only protects the host from tumorigenesis but also shapes tumor immunogenicity is the basis for the cancer immunoediting hypothesis. This process encompasses three stages: elimination, equilibrium, and escape [8]. During the elimination stage, the immune system recognizes and eliminates the most immunogenic cancer cells through cytotoxic mechanisms. In the equilibrium stage, the tumor is held in check via immunosurveillance, although certain tumor cells manage to evade immune responses and promote tumor growth. Through this escape stage, the most aggressive clones adopt different strategies to evade immune recognition and promote the formation of an immunosuppressive TME, contributing to the development of malignant neoplasia [8]. The balance between immune components that provide protective antitumor immunity by targeting immunogenic tumor variants and those that facilitate tumor progression, shaping tumor immunogenicity, determines the magnitude of the immune response generated. This immunogenic capacity not only is critical for disease evolution and prognosis but also holds significant predictive value, influencing clinical responses to therapeutic regimens [9].

2.1.1. Tumor-Infiltrating Lymphocytes (TILs)

Tumor-infiltrating lymphocytes (TILs) are white blood cells that have migrated from the bloodstream into the tumor. They comprise various immunophenotypic and functionally distinct subpopulations, including CD8+ cytotoxic T cells, CD4+ helper T cells, B cells, and natural killer (NK) cells [10]. There is evidence that the infiltrating lymphocyte population differs throughout the course of the disease, being more abundant in early stages and at the onset of metastatic disease compared to advanced multi-treated stages [11].

Different studies have evaluated the prognostic value of TILs using immune and hematoxylin and eosin (H&E) staining, flow cytometry, and/or gene expression analysis. The data consistently demonstrate that TIL infiltration is associated with improved clinical outcomes [10]. Tumor infiltration via TILs correlates with longer overall survival (OS) independently of other clinical–pathological parameters [12]. The improved prognostic impact of TILs is closely linked to an increase in the density of cytotoxic T lymphocytes, or CD8+ lymphocytes, mainly in ER- tumors [13]. In fact, the clinical utility of using TILs as prognostic biomarkers in TN is well established, and the International Immuno-Oncology Biomarker Working Group has provided a standardized and reproducible method for assessing TIL density in BC [14]. Although a high percentage of TILs suggests a favorable prognosis for ER- tumors, their impact on ER+ tumors remains unknown. Several studies have retrospectively studied the role of TIL infiltration in the prognosis of these patients, but to date, no positive impact has been shown on OS, disease-free survival (DFS), or breast-cancer-specific survival. In large cohorts of patients with ER+ tumors, actually, high levels of CD8+ TILs have been associated with an unfavorable prognosis [15][16].

The clinical utility of TIL infiltration is not limited to its prognostic value. Different studies have shown that assessing the quantity/density of TILs can be used as a predictive factor for specific therapeutic treatments, especially in ER-

tumors.

In summary, TILs exhibit functional and phenotypic diversity. Therefore, a panel of parameters, including TIL counts, TIL subsets, their tumor reactivity, and functional states, should be considered to define truly “hot” tumors that may help predict a better prognosis following cancer therapy.

2.1.2. Tumor-Associated Macrophages

Tumor-associated macrophages (TAMs) play a role in tumor biology by mediating tumor growth and progression, as well as contributing to therapy resistance [17]. In breast cancer, resident macrophages and the recruitment of circulating monocytes support TAM accumulation [18]. TAMs have historically been divided into two categories: M1 and M2. M1 refers to macrophages that undergo classical activation via interferon-γ (IFNγ) with either lipopolysaccharide (LPS) or TNF, whereas M2 refers to macrophages that undergo alternative activation via IL-4 [19]. M1 or M2 polarized macrophages have opposing effects on tumor progression. Evidence suggests that an increase in M1 macrophages in the TME is associated with reduced tumor aggressiveness, while an increase in M2 macrophages is related to tumor growth and poor cancer prognosis [20].

In the early 1970s, Wood and Gollahon observed the presence of macrophages in the breast TME, which determined the risk of disease progression and therapeutic resistance [21]. Since then, multiple clinical studies have supported the value of enumerating TAMs for prognosis and/or predicting outcomes [22][23][24].

The TAM status also predicts sensitivity to chemotherapy and radiation therapy. When leukocyte complexity in breast cancer tissue was evaluated, Ruffell, et al. found that, in patients who had not received chemotherapy, macrophages were predominantly present in non-adjacent normal tissues but not in breast tissues [25].

2.2. Cancer-Associated Fibroblasts (CAFs) and the Extracellular Matrix (ECM)

Cancer-associated fibroblasts (CAFs) are one of the most abundant stromal components in the TME. Multiple studies have demonstrated that CAFs play a prominent role in cancer pathogenesis, which has significant clinical implications [26]. Activated CAFs show migratory and proliferative properties, unlike their inactive counterparts. Their most distinctive feature is the high capacity of synthesis and remodeling of the extracellular matrix (ECM) during the desmoplastic reaction [27][28]. In the desmoplastic reaction, activated fibroblasts abundantly synthesize various types of collagens, hyaluronan, fibronectins, and laminins that constitute the ECM and basement membrane [29]. The deposition of these components in the tumor stroma can act as a physical barrier against immune infiltration or as a structural scaffold for intercellular interaction, thus modulating tumorigenesis [30].

Similar to other cancer types, most studies have applied IHC to identify potential biomarkers derived from CAFs in BC. These studies suggest that the nature and quantity of CAFs have direct prognostic relevance in these patients. For instance, a higher proportion of αSMA-positive myofibroblasts has been associated with increased tumor cell proliferation and shorter recurrence-free survival and breast-cancer-specific survival [31]. Finak, et al. used laser capture microdissection to isolate fibroblasts from normal breast tissue and breast cancer to establish a “CAF gene

signature" consisting of 26 genes associated with an adverse outcome in four published datasets comprising a total of 1021 cancerous tissues [32]. Researchers have also focused on specific molecular subtypes of BC. Roman-Perez, et al. defined an "active stroma signature" composed of genes involved in cell motility and fibrosis activation, which was associated with poor prognosis in 43 ER+ samples [33]. On the other hand, the study by Beck, et al. suggested that an activated stroma does not always indicate a more aggressive clinical behavior. They constructed a gene signature derived from desmoid-type fibromatosis, a type of soft tissue tumor. The signature was applied to identify a fibrotic stromal reaction associated with a favorable prognosis in ER+ breast cancer patients [34].

The evaluation of CAFs in BC can also serve as a predictive factor. In this regard, Yamashita, et al. identified that α-SMA expression in myofibroblasts is not only associated with a worse prognosis but also serves as an independent predictor of metastasis in patients with invasive breast cancer [35]. Interestingly, a pilot study for the detection of circulating CAFs in the peripheral blood of patients with advanced breast cancer determined that circulating CAFs were present in 88% of patients with metastasis compared to 33% of patients with localized tumors [36].

2.3. The Tumor Vasculature

Angiogenesis is a fundamental biological process that involves the formation of new blood vessels from pre-existing ones. It plays a crucial role in different physiological and pathological conditions, including wound healing, embryonic development, and tumor growth. In the context of cancer, angiogenesis is a critical factor that supports tumor progression, invasion, and metastasis. The process of angiogenesis involves several steps. First, endothelial cells, the building blocks of blood vessels, are activated and start to migrate towards the tumor under the influence of pro-angiogenic factors. Then, these endothelial cells proliferate and form tube-like structures, eventually connecting to the existing vasculature to create functional blood vessels that supply nutrients and oxygen to the tumor. However, as they grow and expand, tumors face a state of hypoxia or low oxygen levels.

Hypoxia induces the expression of hypoxia-inducible factor (HIF), which upregulates a series of oncogenes associated with an aggressive neoplastic cell phenotype [37]. In particular, the overexpression of HIF-1α protein has been identified in different types of tumors, where high levels influence the growth rate and metastatic potential of these tumors. In BC, the frequency of HIF-1α-positive cells increases with the clinical stage and is associated with a worse prognosis [38].

2.4. Adipocytes

Adipocytes are the primary cellular component of adipose tissue, and they play a crucial role in maintaining the energy balance. The dysregulation of adipocyte function leads to overweight and obesity. Various studies have demonstrated a relationship between obesity and a worse prognosis for breast cancer. This includes a prospective study of nearly 500,000 women, which established a progressive escalation in the risk of breast cancer mortality with each successive increase in BMI category [39].

3. The TME as a Therapeutic Target

3.1. Exploiting the Immune System for Therapeutic Benefit

3.1.1. Immune Checkpoint Inhibitors

Immune checkpoints are essential for regulating the immune response and preventing tissue damage. When proteins on the surfaces of T cells bind to proteins in other cells, like tumor cells, these checkpoints become activated to restrict the immune response. However, this signaling can also create an environment that allows tumor cells to evade destruction via the immune system. For over a decade, it has been shown that blocking these immune checkpoints with monoclonal antibodies can trigger effective anti-tumor responses in different types of cancer. A variety of immune checkpoint inhibitor (ICI) proteins have been proposed as therapeutic targets in cancer treatments. The most clinically developed ones include programmed cell death protein 1 (PD-1), programmed cell death ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) [40]. In breast cancer, the TN subtype presents characteristics that make it more susceptible to immunotherapy, such as the increased infiltration of T lymphocytes and elevated levels of protein expression, such as PD-L1, in tumor cells and the immune system.

In general, schedules combining PD-1/L1 inhibitors and chemotherapy have shown greater success than ICIs as a monotherapy. For instance, the IMpassion130 trial, which included previously untreated mTNBC patients, demonstrated that adding atezolizumab to nab-paclitaxel resulted in a clinically significant improvement in OS by 7 months in the PD-L1 positive subgroup [41][42]. These results led to the FDA's and European Commission's approval of atezolizumab and nab-paclitaxel for mTNBC patients with PD-L1 positivity, marking the first immunotherapy approval in breast cancer. Subsequently, the KEYNOTE program showed that the use of pembrolizumab, a monoclonal antibody targeting PD-1, in combination with chemotherapy, led to clinically significant improvements in different clinical settings (KEYNOTE-355 and KEYNOTE-522 [43][44]).

3.1.2. Modulation of TAMs for Therapeutic Applications

With a better understanding of cancer immunology, different strategies are being explored to modulate TAMs for therapeutic purposes. For instance, bisphosphonates, compounds with a high affinity for hydroxyapatite, are used in the treatment of bone diseases such as osteoporosis and Paget's disease and for managing bone metastases. However, preclinical studies in murine models of breast cancer have suggested that they may also have an extra-skeletal therapeutic effect [45][46]. In this scenario, zoledronic acid, a bisphosphonate, attaches to microcalcifications present in breast tumors. Subsequently, it is phagocytosed via TAMs, inducing apoptosis and promoting the repolarization of M2 macrophages to M1.

3.2. Harnessing the Tumor Microenvironment Crosstalk as a Therapeutic Target

In ER+ breast cancer, estrogen provides a crucial mitogenic signal. Estradiol (E2) is synthesized in the ovaries and extragonadal sites, with extragonadal synthesis becoming dominant after menopause. In postmenopausal women, locally produced estrogen within fibroblasts and adipocytes represents the main source of E2. The enzyme

aromatase plays a key role in E2 biosynthesis, making it an excellent target for microenvironment-directed therapy [47][48]. Third-generation aromatase inhibitors—anastrozole, letrozole, and exemestane—can inhibit aromatization in the whole body by up to 98%. Aromatase inhibitors have been shown to be even more effective than tamoxifen in preventing breast cancer recurrence in postmenopausal women [49]. These drugs are approved based on data from randomized clinical trials in both adjuvant [50][51][52][53] and metastatic settings [54][55][56], as well as in combination with other drugs like CDK4/6 inhibitors [57][58][59], olaparib [60], or everolimus [61], with similar efficacy and toxicity. Other hormonal treatments, which are not targeted at the microenvironment, act directly on the ER: selective ER modulators (tamoxifen and raloxifene) or selective ER downregulators (new SERDs).

In addition, in response to the hypoxic microenvironment, tumor cells release pro-angiogenic factors, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), which promote the sprouting of new blood vessels from nearby vessels. Understanding the mechanisms of angiogenesis has led to the development of anti-angiogenic therapies, a promising approach to cancer treatment. Bevacizumab, a monoclonal antibody targeting VEGF, has been approved for use in several cancers, including colorectal and lung cancer [62][63]. Since many neoplasms are highly vascular and rely on a solid blood supply to maintain cellular viability, bevacizumab has been studied in combination with various cytotoxic chemotherapy regimens. In 2008, its use was approved in combination with paclitaxel for HER2-negative mBC [64]. However, due to considerable controversy, this approval was revoked in 2010 due to safety concerns, including an increase in thrombosis cases, and a lack of overall survival improvement in a large number of patients [65].

4. Conclusions

Overall, the TME holds significant prognostic and predictive value in breast cancer. Its composition and interaction with tumor cells play a key role in tumorigenesis, progression, and treatment resistance in breast cancer. As stated above, tumors with a high content of immune cells often exhibit a better response to immunotherapies. Moreover, specific TME markers, such as blood vessel density or TIL infiltration, have been shown to be related to patient survival. Therefore, TME analysis can provide valuable information for selecting more effective treatments and identifying patients with a higher risk of recurrence.

References

1. Sung, P.H.; Ferlay, P.J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, P.A.; Bray, F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 2018, 68, 394–424.
2. Simpson, J.F.; Gray, R.; Dressler, L.G.; Cobau, C.D.; Falkson, C.I.; Gilchrist, K.W.; Pandya, K.J.; Page, D.L.; Robert, N.J. Prognostic Value of Histologic Grade and Proliferative Activity in Axillary Node–Positive Breast Cancer: Results from the Eastern Cooperative Oncology Group Companion Study, EST 4189. J. Clin. Oncol. 2000, 18, 2059–2069.

3. Ignatiadis, M.; Sotiriou, C. Luminal breast cancer: From biology to treatment. Nat. Rev. Clin. Oncol. 2013, 10, 494–506.
4. Oh, D.Y.; Bang, Y.J. HER2-targeted therapies—A role beyond breast cancer. Nat. Rev. Clin. Oncol. 2020, 17, 33–48.
5. Yerushalmi, R.; Woods, R.; Ravdin, P.M.; Hayes, M.M.; Gelmon, K.A. Ki67 in breast cancer: Prognostic and predictive potential. Lancet Oncol. 2010, 11, 174–183.
6. Kalinsky, K.; Barlow, W.E.; Gralow, J.R.; Meric-Bernstam, F.; Albain, K.S.; Hayes, D.F.; Lin, N.U.; Perez, E.A.; Goldstein, L.J.; Chia, S.K.L.; et al. 21-Gene Assay to Inform Chemotherapy Benefit in Node-Positive Breast Cancer. New Engl. J. Med. 2021, 385, 2336–2347.
7. Fico, F.; Santamaria-Martínez, A. The tumor microenvironment as a driving force of breast cancer stem cell plasticity. Cancers 2020, 12, 3863.
8. Gonzalez, H.; Hagerling, C.; Werb, Z. Roles of the immune system in cancer: From tumor initiation to metastatic progression. Genes Dev. 2018, 32, 1267–1284.
9. Baxevanis, C.N.; Fortis, S.P.; Perez, S.A. The balance between breast cancer and the immune system: Challenges for prognosis and clinical benefit from immunotherapies. Semin. Cancer Biol. 2019, 72, 76–89.
10. Savas, P.; Salgado, R.; Denkert, C.; Sotiriou, C.; Darcy, P.K.; Smyth, M.J.; Loi, S. Clinical relevance of host immunity in breast cancer: From TILs to the clinic. Nat. Rev. Clin. Oncol. 2016, 13, 228–241.
11. Loi, S.; Drubay, D.; Adams, S.; Pruneri, G.; Francis, A.P.; Lacroix-Triki, M.; Joensuu, H.; Dieci, M.V.; Badve, S.; Demaria, S.; et al. Tumor-Infiltrating Lymphocytes and Prognosis: A Pooled Individual Patient Analysis of Early-Stage Triple-Negative Breast Cancers. J. Clin. Oncol. 2019, 37, 559–569.
12. Mahmoud, S.M.A.; Paish, E.C.; Powe, D.G.; Macmillan, R.D.; Grainge, M.J.; Lee, A.H.S.; Ellis, I.O.; Green, A.R. Tumor-Infiltrating CD8+ Lymphocytes Predict Clinical Outcome in Breast Cancer. J. Clin. Oncol. 2011, 29, 1949–1955.
13. Denkert, C.; Wienert, S.; Poterie, A.; Loibl, S.; Budczies, J.; Badve, S.; Bago-Horvath, Z.; Bane, A.; Bedri, S.; Brock, J.; et al. Standardized evaluation of tumor-infiltrating lymphocytes in breast cancer: Results of the ring studies of the international immuno-oncology biomarker working group. Mod. Pathol. 2016, 29, 1155–1164.
14. Salgado, R.; Denkert, C.; Demaria, S.; Sirtaine, N.; Klauschen, F.; Pruneri, G.; Wienert, S.; Van den Eynden, G.; Baehner, F.L.; Penault-Llorca, F.; et al. The evaluation of tumor-infiltrating lymphocytes (TILS) in breast cancer: Recommendations by an International TILS Working Group 2014. Ann. Oncol. 2015, 26, 259–271.

15. Baker, K.; Lachapelle, J.; Zlobec, I.; Bismar, T.A.; Terracciano, L.; Foulkes, W.D. Prognostic significance of CD8+ T lymphocytes in breast cancer depends upon both oestrogen receptor status and histological grade. Histopathology 2011, 58, 1107–1116.
16. Ali, H.R.; Provenzano, E.; Dawson, S.J.; Blows, F.M.; Liu, B.; Shah, M.; Earl, H.M.; Poole, C.J.; Hiller, L.; Dunn, J.A.; et al. Association between CD8+ T-cell infiltration and breast cancer survival in 12,439 patients. Ann. Oncol. 2014, 25, 1536–1543.
17. Mantovani, A.; Marchesi, F.; Malesci, A.; Laghi, L.; Allavena, P. Tumour-associated macrophages as treatment targets in oncology. Nat. Rev. Clin. Oncol. 2017, 14, 399–416.
18. Franklin, R.A.; Liao, W.; Sarkar, A.; Kim, M.V.; Bivona, M.R.; Liu, K.; Pamer, E.G.; Li, M.O. The cellular and molecular origin of tumor-associated macrophages. Science 2014, 344, 921–925.
19. Boutilier, A.J.; Elsawa, S.F. Macrophage polarization states in the tumor microenvironment. Int. J. Mol. Sci. 2021, 22, 6995.
20. Komohara, Y.; Jinushi, M.; Takeya, M. Clinical significance of macrophage heterogeneity in human malignant tumors. Cancer Sci. 2013, 105, 1–8.
21. Wood, G.W.; Gollahon, K.A. Detection and Quantitation of Macrophage Infiltration Into Primary Human Tumors With the Use of Cell-Surface Markers. JNCI J. Natl. Cancer Inst. 1977, 59, 1081–1087.
22. Leek, R.; Lewis, C.; Whitehouse, R.; Greenall, M.; Clarke, J.; Harris, A.L. Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. AACR 1996, 56, 4625–4629.
23. Tsutsui, S.; Yasuda, K.; Suzuki, K.; Tahara, K.; Higashi, H.; Era, S. Macrophage infiltration and its prognostic implications in breast cancer: The relationship with VEGF expression and microvessel density. Oncol. Rep. 2005, 14, 425–431.
24. Mahmoud, S.M.A.; Lee, A.H.S.; Paish, E.C.; Macmillan, R.D.; Ellis, I.O.; Green, A.R. Tumour-Infiltrating Macrophages And Clinical Outcome In Breast Cancer. J. Clin. Pathol. 2012, 65, 159–163.
25. Ruffell, B.; Au, A.; Rugo, H.S.; Esserman, L.J.; Hwang, E.S.; Coussens, L.M. Leukocyte composition of human breast cancer. Proc. Natl. Acad. Sci. USA 2011, 109, 2796–2801.
26. Su, S.; Chen, J.; Yao, H.; Liu, J.; Yu, S.; Lao, L.; Wang, M.; Luo, M.; Xing, Y.; Chen, F.; et al. CD10+GPR77+ Cancer-Associated Fibroblasts Promote Cancer Formation and Chemoresistance by Sustaining Cancer Stemness. Cell 2018, 172, 841–856e16.
27. Tsujino, T.; Seshimo, I.; Yamamoto, H.; Chew, Y.N.; Ezumi, K.; Takemasa, I.; Ikeda, M.; Sekimoto, M.; Matsuura, N.; Monden, M.; et al. Stromal myofibroblasts predict disease recurrence for colorectal cancer. Clin. Cancer Res. 2007, 13, 2082–2090.

28. Calvo, F.; Ege, N.; Grande-Garcia, A.; Hooper, S.; Jenkins, R.P.; Chaudhry, S.I.; Harrington, K.; Williamson, P.; Moeendarbary, E.; Charras, G.; et al. Mechanotransduction and YAP-dependent matrix remodelling is required for the generation and maintenance of cancer-associated fibroblasts. Nat. Cell Biol. 2013, 15, 637–646.
29. Tomasek, J.J.; Gabbiani, G.; Hinz, B.; Chaponnier, C.; Brown, R.A. Myofibroblasts and mechano: Regulation of connective tissue remodelling. Nat. Rev. Mol. Cell Biol. 2002, 3, 349–363.
30. Cukierman, E.; Bassi, D.E. Physico-mechanical aspects of extracellular matrix influences on tumorigenic behaviors. Semin. Cancer Biol. 2010, 20, 139–145.
31. Surowiak, P.; Surowiak, P.; Murawa, D.; Materna, V.; Maciejczyk, A.; Pudelko, M.; Ciesla, S.; Breborowicz, J.; Murawa, P.; Zabel, M.; et al. Occurence of stromal myofibroblasts in the invasive ductal breast cancer tissue is an unfavourable prognostic factor. Anticancer. Res. 2007, 27, 2917–2924.
32. Finak, G.; Bertos, N.; Pepin, F.; Sadekova, S.; Souleimanova, M.; Zhao, H.; Chen, H.; Omeroglu, G.; Meterissian, S.; Omeroglu, A.; et al. Stromal gene expression predicts clinical outcome in breast cancer. Nat. Med. 2008, 14, 518–527.
33. Román-Pérez, E.; Casbas-Hernández, P.; Pirone, J.R.; Rein, J.; Carey, L.A.; Lubet, R.A.; Mani, S.A.; Amos, K.D.; Troester, M.A. Gene expression in extratumoral microenvironment predicts clinical outcome in breast cancer patients. Breast Cancer Res. 2012, 14, R51.
34. Beck, A.H.; Espinosa, I.; Gilks, C.B.; Van De Rijn, M.; West, R.B. The fibromatosis signature defines a robust stromal response in breast carcinoma. Lab. Investig. 2008, 88, 591–601.
35. Yamashita, M.; Ogawa, T.; Zhang, X.; Hanamura, N.; Kashikura, Y.; Takamura, M.; Yoneda, M.; Shiraishi, T. Role of stromal myofibroblasts in invasive breast cancer: Stromal expression of alpha-smooth muscle actin correlates with worse clinical outcome. Breast Cancer 2012, 19, 170–176.
36. Ao, Z.; Shah, S.H.; Machlin, L.M.; Parajuli, R.; Miller, P.C.; Rawal, S.; Williams, A.J.; Cote, R.J.; Lippman, M.E.; Datar, R.H.; et al. Identification of cancer-associated fibroblasts in circulating blood from patients with metastatic breast cancer. Cancer Res. 2015, 75, 4681–4687.
37. de Heer, E.C.; Jalving, M.; Harris, A.L. HIFs, angiogenesis, and metabolism: Elusive enemies in breast cancer. Clin. Investig. 2020, 130, 5074–5087.
38. Shamis, S.A.K.; McMillan, D.C.; Edwards, J. The relationship between hypoxia-inducible factor 1α (HIF-1α) and patient survival in breast cancer: Systematic review and meta-analysis. Crit. Rev. Oncol. 2021, 159, 103231.
39. Calle, E.E.; Rodriguez, C.; Walker-Thurmond, K.; Thun, M.J. Overweight, Obesity, and Mortality from Cancer in a Prospectively Studied Cohort of U.S. Adults. N. Engl. J. Med. 2003, 348, 1625–1638.

40. Gaynor, N.; Crown, J.; Collins, D.M. Immune checkpoint inhibitors: Key trials and an emerging role in breast cancer. Semin. Cancer Biol. 2022, 79, 44–57.
41. Schmid, P.; Adams, S.; Rugo, H.S.; Schneeweiss, A.; Barrios, C.H.; Iwata, H.; Diéras, V.; Hegg, R.; Im, S.A.; Shaw Wright, G.; et al. Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. N. Engl. J. Med. 2018, 379, 2108–2121.
42. Schmid, P.; Adams, S.; Rugo, H.S.; Schneeweiss, A.; Barrios, C.H.; Iwata, H.; Dieras, V.; Henschel, V.; Molinero, L.; Chui, S.Y.; et al. IMpassion130: Updated overall survival (OS) from a global, randomized, double-blind, placebo-controlled, Phase III study of atezolizumab (atezo) + nab- paclitaxel (nP) in previously untreated locally advanced or metastatic triple-negative breast cancer (mTNBC). J. Clin. Oncol. 2019, 37, 1003.
43. Schmid, P.; Cortes, J.; Pusztai, L.; McArthur, H.; Kümmel, S.; Bergh, J.; Denkert, C.; Park, Y.H.; Hui, R.; Harbeck, N.; et al. Pembrolizumab for Early Triple-Negative Breast Cancer. N. Engl. J. Med. 2020, 382, 810–821.
44. Cortes, J.; Rugo, H.S.; Cescon, D.W.; Im, S.A.; Yusof, M.M.; Gallardo, C.; Lipatov, O.; Barrios, C.H.; Perez-Garcia, J.; Iwata, H.; et al. Pembrolizumab plus Chemotherapy in Advanced Triple-Negative Breast Cancer. N. Engl. J. Med. 2022, 387, 217–226.
45. Locati, M.; Curtale, G.; Mantovani, A. Diversity, Mechanisms, and Significance of Macrophage Plasticity. Annu. Rev. Pathol. Mech. Dis. 2020, 15, 123–147.
46. Mantovani, A.; Sozzani, S.; Locati, M.; Allavena, P.; Sica, A. Macrophage polarization: Tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol. 2002, 23, 549–555.
47. Santen, R.J.; Santner, S.J.; Pauley, R.J.; Tait, L.; Kaseta, J.; Demers, L.M.; Hamilton, C.; Yue, W.; Wang, J.P. Estrogen production via the aromatase enzyme in breast carcinoma: Which cell type is responsible? J. Steroid Biochem. Mol. Biol. 1997, 61, 267–271.
48. Santner, S.J.; Pauley, R.J.; Tait, L.; Kaseta, J.; Santen, R.J. Aromatase Activity and Expression in Breast Cancer and Benign Breast Tissue Stromal Cells 1. J. Clin. Endocrinol. Metab. 1997, 82, 200–208.
49. Cuzick, J.; Sasieni, P.; Howell, A. Should aromatase inhibitors be used as initial adjuvant treatment or sequenced after tamoxifen? Br. J. Cancer 2006, 94, 460–464.
50. Bradley, R.; Burrett, J.; Clarke, M.; Davies, C.; Duane, F.; Evans, V.; Gettins, L.; Godwin, J.; Gray, R.; Liu, H.; et al. Aromatase inhibitors versus tamoxifen in early breast cancer: Patient-level meta-analysis of the randomised trials. Lancet 2015, 386, 1341–1352.
51. Regan, M.M.; Neven, P.; Giobbie-Hurder, A.; Goldhirsch, A.; Ejlertsen, B.; Mauriac, L.; Forbes, J.F.; Smith, I.; Láng, I.; Wardley, A.; et al. Assessment of letrozole and tamoxifen alone and in sequence for postmenopausal women with steroid hormone receptor-positive breast cancer: The

BIG 1-98 randomised clinical trial at 81 years median follow-up. Lancet Oncol. 2011, 12, 1101–1108.

52. Goss, P.E.; Ingle, J.N.; Pritchard, K.I.; Ellis, M.J.; Sledge, G.W.; Budd, G.T.; Rabaglio, M.; Ansari, R.H.; Johnson, D.B.; Tozer, R.; et al. Exemestane versus anastrozole in postmenopausal women with early breast cancer: NCIC CTG MA.27—A randomized controlled phase III trial. J. Clin. Oncol. 2013, 31, 1398–1404.
53. Smith, I.; Yardley, D.; Burris, H.; De Boer, R.; Amadori, D.; McIntyre, K.; Ejlertsen, B.; Gnant, M.; Jonat, W.; Pritchard, K.I.; et al. Comparative efficacy and safety of adjuvant letrozole versus anastrozole in postmenopausal patients with hormone receptor-positive, node-positive early breast cancer: Final results of the randomized phase III femara versus anastrozole clinical evaluation (FACE) trial. J. Clin. Oncol. 2017, 35, 1041–1048.
54. Mauri, D.; Pavlidis, N.; Polyzos, N.P.; Ioannidis, J.P.A. Survival with aromatase inhibitors and inactivators versus standard hormonal therapy in advanced breast cancer: Meta-analysis. J. Natl. Cancer Inst. 2006, 98, 1285–1291.
55. Campos, S.; Guastalla, J.P.; Subar, M.; Abreu, P.; Winer, E.; Cameron, D. A comparative study of exemestane versus anastrozole in patients with postmenopausal breast cancer with visceral metastases. Clin. Breast Cancer 2009, 9, 39–44.
56. Geisler, J.; Haynes, B.; Anker, G.; Dowsett, M.; Lønning, P.E. Influence of Letrozole and Anastrozole on Total Body Aromatization and Plasma Estrogen Levels in Postmenopausal Breast Cancer Patients Evaluated in a Randomized, Cross-Over Study. J. Clin. Oncol. 2002, 20, 751–757.
57. Finn, R.S.; Martin, M.; Rugo, H.S.; Jones, S.; Im, S.A.; Gelmon, K.; Harbeck, N.; Lipatov, O.N.; Walshe, J.M.; Moulder, S.; et al. Palbociclib and Letrozole in Advanced Breast Cancer. N. Engl. J. Med. 2016, 375, 1925–1936.
58. Im, S.-A.; Im, S.A.; Lu, Y.S.; Bardia, A.; Harbeck, N.; Colleoni, M.; Franke, F.; Chow, L.; Sohn, J.; Lee, K.S.; et al. Overall Survival with Ribociclib plus Endocrine Therapy in Breast Cancer. N. Engl. J. Med. 2019, 381, 307–316.
59. Johnston, S.; Martin, M.; Di Leo, A.; Im, S.A.; Awada, A.; Forrester, T.; Frenzel, M.; Hardebeck, M.C.; Cox, J.; Barriga, S.; et al. MONARCH 3 final PFS: A randomized study of abemaciclib as initial therapy for advanced breast cancer. Npj Breast Cancer 2019, 5, 5.
60. Tutt, A.N.J.; Garber, J.E.; Kaufman, B.; Viale, G.; Fumagalli, D.; Rastogi, P.; Gelber, R.D.; de Azambuja, E.; Fielding, A.; Balmaña, J.; et al. Adjuvant Olaparib for Patients with BRCA1—Or BRCA2 -Mutated Breast Cancer. N. Engl. J. Med. 2021, 384, 2394–2405.
61. Baselga, J.; Campone, M.; Piccart, M.; Burris, H.A.; Rugo, H.S.; Sahmoud, T.; Noguchi, S.; Gnant, M.; Pritchard, K.I.; Lebrun, F.; et al. Everolimus in Postmenopausal Hormone-Receptor–

Positive Advanced Breast Cancer. N. Engl. J. Med. 2012, 366, 520–529.

62. Bear, H.D.; Tang, G.; Rastogi, P.; Geyer, C.E.; Liu, Q.; Robidoux, A.; Baez-Diaz, L.; Brufsky, A.M.; Mehta, R.S.; Fehrenbacher, L.; et al. Neoadjuvant plus adjuvant bevacizumab in early breast cancer (NSABP B-40): Secondary outcomes of a phase 3, randomised controlled trial. Lancet Oncol. 2015, 16, 1037–1048.
63. von Minckwitz, G.; Loibl, S.; Untch, M.; Eidtmann, H.; Rezai, M.; Fasching, P.A.; Tesch, H.; Eggemann, H.; Schrader, I.; Kittel, K.; et al. Survival after neoadjuvant chemotherapy with or without bevacizumab or everolimus for HER2-negative primary breast cancer (GBG 44-GeparQuinto). Ann. Oncol. 2014, 25, 2363–2372.
64. Miller, K.; Miller, K.; Wang, M.; Gralow, J.; Dickler, M.; Cobleigh, M.; Perez, E.A.; Shenkier, T.; Cella, D.; Davidson, N.E. Paclitaxel plus Bevacizumab versus Paclitaxel Alone for Metastatic Breast Cancer. N. Engl. J. Med. 2007, 357, 2666–2676.
65. Gray, R.; Bhattacharya, S.; Bowden, C.; Miller, K.; Comis, R.L. Independent review of E2100: A phase III trial of bevacizumab plus paclitaxel versus paclitaxel in women with metastatic breast cancer. J. Clin. Oncol. 2009, 27, 4966–4972.