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Advancing breast cancer therapy in the era of molecular diagnostics☆

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ABSTRACT

Advances in cancer biology and drug development now enable treatments tailored to individual tumor profile. Targeting specific molecular alterations marked a significant step forward in cancer care, including breast cancer. Access to these therapies is improving thanks to the implementation of molecular tumor boards and efforts to provide molecular diagnostics at sustainable costs for all. In this context, we highlight recent progress in breast cancer therapy, focusing on biomarker-driven approaches, immunotherapy, and precision medicine paving the way for increasingly personalized and effective options.

1. Introduction

Breast cancer affects over two million people annually and is the leading cause of cancer-related death among women worldwide [1]. Originally categorized based on gene expression profiles [2], breast cancer intrinsic subtypes are now identified in clinical practice using surrogate markers. Luminal A and B tumors are hormone receptor-positive (HR-positive) and differ in proliferation rates, with luminal B exhibiting increased levels of Ki67; HER2-positive tumors are characterized by HER2 overexpression and/or gene amplification, whereas triple-negative breast cancer (TNBC) is defined by the absence of both HR and HER2 [3]. Treatment has traditionally involved surgery, chemotherapy, and radiation. However, advances in molecular profiling and drug development have expanded opportunities, allowing therapies based on the individual tumor molecular profile [4]. Actionable molecular alterations, either at the somatic (e.g., *PIK3CA*, *AKT*, *ESR1*) or germline (e.g., *BRCA1 and BRCA 2*) level, have already been implemented in clinical practice, while representing only the tip of an iceberg [5]. Comprehensive gene profiling shows that up to two-thirds of metastatic patients have tumors with molecular alterations, which could inform treatment strategies [6]. Additionally, the development of immune checkpoint inhibitors has broadened the scope of personalized treatment by activating the individual immune system against cancer [7–9]. Herein, we discuss recent advances in breast cancer treatment, focusing on biomarker-driven approaches, immunotherapy, and precision medicine for personalized strategies.

2. Targeted therapy

This section aims to provide a brief overview of major molecular

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alterations in breast cancer and their corresponding matched drugs (Table 1).

2.1. PIK3CA

Aberrant activation of the phosphatidylinositol 3-kinase (PI3K) pathway leads to uncontrolled tumor cell growth and drug resistance. Somatic alteration of the *PIK3CA* gene, which encodes the p110α catalytic subunit of PI3K, occurs in 40 % of HR-positive breast cancer [10]. Most of these mutations cluster in the helical and kinase domains, specifically the E545K, E542K, and H1047R hotspot mutations, which increase PI3K activity resulting in constitutive phosphorylation of AKT and its downstream effectors. Other alterations in the pathway include *PTEN* loss-of-function (2–4 %) and *AKT1* activating mutations (2–3 %). Similar patterns have been observed in HER2-positive breast cancer. In contrast, TNBC is more frequently associated with *PTEN* (30–50 %) than with *PIK3CA* mutations (<10 %) [10].

Table 1
Breast cancer targets and matched therapeutics.

TARGET	MATCHED THERAPY	
	Already available	In development
PIK3CA	**α-specific PI3K inhibitor**	**Isoform-specific PI3K inhibitors**
	Alpelisib	HS-10352
		Inavolisib
		MEN1611
		OKI-219
		RLY-2608
		STX-478
		Tenalesib
		TOS-358
		Dual PI3K and mTOR inhibitors
		Gedatolisib
AKT	**Pan AKT inhibitor**	**Pan AKT inhibitor**
	Capivasertib	Ipatasertib
ESR1	**SERD**	**SERD**
	Elacestrant	Amcenestrant
		Borestrant
		Camizestrant
		Giredestrant
		Imlunestrant,
		Rintodestrant
		Taragarestrant
BRCA1/2	**Parp inhibitors**	**Parp inhibitors**
	Olaparib	Niraparib
	Talazoparib	Pamiparib
		Rucaparib
		Veliparib
HER2	**Anti-HER2 mAb**	**Anti-HER2 mAb**
	Trastuzumab	Zanidantamab
	Pertuzumab	**TKIs**
	Margetuximab	Pyrotinib
	TKIs	Poziotinib
	Lapatinib	**Anti-HER2 ADCs**
	Neratinib	A166 (ZW49, SHR-A1811)
	Tucatinib	ARX788
	Anti-HER2 ADCs	SYD985
	TDM-1	RC48
	T-DXd	**Anti-HER3 ADCs**
		Patritumab deruxtecan (HER3-DXd)
		SHR-A2009
PD−1/PD−L1 axis	Pembrolizumab	Adebrelimab
	Atezolizumab	Camrelizumab
		Cemiplimab
		Dostarlimab
		Durvalumab
		SHR1701

Abbreviations: ADC, antibody-drug conjugate; mAb, monoclonal antibody; HER2, human epidermal growth factor receptor 2; PIK3CA, Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.
Source: clinicaltrials.gov, as assessed on November 12, 2024

Alpelisib is a potent and selective α-specific PI3K inhibitor and earned approval for postmenopausal HR-positive/HER2-negative *PIK3CA*-mutated advanced breast cancer patients progressing on endocrine therapy. Based on the SOLAR-1 study, the combination of alpelisib plus fulvestrant showed a significant improvement in median progression-free survival (PFS) over fulvestrant alone, 11 *versus* (vs.) 5.7 months (Hazard Ratio [HR] 0.65, 95 % Confidence Interval [CI] 0.50–0.85; $p < 0.001$) [11]. The efficacy and safety of alpelisib was confirmed in the BYLieve study, investigating its combination with fulvestrant after progression to CDK4/6 inhibitors. This trial reinforced previously reported findings, demonstrating that alpelisib resulted in a proportion of patients alive at six months of 50.4 %, albeit with a non negligible 26 % rate of serious adverse events [12]. The phase III EPIK-B5 study is currently ongoing to confirm the efficacy and safety of alpelisib plus fulvestrant in a broader population of patients with HR-positive/HER2-negative/*PIK3CA*-mutated advanced breast cancer who have previously received CDK4/6 inhibitor and aromatase inhibitor treatment [13]. Several other selective α-specific PI3K inhibitors are currently being studied, including inavolisib. When combined with palbociclib and fulvestrant in selected patients with mutated *PIK3CA* tumors, the combination of inavolisib nearly doubled disease control compared to standard therapy, achieving a median PFS of 15 vs. 7.3 months [14]. International guidelines do not currently recommend mTOR inhibitors as a standard treatment in *PI3KCA*-mutated breast cancer. However, subgroup analyses from BOLERO-2 in patients with evaluable PI3K status showed that everolimus combined with exemestane outperforms fulvestrant, with a median PFS of 6.7 vs. 2.76 months (HR = 0.51) in 143 PIK3CA-mutated patients, and 8.25 vs. 4.17 months (HR = 0.37) in 159 wild-type patients [reviewed in 15]. This confirms that everolimus is a mutation-agnostic targeted therapy.

It is important to note that these subgroup analyses were conducted in 302 out of the 724 original patients. More importantly, BOLERO-2 and other everolimus studies were primarily conducted in CDK4/6 inhibitor-naïve patients [reviewed in 15]. No randomized trials have evaluated everolimus after CDK4/6 inhibitors in PI3KCA-mutated breast cancer. A recently reported single-arm phase II study in 57 patients receiving everolimus plus fulvestrant showed a median PFS of 6.9 months and a median OS of 38.3 months. In the PIK3CA-mutated subgroup, the median PFS was 3.1 months, while median OS was not reached [reviewed in 15]. Given the limited data on everolimus in PIK3CA-altered patients following CDK4/6i treatment, *PIK3CA* or *AKT* inhibitors are preferred over everolimus. However, when and where these inhibitors are unavailable, everolimus plus fulvestrant remains a viable option. While we are writing, the FDA approved the combination of inavolisib plus palbociclib and fulvestrant for patients with endocrine-resistant, PIK3CA-mutated, HR-positive and HER2-negative advanced breast cancer, based on the INAVO120 trial (NCT04191499) [15].This approval applies to patients with either metastatic progression or disease within 12 months of adjuvant endocrine therapy. The European Medicines Agency EMA is currently reviewing the data for potential approval. It is estimated that 20 %–40 % of patients exhibit endocrine-resistant disease at the time of initiating first-line treatment, suggesting that a portion of patients with PIK3CA-mutant tumors could benefit from this upfront strategy.

The recent ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT) classifies *PIK3CA* mutations in tier “IA” to support the prescription of α-specific PI3K inhibitors [16]. However, the optimal method for detecting these mutations is still undefined. Comprehensive gene profiling identifies *PIK3CA* mutations in almost 35 % of metastatic breast cancer patients, 20 % more than qPCR used in the Therascreen® PIK3CA companion test for alpelisib [13]. Notably, PTEN loss is a key resistance mechanism to α-specific PI3K inhibitors, causing a switch from PI3Kα to PI3Kβ dependency, and leading to continuous signaling and therapy escape [reviewed in 9]. PTEN loss is common in both patients who do not respond at all to treatment (25 %), and those who

eventually progress after initial response.

2.2. AKT

AKT inhibitors have been developed and continue to be refined to overcome endocrine resistance. Over time, these drugs have become more selective and have reduced off-target effects, especially in the latest generations. Capivasertib, for instance, selectively inhibits all *AKT* isoforms (*AKT1, AKT2,* and *AKT3*), blocking cell growth by disrupting downstream signaling [10]. The phase II FAKTION study demonstrated that capivasertib combined with fulvestrant significantly improved both PFS and overall survival (OS) compared to fulvestrant alone in HR-positive/HER2-negative breast cancer patients, especially in those with PI3K pathway alterations [17]. The CAPItello-291 study confirmed an improvement of PFS by 40 % overall, and 50 % in selected cases for dysregulated PI3K pathway. These findings led to the approval of capivasertib in combination with fulvestrant for advanced breast cancer patients with one or more *PIK3CA/AKT1/PTEN* alterations following recurrence or progression on or after endocrine therapy [18].

As such, for patients with activating *PIK3CA* mutations, either capivasertib or alpelisib are viable options. In the absence of a direct comparative clinical trial, the decision is based on pragmatic factors such as drug availability and the toxicity profile, with capivasertib likely offering better safety due to fewer cases of hyperglycemia.

2.3. ESR1

ESR1 mutations are a key mechanism of drug resistance, occurring in about 30 % of patients receiving endocrine therapy.

ESR1 mutations are most frequently acquired when aromatase inhibitors are used to treat advanced breast cancer. These mutations are more frequently selected in patients that progress after sensitivity to prior aromatase inhibitor therapy, and are relatively rare in patients with intrinsic endocrine resistance [19].

In metastatic disease, liquid biopsy is able to detect *ESR1* mutations up to a 25 % higher rate than solid biopsies due to their polyclonal nature [20]. The phase III PADA-1 study demonstrated that switching to fulvestrant and palbociclib upon detection of rising *ESR1* mutations significantly improved PFS from 5.7 to 11.9 months compared to continuing palbociclib with an aromatase inhibitor [21]. These findings have been fundamental for the development of oral selective estrogen receptor degrader (SERDs). Currently, elacestrant is the only SERD approved for clinical practice, demonstrating a 30 % improvement in PFS over standard therapy overall, and a 45 % improvement in patients with *ESR1* mutations [22]. Building on these findings, the SERENA-6 study is testing longitudinal monitoring of *ESR1* mutations during first-line treatment, with a switch to the novel SERD camizestrant upon mutation detection [23]. Safety data and early activity results are also available for imlunestrant, with first-line metastatic results expected soon [24].

2.4. PARP

2.4.1. Studies in patients with germline pathogenic or likely pathogenic variants of BRCA1 and BRCA2 genes (gBRCA)

Following the pivotal results of the phase III OlympiAD study, olaparib became the first approved PARP inhibitor in 2018. In this study, patients with HER2-negative breast cancer and g*BRCA* were randomized to receive either olaparib or standard chemotherapy (i.e., capecitabine, eribulin, or vinorelbine). Olaparib demonstrated a significant benefit, with a median PFS of 7 months, exceeding the 4.2 months observed with standard chemotherapy [25]. Although olaparib did not demonstrate a statistically significant improvement in OS, a potentially meaningful OS benefit was observed in patients who had not undergone chemotherapy for metastatic disease. More recently, olaparib has shown additional benefits in invasive disease-free survival (85.9 %) and distant disease-free survival (87.5 %) and OS (89.8 %) in the adjuvant setting for patients with high-risk, g*BRCA*-mutated, HER2-negative breast cancer who had undergone neoadjuvant or adjuvant therapy [26].

In addition, in the phase III EMBRACA trial, talazoparib demonstrated an improvement in median PFS (8.6 months vs. 5.6 months) and overall response rate (ORR) (62.6 % vs. 27.2 %) in patients with locally advanced or metastatic g*BRCA*-mutated, HER2-negative breast cancer, compared to standard treatment. However, the trial did not show any survival benefit [27]. Other PARP inhibitors, including rucaparib, veliparib, and niraparib, are currently being investigated, though none have received approval for clinical use. It is important to note that, to date, somatic multigene sequencing cannot replace germline testing for *BRCA1* and *BRCA2* mutations [28], and international guidelines recommend germline testing for *BRCA1* and *BRCA2* for all patients that are eligible to receive PARP inhibitors both in adjuvant and metastatic settings.

2.4.2. Studies in patients with somatic BRCA1/2 (sBRCA) mutations and other homologues recombination repair (HRR) genes

In the TBCRC-048 phase II study, olaparib demonstrated a promising ORR of 82 % in patients with germline PALB2 (gPALB2) mutations and 50 % in those with somatic BRCA1/2 (sBRCA1/2) mutations, with a median progression-free survival (PFS) of 13.3 months (90 % CI, 12 months to not available/computable [NA]) and 6.3 months (90 % CI, 4.4 months to NA), respectively [29]. In a phase II study of talazoparib in patients with advanced cancer harboring somatic BRCA1/2 mutations, the clinical benefit rate (CBR) was 28.6 % in those with breast cancer [30]. These preliminary results suggest that olaparib and talazoparib are promising treatment options for patients with somatic BRCA1/2 and gPALB2 mutations, thereby expanding the pool of metastatic breast cancer patients who could benefit from PARP inhibitors.

2.5. HER2

It is beyond the scope of this work to review the history of anti-HER2 treatment from the introduction of trastuzumab to the development of tyrosine kinase inhibitors (TKIs) and dual blockade strategies. However, before discussing novel anti-HER2 agents, it is noteworthy to recall that at a median follow-up of 8 years, the CLEOPATRA trial demonstrated a remarkable 16.3 month improvement in median OS (57.1 vs. 40.8 months, HR 0.69, 95 %CI 0.58–0.82) in favor of dual anti-HER2 blockade [31].

2.5.1. Antibody-drug conjugates

Antibody-drug conjugates (ADCs) are targeted therapies that reduce the non-specific cytotoxicity of traditional chemotherapy. These biological compounds consist of monoclonal antibodies (mAbs) linked to cytotoxic agents via bioactive linkers. Three generations of ADCs have been developed, with each improving upon the last. The first-generation gemtuzumab ozogamicin was withdrawn due to instability and toxicity. Second-generation ADCs, like trastuzumab emtansine (T-DM1), used more stable linkers, improving anti-tumor activity and safety in both metastatic and adjuvant settings. Third-generation ADCs, such as trastuzumab deruxtecan (T-DXd), feature stable linkers, a high drug-to-antibody ratio (DAR), and a topoisomerase I inhibitor (DXd) as the cytotoxic component reviewed in [32,33].

The phase II DESTINY-Breast01 study demonstrated T-DXd efficacy in HER2-positive metastatic breast cancer, with an overall response rate (ORR) of 60 % and a median PFS of 16.4 months in heavily pretreated patients [34]. This led to further evaluation in the DESTINY-Breast03 trial, which showed a median PFS of 29 months for T-DXd compared to 7.2 months for TDM-1, and a median OS of 52.6 vs. 42.7 months, respectively, despite a higher incidence of interstitial lung disease with T-DXd [35]. T-DXd was established as the preferred second-line treatment for advanced HER2-positive breast cancer after progression on taxanes and trastuzumab. The DESTINY-Breast04 trial showed superior

PFS and OS for T-DXd over chemotherapy in HER2-low (defined as 1+ by IHC, or 2+ by IHC in absence of gene amplification) advanced breast cancer [36]. Additionally, T-DXd demonstrated benefits in PFS and intracranial ORR in patients with stable, asymptomatic brain metastases [37]. The DEBBRAH and TUXEDO trials further explored T-DXd intracranial activity in HER2-positive and HER2-low patients, reporting promising results [38,39]. Ongoing studies, such as DESTINY-Breast07 and DESTINY-Breast09, will clarify its role in first-line metastatic settings and early-stage high-risk breast cancer [40,41]. The technology of ADCs has been applied to other antigens, including TROP2, whose discussion is beyond the scope of this overview because research on evaluating this target to select patients is still in its early-stages.

2.5.2. Tyrosine kinase inhibitors

Activating HER2 mutations drive ~5 % of metastatic breast cancer cases, primarily in the tyrosine kinase domain, often without HER2 gene amplification. These mutations are common in invasive lobular carcinoma and are linked to poor prognosis and resistance to endocrine therapy in HR-positive breast cancer [42]. The pan-HER TKI neratinib has shown anti-tumor activity in *HER2* mutant breast cancer, with NCCN recommending its use in metastatic cases harboring *HER2* mutations. However, clinical responses are often short-lived, with secondary *HER2* mutations emerging during progression, reducing sensitivity to neratinib. These secondary mutations enhance HER2 signaling through PI3K/Akt and MEK/ERK pathways, raising the threshold for HER2 TKI inhibition. HER2 double mutants are highly sensitive to nanomolar doses of HER2 and MEK inhibitors in combination [43]. The neratinib-trametinib combination has shown strong efficacy in HER2-amplified xenografts and is in clinical trials for *HER2*-mutant or amplified tumors. In addition, a recently published study evaluating tucatinib in combination with trastuzumab, with or without fulvestrant, showed an ORR of 41.9 % (90 % CI 26.9–58.2) and a PFS of 9.5 months (90 % CI 5.4–13.8) in 31 heavily pretreated HER2-negative breast cancer patients with HER2 mutations [44]. Other HER2 TKIs, like poziotinib or mobocertinib, may also enable sufficient inhibition to overcome resistance [43].

2.6. HER2 classifiers

HER2DX is a genomic tool designed to guide the treatment of HER2-positive early-stage breast cancer by combining data on immune response, tumor characteristics, and clinical factors [45]. It provides three key scores: long-term prognosis (risk score), likelihood of achieving pathological complete response (pCR score), and ERBB2 mRNA expression (ERBB2 score). The tool has been evaluated in approximately 2000 patients and has shown clinical value in both neoadjuvant and adjuvant settings [reviewed in 46]. Currently, it is being prospectively evaluated in the DEFINITIVE trial and retrospectively in the CompassHER2 trial.

In addition, the S18 signature and its reduction to the 5-gene S5, is a gene-expression-based classifier generated by principal component analysis of the genes that do not change their expression during treatment with anti-HER2 therapies. It provides valuable information beyond clinical variables on the long-term prognosis of patients treated with trastuzumab alone or in dual blockade with a TKI [46]. This signature demonstrated its prognostic value in the NSABP-B31 study [47] and is currently being evaluated in real-world patients undergoing therapy based on trastuzumab plus pertuzumab.

3. Immunotherapy

Breast cancer can evade immune response by reducing neoantigen expression, downregulating antigen-presenting cells, and releasing immunosuppressive cytokines [48]. Immune checkpoints including the PD-1/PD-L1 and CTLA-4 axis regulate immune tolerance and T-cell activation. Checkpoint inhibition can restore T-cell activity, enhancing anti-tumor immunity [48] especially in TNBC that presents tumor-infiltrating lymphocytes (TILs) and PD-L1 expression [49]. The results of the most advanced immunotherapy studies are presented below, while Table 2 provides a comprehensive list of all studies.

Evaluating PD-L1 expression on tumor and/or immune cells is essential, as regulatory agencies require an approved IHC PD-L1 assay for each immunotherapeutic agent. For pembrolizumab, the 22C3 pharmDx assay is used with the combined positive score (CPS, positive if $\geq$ 10), while for atezolizumab, the Ventana SP142 assay is used with the immune cell (IC, positive if $\geq$ 5 %) score. Beyond its indications for specific types of tumors, anti-PD-1 immunotherapy has also received tumor-agnostic approval for patients harboring certain molecular alterations or genomic signatures [50]. These include microsatellite instability (MSI) and a high tumor mutational burden (TMB), defined as greater than 10 mutations per megabase. Despite this broadened scope of approval, the prevalence of such alterations within the context of breast cancer remains exceptionally low [51], limiting the applicability of this therapeutic approach for the majority of breast cancer patients.

3.1. Anti PD-1

Pembrolizumab, a humanized IgG4 monoclonal antibody, targets the PD-1 receptor, blocking its interaction with PD-L1. The pivotal KEYNOTE-355 trial demonstrated a substantial PFS benefit in patients with a CPS (the number of PD L1-positive cells by the total number of tumor cells multiplying by 100) $\geq$ 10, where the addition of pembrolizumab to chemotherapy extended median PFS from 5.6 to 9.7 months compared to chemotherapy alone [52]. Further OS analysis confirmed a benefit in the CPS$\geq$ 10 group (23.0 vs. 16.1 months), though no survival advantage was observed in patients with CPS$\geq$ 1 or in the intention-to-treat population [9]. Additionally, the KEYNOTE-522 trial established pembrolizumab efficacy in early-stage TNBC, significantly improving pathological complete response (pCR, 64.8 % vs. 51.2 %) and event-free survival (EFS, 84.3 % vs. 76.8 %), leading to pembrolizumab first approval for early-stage TNBC [8,53], regardless of PD-L1 expression.

3.2. Anti PD-L1

Atezolizumab, a IgG1 monoclonal antibody blocking PD-L1, showed promise in the IMpassion130 trial, which demonstrated longer PFS in PD-L1-positive metastatic TNBC treated with atezolizumab plus nab-paclitaxel (7.5 vs. 5.0 months) [7]. Although an exploratory unplanned analysis showed prolonged survival in the PD-L1 subgroup (25.4 vs. 17.9 months), the study did not achieve OS significance in the intention-to-treat population, leading to the withdrawal of atezolizumab U.S. approval for this indication [54]. The IMpassion031 trial demonstrated that adding atezolizumab to neoadjuvant chemotherapy increased pCR from 41 % in the placebo group to 58 %. Among PD-L1-positive patients, pCR was even higher, reaching 69 % with atezolizumab vs. 49 % with placebo [55]. More recently the NSABP B-59/GBG-96-GeparDouze trials reported that adding atezolizumab to neoadjuvant chemotherapy followed by adjuvant atezolizumab did not significantly prolong EFS (HR 0.8, 95 % CI 0.62–1.03; $p = 0.08$). Despite failing its primary objective, the study demonstrated that pCR increased from 57 % to 63 % (adjusted $p = 0.0091$) [56].

Durvalumab is another PD-L1 inhibitor showing promising results in the treatment of early-stage TNBC. In the phase II GeparNuevo trial, the addition of durvalumab to anthracycline/taxane-based neoadjuvant chemotherapy resulted in a modest improvement in pCR, increasing from 44 % to 53 % [57]. However, significant improvements were observed in 3-year invasive disease-free survival, distant disease-free survival, and OS [58]. In a separate phase II trial, durvalumab combined with nab-paclitaxel and doxorubicin/cyclophosphamide achieved a pCR of 44 % in PD-L1-positive cases, suggesting that durvalumab may be especially effective in this subset [59]. This finding is supported by

Table 2
Clinical trials with immune checkpoint inhibitors in triple-negative breast cancer.

Study	Phase	Stage	Patients (*N*)	Treatment	Primary end-point	Secondary end-points
KEYNOTE-119 NCT02555657	III	Metastatic	622	Pembrolizumab vs. TPC[a]	OS CPS≥10 12.7 vs.11.6 mos (not significant)	PFS 2.1 vs. 3.3 mos (HR 1.6, 1.33–1.92) Severe AEs 14 % vs. 36 %
KEYNOTE-355 NCT02819518	III	Metastatic	847	Pembrolizumab + TPC[b] vs. placebo + TPC	PFS CPS≥ 10 9.7 vs. 5.6 mos (1-sided p = 0.0012)	OS (CPS≥ 10) 23.0 vs. 16.1 mos (HR 0.54, 0.28–1.04)
KEYNOTE-522 NCT03036488	III	EBC	1174	Taxane/carboplatin and anthracycline plus either pembrolizumab or placebo followed by adjuvant pembrolizumab or placebo	pCR 64.8 % vs. 51.2 % (p < 0.001)	EFS 84.3 % vs. 76.8 % (HR 0.63, p < 0.001)
IMPassion130 NCT02425891	III	Metastatic	902	nab-paclitaxel plus either atezolizumab or placebo	PFS in PD-L1+ 7.5 vs. 5.0 mos (p < 0.001)	OS in ITT 21.3 vs. 17.6 mos (p = 0.08) OS in PD-L1+ 25.4 vs. 17.9 mos (HR 0.62, 0.45–0.86)
IMPassio031 NCT03197935	III	EBC	333	nab-paclitaxel, doxorubicin/cyclophosphamide plus either atezolizumab or placebo followed by adjuvant atezolizumab[c]	pCR 58 % vs. 41 % (1-sided p = 0.004)	pCR in PD-L1+ 69 % vs. 49 % (not significant)
TONIC NCT02499367	II	Metastatic	67	Nivolumab after induction doxorubicin or cisplatin	ORR 35 % doxorubicin 23 % cisplatin	Up-regulation of PD-1/PD-L1 and T cell cytotoxicity
DART SWOG S1609 NCT02834013	II	Metaplastic Metastatic	33	Nivolumab + ipilimumab	ORR 18 %	mPFS 2 mos mOS 12 mos irAEs, 47 %
GeparNuevo NCT02685059	II	EBC	174	Neoadjuvant nab-paclitaxel, epirubicin and cyclophosphamide plus either durvalumab or placebo	pCR 53.4 % vs. 44.2 % (not significant)	iDFS 85.6 % vs. 77.2 %, DDFS 91.7 % vs. 78.4 % OS 95.2 % vs. 83.5 % (all significant)
SAFIR02-BREAST IMMUNO NCT02299999	II	Metastatic	199	Durvalumab vs. chemotherapy[d]	PFS 4.5 vs. 6.2 mos (not significant)	OS benefit in PD-L1+ and PD-L1- TNBC (exploratory analysis)

Abbreviations: AE, adverse event; irAE, immune-related adverse event; CPS, combined positive score; pCR, pathological complete response; DDFS, distant disease-free survival; iDFS, invasive disease-free survival; ITT, intention to treat; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; [a] TPC: capecitabine, eribulin, gemcitabine or vinorelbine. [b]TPC: nab-paclitaxel, paclitaxel or carboplatin plus gemcitabine. [c] After surgery, patients and study personnel were unmasked to treatment assignment: patients in the experimental arm continued to receive adjuvant atezolizumab, whereas patients in the control arm were monitored. [d]Durvalumab or chemotherapy were given as maintenance.

the SAFIR02-BREAST IMMUNO data from the metastatic setting, where durvalumab demonstrated a survival benefit as a single-agent maintenance in both PD-L1-positive and PD-L1-negative TNBC, further supporting the exploration of its use following chemotherapy [60].

As a final note, recent investigations have provided insights into the role of immunotherapy in HR-positive/HER2-negative breast cancer. The KEYNOTE-756 and CheckMate 7FL trials assessed the efficacy of adding pembrolizumab and nivolumab to neoadjuvant anthracycline/taxane-based chemotherapy, followed by adjuvant endocrine therapy [61,62]. Both studies demonstrated significantly higher pCR with immunotherapy, with improvements of 10.5 % for pembrolizumab and 8.5 % for nivolumab. Notably, patients with PD-L1-positive tumors derived the greatest benefit from pembrolizumab, with a pCR of 44.3 %, compared to 20.2 % in the placebo. The I-SPY2 trial further supported these findings, particularly in high-risk patients [63].

Currently, testing for PD-L1 expression is required to prescribe pembrolizumab and atezolizumab in the metastatic setting. However, neither marker alone reliably identifies patients most likely to benefit [64]. As a result, research is expanding to include additional biomarkers, such as tumor mutational burden, microsatellite instability, and circulating tumor DNA, to improve patient selection [64].

4. Generic drugs

Chemotherapeutic agents are classified based on their mechanism of action into antimetabolites, such as methotrexate and 5-fluorouracil, which disrupt DNA synthesis by mimicking normal metabolic molecules, causing replication errors; DNA alkylating agents like platinum-based chemotherapies form covalent bonds with DNA, resulting in cross-linking and strand breaks; antimitotic drugs like taxanes, prevent proper cell division by stabilizing microtubules; and anthracyclines intercalate into DNA and inhibit topoisomerase II, blocking replication and promoting oxidative stress reviewed in [65]. Chemotherapy acts in a nonspecific manner on rapidly dividing cells; though emerging evidence suggests that chemotherapy activity varies depending on the intrinsic breast cancer subtype and the presence of specific biomarkers. Herein, we focus on BRCA deficiency and triple negative breast cancer.

4.1. Germline and somatic BRCA and PALB2 mutations

Germline *BRCA1* and *BRCA2* (g*BRCA*) and *PALB2* (g*PALB2*) mutations impair DNA repair and increase cell vulnerability to treatments that cause DNA damage. However, studies on the efficacy of chemotherapy in patients with g*BRCA* mutations have yielded inconsistent results. In the neoadjuvant setting, Byrski et al. reported a pCR of up to 83 % in 25 patients receiving cisplatin [66], and Silver et al. showed that all TNBC with g*BRCA1* mutations and low BRCA1 mRNA expression had pCR when treated with for courses of cisplatin [67]. In contrast, the INFORM study, which randomized 117 BRCA mutation carriers to receive either cisplatin or doxorubicin/cyclophosphamide, showed a pCR of only 18 % for cisplatin and 26 % for doxorubicin/cyclophosphamide [68]. The combination of carboplatin and docetaxel in TNBC patients provided a pCR of 55 % without any

statistically significant difference between g*BRCA* and wild type groups [69]. In the BRIGHTNESS trial, carboplatin increased the pCR from 41 % to 50 % in *BRCA* mutation carriers, and from 29 % to 59 % in wild-type patients [70]. Of note, the improvement in EFS with carboplatin did not differ significantly between mutated and wild-type patients. Similarly, the GeparSixto trial showed that the absolute improvement in disease-free survival at 35 months was greater in *BRCA* wild-type patients (85 % vs. 74 %) compared to mutated patients (86 % vs. 82 %) [71]. These findings were unexpected, especially given the results from the TNT trial in the metastatic setting, where carboplatin demonstrated superiority over docetaxel in *BRCA1* and *BRCA2* mutation carriers [72].

As BRCA proteins affect sensitivity to antimicrotubule agents *in vitro*, concerns have arisen about the taxane response in BRCA-deficient cases, with several studies addressing this issue with scattered results. Specifically, HR-negative breast cancer patients with *BRCA1* mutations show reduced response to taxanes compared to non-BRCA1 mutation carriers, while HR-positive breast cancer patients with BRCA1/2 mutations respond to taxanes similarly to sporadic patients [73]. Moving beyond retrospective and observational studies, extended studies such as those by Arun et al. found that *BRCA1* mutation carriers had a higher pCR (46 % vs. 22 %) compared to patients without BRCA1 mutations when treated with taxane combinations [74]. However, these studies also included anthracyclines, making it difficult to isolate the specific contribution of each drug to the outcome, with some suggesting that the large difference may be due to anthracyclines rather than taxanes. Additionally, the randomized study by Tutt et al., which demonstrated increased sensitivity to carboplatin in metastatic TNBC with *BRCA* mutations, also showed a similar response rate to docetaxel in BRCA1-mutated compared to wild-type cases [72].

Hence, in metastatic BRCA1/2 mutated breast cancer, carboplatin demonstrates superior efficacy compared to taxanes, although taxanes exhibit similar activity in both *BRCA*-mutated and wild-type patients. In early-stage breast cancer, platinum-based therapies offer some improvement in pCR rates but do not translate into long-term survival benefits. The design of randomized trials including or excluding platinum agents specifically for *BRCA*-mutated patients remains an unmet need to address this issue conclusively. A Cochrane systematic review on the use of platinum in TNBC revealed a statistically significant advantage in pCR and Disease Free Survival (DFS) or patients with wild-type *BRCA* but not for those with *gBRCA* [75]. Nevertheless, platinum agents have become a standard clinical practice due to their capacity to enhance sensitivity to immunotherapy.

Another caveat in g*BRCA* mutated patients with HR-positive/HER2-negative tumors is the use of CDK4/6 inhibitors. Retrospective data in the metastatic setting suggest poorer outcomes with CDK4/6 inhibitors in mutated patients compared to those with wild-type BRCA. Since the BRCA gene is located on chromosome 13q, near the RB1 gene locus, it has been hypothesized that selective negative therapeutic pressure from prior treatments could lead to biallelic loss of RB1, potentially driving resistance to CDK4/6 inhibitors [76,77]. Theoretically, the optimal treatment sequence for BRCA-mutated patients could involve the use of PARP inhibitors prior to CDK4/6 inhibitor therapy, aiming to intercept deleterious RB1-loss trajectories and suppress the emergence of resistance.

However, given the small number of gBRCA-mutated patients included in these studies, caution is warranted in drawing definitive conclusions about potential resistance mechanisms induced by drug exposure and the optimal treatment sequence.

In addition, data from the MonarchE trial in early-stage disease indicate a comparable benefit of adjuvant CDK4/6 inhibitors, regardless of BRCA status [78]. Further studies are needed to address these important questions.

The GeparOLA phase II study compared neoadjuvant treatment with carboplatin and paclitaxel to the combination of olaparib and paclitaxel, both followed by epirubicin/cyclophosphamide. Among 107 patients, 77 of them with TNBC, harboring g*BRCA* mutations or tumors exhibiting HRD or s*BRCA* mutations, the pCR was 55.1 % with olaparib compared to 48.6 % with carboplatin. After 49.8 months of follow-up, the 4-year DFS was 76 % with olaparib *versus* 88.5 % with carboplatin [79]. *PALB2* deletion was associated with a favorable survival in a recent retrospective study on 66 patients with *BRCA1/2* and *PALB2* somatic mutations treated with chemotherapy [80].

4.2. TNBC

The inclusion of carboplatin in neoadjuvant therapy for triple-negative (TNBC) is supported by multiple trials showing improved pCR, with some suggesting better EFS. For instance, in the BRIGHTNESS trial, patients receiving weekly paclitaxel followed by anthracycline-containing chemotherapy showed that adding carboplatin increased the pCR from 40 % to 54 %, five-year EFS from 64 % to 71 %, and 5-year OS from 67 % to 74 % [70]. Similarly, the CALGB/Alliance 40603 trial, which included 443 patients, found that adding carboplatin to paclitaxel followed by anthracycline chemotherapy raised pCR from 41 % to 54 %. However, carboplatin failed to improve 5-year EFS [81]. Finally, for TNBC patients, capecitabine has shown improved outcomes in cases of residual disease after neoadjuvant chemotherapy. In the GICAM/CIBOMA trial, Asleh et al. found that the non-basal molecular subtype was the strongest predictor of benefit from salvage adjuvant capecitabine [82].

5. Precision oncology

Precision oncology is an innovative approach aimed at selecting treatments based on the molecular characteristics of a tumor at the individual level. Its implementation requires the identification of key molecular targets and the availability of suitable therapies. Initially, classification scales were developed to categorize gene alterations, i.e., mutations, amplifications, and translocations, using a combined index of data strength and clinical relevance. More recently, additional factors, including data robustness, clinical targetability, known or predicted pathogenicity, and prognostic or predictive significance, have been incorporated reviewed in [32,33,83]. As a result, the ESMO ESCAT scale provides the latest comprehensive framework for classifying genomic alterations into six tiers based on clinical evidence [84]. The top tiers I and II represent the forefront of precision oncology, as they include alterations associated with approved drugs or showing promising results in subgroup analyses.

The first application of precision medicine in breast cancer dates back to 2015 when the phase II SHIVA trial screened 741 patients with treatment-refractory metastases, including breast cancer, for molecular alterations in the hormone receptor, PI3K/Akt/mTOR, and RAF/MEK pathways [85]. Remarkably, 26 % of patients received molecularly-guided therapies, indicating that precision oncology is feasible for approximately 1 in 4 cancer patients. This was the first reported success rate of its kind and continues to be relevant today, highlighting the potential for integrating molecular data into clinical decision-making. However, PFS was not significantly improved in patients treated with molecularly guided therapies compared to physician choices (2.3 vs. 2.0 months, HR 0.88, $p = 0.41$). This initially disappointing result is now viewed with less concern, given that the availability of targeted agents was still limited at the time the study was conducted. In addition, the study was designed to detect a 30 % improvement in PFS at six months, suggesting that the sample size may have been insufficient to evaluate any smaller yet clinically relevant benefit of molecularly guided therapy.

In fact, the SAFIR02-BREAST and SAFIR-PI3K phase II trials demonstrated more favorable outcomes in 1462 patients with HER2-negative metastatic breast cancer [86]. After completing 6–8 cycles of chemotherapy, patients without disease progression and harboring a targetable genomic alteration were randomized to receive either maintenance targeted therapy or standard chemotherapy. In patients with

ESCAT I/II genomic alterations, median PFS was significantly longer with targeted therapy (9.1 months) than with chemotherapy (2.8 months) (HR 0.41, $p < 0.001$). Notably, no benefit was observed when targeted therapy was administered without ESCAT guidance, highlighting that precision oncology enhances patient outcomes when actionable gene alterations are also clinically relevant.

More recently, the ROME trial provided compelling evidence for a contemporary approach to precision oncology, which includes comprehensive gene profiling and molecular tumor board (MTB) recommendations that can impact patient outcomes [87]. This large-scale study demonstrated that molecularly guided therapy achieved higher objective response rates and 12-month PFS of 17 % and 22.3 %, respectively, compared to 9.5 % and 7.7 % of standard of care, all while maintaining a favorable tolerability profile. Additionally, a high tumor mutational burden was confirmed as an important marker of response to immunotherapy.

In parallel with these randomized clinical trials aimed at comparing the agnostic approach to the traditional approach, a wealth of real-world data on breast cancer care is emerging. Among these is the SOLTI-1301 AGATA study, a large cohort study that enrolled 305 patients with advanced breast cancer. Among the evaluated patients, at least one somatic mutation was identified in 63 %, although only 11 % were eventually treated accordingly, primarily through clinical trials. Among those treated, 46.2 % achieved a PFS of at least 6 months. The most common identified mutations were *PIK3CA* (34 %) and *TP53* (22 %), while *AKT1* occurred more frequently in metastatic rather than primary tumors (9 % vs. 2 %, $p = 0.01$), emphasizing that tumors may develop new mutations during progression [88] (Table 3, Fig. 1). Among less common alterations, fusions involving *NTRK* genes result in the production of chimeric Trk proteins with constitutively active or overexpressed kinase function, driving oncogenic potential. Larotrectinib, a selective pan-Trk inhibitor, showed efficacy in the LOXO-101 trial. A total of 55 patients received treatment, including one with breast cancer (2 %). The ORR was 75 %, 95 % CI (61–85). After one year, 71 % of responses were ongoing, and 55 % of patients remained progression-free [89].

Table 3
Precision medicine in breast cancer.

Study	Phase	Patients enrolled, (%) breast cancer	Intervention	Major findings
SHIVA	II	741 (20 %)	Molecularly-informed therapy (n = 99) vs. Soc (n = 96)	PFS 2.3 vs. 2.0 mos (HR 0.88, p = 0.41)
SAFIR02-BREAST and SAFIR-PI3K	II	1462 (100 %)	Molecularly-informed therapy (n = 157) vs. SoC (n = 81)	PFS 9.1 vs. 2.8 mos (HR 0.41, p < 0.001)
ROME trial	II	1200 (5 %)	Molecularly-informed therapy based on MTB (n = 200) vs. SoC (n = 200)	ORR 17 % vs 9.5 %, p = 0.026 PFS 22.3 % vs 7.7 % mos (HR 0.64, p < 0.001)
AGATA SOLTI-1301	Real-world data	305 (100 %)	N/A	*PIK3CA* (34 %), *TP53* (22 %), *AKT1* mutations more frequent in metastatic tissue. PFS≥ 6 months in 46.2 % of patients receiving molecularly-informed therapy

Abbreviations HR, hazard ratio; mos, months; MTB, molecular tumor board; NGS, next-generation sequencing; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; SoC, standard of care; hTMB, high tumor mutational burden.

6. Individual response to guide therapy

The optimal context for evaluating treatment is when an intermediate and early surrogate of efficacy is available, and allows the patient to serve as its own control. This concept has been historically pursued in the neoadjuvant setting. Pioneering studies, such as the Aberdeen and GeparTrio trials first showed the potential of modifying treatment based on early clinical response reporting improved clinical outcomes in patients receiving response-guided chemotherapy compared to those treated with conventional chemotherapy.

Advances in imaging techniques have further refined this approach [90,91]. The PHERGain study, a phase 2 trial, utilized 18-fluorine-fluorodeoxyglucose-PET (FDG-PET) scans to assess early tumor response and guide treatment decisions in HER2-positive early breast cancer patients [92]. Those who were PET responders continued with trastuzumab and pertuzumab, while non-responders switched to chemotherapy. The results were remarkable, with PET responders achieving a 94.8 % invasive disease-free survival (iDFS) rate at three years. Additionally, PET-guided therapy identified patients who could safely avoid chemotherapy, minimizing unnecessary treatments and reducing side effects. This study highlights the promise of advanced imaging techniques in guiding personalized treatment strategies, optimizing outcomes, and reducing chemotherapy-related toxicities.

In HR-positive breast cancer, the PEPI score has been explored in trials like ACOSOG Z1031 to guide decisions between neoadjuvant endocrine therapy or chemotherapy [93]. In this trial, patients with a PEPI score of 0 exhibited a low 5-year risk of relapse (3.6 %) without chemotherapy. The ALTERNATE trial further tested this concept, comparing the efficacy of aromatase inhibitors, SERD, and their combination [94]. The mPEPI score, a modified version of the PEPI score excluding the Allred ER score, was introduced to identify patients with low relapse risk (mPEPI 0) who could safely avoid chemotherapy after neoadjuvant hormonal therapy. Despite no improvement in event-free survival with fulvestrant or the combination, the mPEPI score proved useful for identifying patients who could avoid chemotherapy, supporting chemotherapy de-escalation for low-risk patients.

More recently, molecular diagnostics have allowed for treatment changes based on individual responses, even in metastatic settings. As already mentioned, the PADA-1 trial assessed the clinical utility of monitoring ESR1 mutations in patients with estrogen receptor-positive, HER2-negative advanced breast cancer treated with aromatase inhibitors and palbociclib [21]. The study found that switching to fulvestrant and palbociclib upon detecting rising ESR1 mutations led to significantly improved PFS compared to continuing with the same regimen. Similarly, the SERENA-6 trial (NCT04964934) is investigating the efficacy of switching to camizestrant upon detecting ESR1 mutations in circulating tumor DNA [23]. This strategy aims to treat ESR1 mutant clones and delay the need for chemotherapy, with the primary endpoint being PFS. Secondary endpoints include chemotherapy-free survival, overall survival, and safety.

All these studies underscore the growing role of personalized treatment approaches in breast cancer, leveraging early response data, molecular markers, and advanced imaging techniques to guide therapy and reduce unnecessary treatments, especially in hormone receptor-positive and HER2-negative breast cancer.

7. Molecular tumor board

MTB is a multidisciplinary team of experts who review the molecular profiles of tumors to recommend personalized treatment strategies. MTBs have the potential to play a crucial role in quality assurance by standardizing the interpretation of molecular data and ensuring

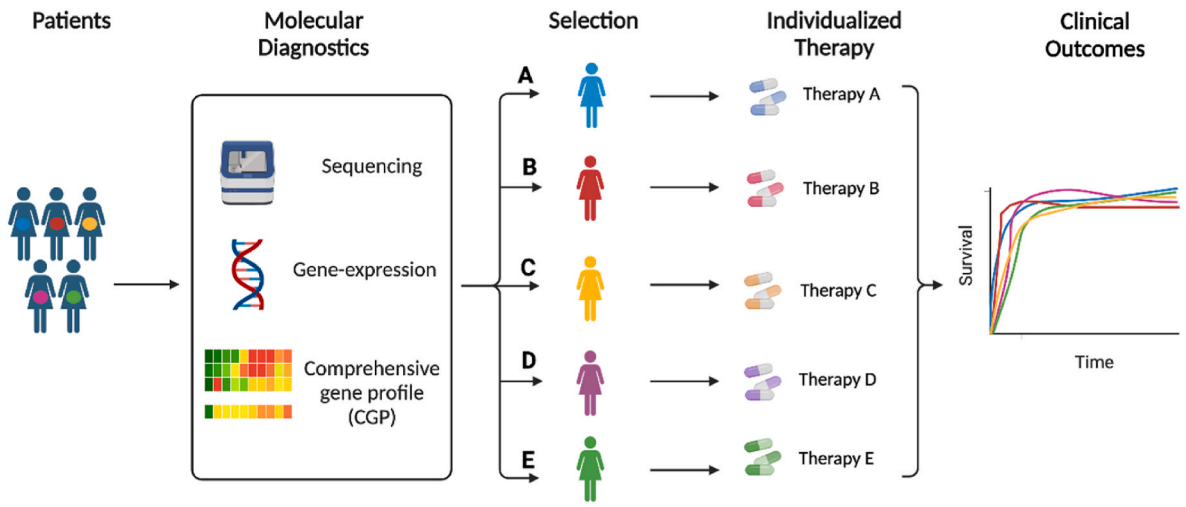

Fig. 1. Precision medicine in breast cancer - The graph illustrates the novel approach to breast cancer patients based on molecular diagnostics to assign individual matched therapies.

appropriate therapeutic recommendations. They also enhance clinician understanding of genomics-driven oncology and improve patient access to phase clinical trials (phase I–III) by identifying eligible candidates through molecular profiling. In a hypothetical scenario, we consider a 64-year-old female diagnosed with advanced HR-positive/HER2-negative breast cancer. This patient might have undergone multiple treatment lines, as patients typically reach the MTB after failing the third line of treatment [95,96]. Next generation sequencing analysis generally reveals an average of four gene alterations per patient, which may vary among individuals but often include recurrent genes such as *TP53, MYC, ERBB2, GATA3, CCND1, PIK3CA, and FGFR1*. While it is common for multiple alterations to be present, actionable mutations are often unique to each case. However, there are instances where unusual combinations may occur. In this hypothetical case, the NGS analysis uncovers two alterations in less common genes: *RET C634R* and *GATA3 P*436fs**11* mutations.

The *RET C634R* mutation is known to play a role in tumorigenesis and has been associated with drug resistance mechanisms in various cancers, including breast cancer. This mutation may activate RET signaling, promoting cell proliferation and survival. Conversely, the *GATA3 P*436fs**11* alteration represents a frameshift mutation that could lead to a truncated GATA3 protein, potentially disrupting its normal tumor suppressor function and contributing to cancer progression.

Given these findings and considering the lack of standard therapies specifically targeting these mutations in breast cancer, a MTB might recommend the off-label use of a multi-kinase inhibitor that also targets RET such as sorafenib. This hypothetical decision would take into account the overall clinical condition of the patient, the potential benefits and the safety profile of targeting the identified mutations.

8. Co-occurring mutations, sequencing, and future directions

The advent of novel therapies has significantly expanded treatment options for metastatic breast cancer, yet optimizing sequencing strategies remains a challenge. Treatment selection is based on hormone receptor status, HER2, and PD-L1 expression, while subsequent therapies consider actionable mutations [97]; 98. However, not all detected alterations translate into effective personalized therapy. According to ESMO ESCAT guidelines, molecular findings must be assessed based on their clinical evidence level. Simply matching a mutation to a drug does not guarantee benefit as decisions must be contextualized within the patient medical history, multidisciplinary discussions, drug efficacy evidence, and, crucially, access to novel treatments.

Therapeutic choices are guided by regulatory approvals and evidence-based medicine. However, multiple therapies may be available for the same indication without direct comparative data. Navigating this complexity requires evaluating clinical history, prior treatment response, and patient preferences. In the absence of head-to-head comparisons -especially regarding overall survival or quality of life - treatment should balance the benefits of progression-free survival with the risk of toxicity.

PI3K inhibitors combined with endocrine therapy are the preferred option for patients with co-occurring *ESR1* and *PIK3CA* alterations following CDK4/6 inhibitor treatment. Elacestrant, in fact, demonstrates limited efficacy in the presence of *PIK3CA* mutations, as shown in a post-hoc analysis from the EMERALD trial, which reported a median progression-free survival (mPFS) of 5.5 months in *PIK3CA*-mutant patients, a result inferior to that of the overall study population. Meanwhile, the efficacy of PI3K inhibitors in *ESR1*-mutated disease remains uncertain due to the lack of dedicated data, underscoring the need for clinical judgment. There is general consensus that elacestrant remains a viable alternative for patients with *PIK3CA* mutations and indolent disease, particularly those who achieved a $\geq$12-month response to CDK4/6 inhibitors, given its favorable tolerability profile.

The choice among capivasertib, inavolisib, and alpelisib should be guided by pivotal trial criteria to ensure alignment with the patient's clinical profile. Additionally, the distinct side-effect profile of each drug must be considered. Capivasertib is associated with less hyperglycemia and has similar or more tolerable side effects compared to alpelisib, which is only recommended for patients with HbA1c $\leq$ 6.5 %. The combination of inavolisib, palbociclib, and fulvestrant represents an appropriate first-line treatment for patients with PIK3CA-altered endocrine-resistant tumors. With several mutant-selective PI3K inhibitors in development, therapeutic options are expected to expand, further emphasizing the importance of clinical trial enrollment.Biomarker-driven studies are also advancing [99]. For instance, the AURORA program aims to characterize molecular aberrations in metastatic breast cancer using molecular screening technology to track disease evolution over time [100]. Finally, the integration of liquid biopsies is transforming breast cancer management, enabling ultra-sensitive, longitudinal detection of tumor DNA through minimally invasive blood tests, thereby facilitating more precise biomarker-driven strategies.

9. Conclusions

As we reflect on the advances in breast cancer treatment, several key points emerge that serve not as conclusions but as insights for future exploration. The integration of molecular profiling and biomarker-

driven therapies has transformed breast cancer treatment, enabling more personalized strategies and improving patient outcomes. However, significant challenges persist in treatment selection and the application of molecular data, particularly regarding the routine use of next-generation sequencing as recommended by ESMO guidelines. Future research should focus on practical aspects of testing, including whether to analyze primary tumors or metastatic lesions, as well as determining the optimal procedures and timing for testing. Technologies like liquid biopsy provide less invasive methods for tracking tumor evolution, while advances in drug development and big data-guided treatment selection offer the potential to enhance clinical decision-making. Lastly, MTBs will play a crucial role in interpreting molecular findings and ensuring that treatments are tailored not only to tumor biology but also to the individual needs and circumstances of each patient.

CRediT authorship contribution statement

Maria Grazia Carnevale: Writing – original draft, Methodology, Formal analysis. **Riccardo Ray Colciago:** Writing – original draft, Methodology, Formal analysis. **Maria Carmen De Santis:** Writing – original draft, Methodology, Formal analysis. **Laura Cortesi:** Writing – review & editing. **Cinzia De Marco:** Writing – review & editing. **Antonio Marra:** Writing – review & editing. **Andrea Vingiani:** Writing – review & editing. **Franco Nolè:** Writing – review & editing. **Giuseppe Curigliano:** Writing – review & editing. **Giancarlo Pruneri:** Writing – review & editing. **Antonio Llombart-Cussac:** Writing – original draft, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Serena Di Cosimo:** Writing – original draft, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Javier Cortes:** Writing – original draft, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization.

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