

Precision Medicine in Advanced Hormone Receptor–Positive Breast Cancer: Integrating Genomic Profiling and Liquid Biopsy Into Clinical Practice

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Abstract: Precision medicine has reshaped the management of advanced hormone receptor–positive, human epidermal growth factor receptor 2–negative breast cancer by coupling treatment with tumor genomics rather than clinicopathologic features alone. In this review, we highlight the role of next-generation sequencing (NGS) in our clinical practice. We summarize the performance characteristics of various NGS platforms and outline the available techniques of NGS. We also describe clinically relevant genomic alterations and targeted treatments.

Introduction

Breast cancer is the most common cancer diagnosed among women in the United States, with an estimated 310,720 new cases in 2024.¹ Despite this increase in the incidence of breast cancer, the mortality rate has decreased by 44% from 1989 to 2022.² Hormone receptor–positive (HR+), human epidermal growth factor receptor 2–negative (HER2–) breast cancer is the most common subtype of breast cancer.² The development of a genomic test called the Prediction Analysis of Microarray of 50 genes (PAM 50) assay by Perou and colleagues led to the first molecular classification of breast cancer, which started the era of molecular genomics in breast cancer. It characterizes breast cancer as luminal A (HR+, HER2–, and low proliferation rates), luminal B (HR+, HER2–, higher proliferation rates than luminal A),

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HER2-enriched (HR+, HER2+, and high proliferation rates), basal-like (HR-, HER2-, and high proliferation rates), and normal-like (similar to normal breast tissue, with low proliferation rates). This stratification so far is used in the prognostic evaluation of breast cancer, with limited use in therapy planning.^{3,4}

Since the development of PAM 50, genomic profiling has become an integral part of the management of advanced breast cancer, and here we aim to review the integration of next-generation sequencing (NGS) in the management of HR+ advanced breast cancer.

Next-Generation Sequencing

It took nearly a quarter of a century to develop DNA sequencing technology after its discovery in 1953.^{5,6} Sanger sequencing, also known as first-generation sequencing, used a “sequencing by synthesis” approach to determine nucleic acid sequences one nucleotide at a time. After automation by Applied Biosystems, this approach was used in the Human Genome Project.⁷ However, the limited throughput of first-generation sequencing led to the development of NGS, which enables massive parallel sequencing and greatly increased data output.⁷ NGS has since driven major advances in cancer research through the precise characterization of cancer genomes and epigenomes.⁸

Various technologies under the umbrella of NGS have been adopted for nucleic acid sequencing in cancer genomics. Whole-genome sequencing (WGS) provides a comprehensive analysis of the entire genome, including coding and noncoding regions. In contrast, whole-exome sequencing (WES) focuses on the protein-coding regions only, which constitute approximately 1% to 2% of the genome. This approach captures approximately 85% of known disease-causing mutations, including most oncogenic driver alterations.^{9,10} These techniques have been applied in early diagnosis, analysis of recurrence, treatment, and research.¹¹ WES may miss important biomarkers like the complex mutational signatures of homologous recombination deficiency, and it provides less accurate estimates of tumor mutational burden (TMB) than WGS, particularly in low-TMB tumors.^{12,13}

Because WGS and WES remain largely cost-prohibitive, focus has shifted to targeted gene panels (TGP), which are focused and affordable.¹⁴⁻¹⁶ In a study by Frampton and colleagues, TGPs identified clinically actionable mutations in 76% of 2221 tumors, a 3-fold higher rate of detection in comparison with other techniques.¹⁷

Liquid biopsy, which is a noninvasive tool for examining body fluids, is increasingly being used to detect biomarkers for disease monitoring and for improving diagnosis and treatment.¹⁸ Various biomarkers in the

physiologic fluids can be examined, including circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), cell-free DNA (cfDNA), and circulating exomes.¹⁹ TGPs employing liquid biopsy are increasingly utilized in oncology for therapy selection, treatment monitoring, and measurable residual disease detection in patients with advanced solid malignancies, particularly when tissue samples are unavailable or insufficient.²⁰⁻²²

Tissue-Based NGS

In 2017, the US Food and Drug Administration (FDA) approved multiple NGS-based assays for use in oncology.^{23,24} These assays enable comprehensive genomic profiling by analyzing hundreds of cancer-related genes in solid tumors with the use of hybrid capture-based enrichment methods.²⁵ Some of the platforms have also been approved as companion diagnostics for targeted therapies and immunotherapies, supporting their role in guiding treatment selection for patients with advanced and recurrent cancers. Since then, additional NGS assays with varying gene coverage, analytical capabilities, and clinical applications have been developed and approved, reflecting the rapid evolution of genomic testing in oncology.

Real-world data and clinical trials have demonstrated that NGS-guided therapy changes management in a clinically meaningful subset of patients. In the SAFIR02-BREAST trial, patients who received targeted treatment had better progression-free survival (PFS; adjusted hazard ratio [HR], 0.41; 90% CI, 0.27-0.61; $P < .001$) if they had level 1 or 2 mutations on the European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of molecular Targets (ESCAT). The improvement with targeted therapy was especially pronounced among patients who had germline *BRCA1/2* mutations.²⁶ Various studies with real-world data have shown that NGS is helpful in recognizing both predictive biomarkers and drug-targetable markers, the most common being *PIK3CA*.²⁷⁻²⁹ NGS has also been instrumental in identifying resistance mutations, such as *ESR1* mutations, that arise after prolonged antiestrogen therapy. These mutations typically occur in the ligand-binding domain and lead to the constitutive activation of estrogen receptor (ER) signaling.³⁰ NGS testing of patients who have been exposed to cyclin-dependent kinase 4/6 (CDK4/6) inhibitors revealed resistance mutations such as *RBI* loss, activating alterations of *AKT1*, *RAS*, *AURKA*, *CCNE2*, *ERBB2*, and *FGFR2*, and loss of ER expression that can affect cell cycle regulatory proteins as well as signaling pathways.³¹ The presence of these mutations provides an opportunity for research and the development of new therapeutics. Various novel therapies are in development that aim to overcome resistance mechanisms, such as by inhibiting *CHEK1* and *AURKA*

or by adding mitogen-activated protein kinase (MAPK) pathway inhibitors to CDK4/6 inhibitors.³¹⁻³³ Preclinical studies have shown that CDK2 inhibition can lead to the resensitization of breast cancer cells to CDK4/6 inhibition, as upregulation of CDK2 is one of the mechanisms of resistance to CDK4/6 inhibition.³⁴ Various ongoing trials of CDK2-inhibiting drugs, both as monotherapy and as part of combination treatment, especially after the progression of cancer in patients on CDK4/6 inhibition, are in the recruitment phase.³⁴

Unlike DNA sequencing, which provides information on genomic alterations, RNA sequencing provides dynamic information about which genes are actively transcribed, offering insights into cellular function and disease mechanisms.³⁵ It is used to characterize the tumor microenvironment, infer immune cell populations, and analyze gene expression signatures relevant to diagnosis, prognosis, and therapeutic response.^{36,37} In addition, RNA sequencing detects more actionable gene fusions than DNA-based methods do, particularly in non-small cell lung cancer, sarcomas, and low-grade gliomas.³⁸⁻⁴⁰ In breast cancer, RNA sequencing aids in the quantification of *ESR1* messenger RNA.^{41,42}

Liquid NGS

Liquid NGS provides a noninvasive tool for genomic profiling. Various types of liquid NGS biomarkers can be tested. CTCs are used primarily in HR+ breast cancer as a predictive and prognostic marker. The number of CTCs is associated with prognosis, with higher CTC counts being linked to poorer prognosis. In addition, a single positive CTC assay result after the completion of treatment is predictive of late clinical recurrence.⁴³⁻⁴⁵

The monitoring of ctDNA has been utilized to evaluate therapy response, with rising ctDNA levels being associated with progression.⁴⁶ Research also suggests that personalized ctDNA monitoring can make it possible to decrease imaging frequency.⁴⁶ In the SOLAR-1 trial, ctDNA was used to determine PFS.⁴⁷ Studies have shown a concordance rate of up to 90% between blood-based and tissue-based NGS.⁴⁸⁻⁵⁰ Quantitative ctDNA testing helps to monitor disease, and a rise in ctDNA points toward progression. In addition, ctDNA is helpful in identifying early genomic alterations. For example, *ESR1* and *PIK3CA* mutations can be detected early with ctDNA.^{51,52} The SERENA-6 trial demonstrated the usefulness of ctDNA in the early detection of *ESR1* mutations, which led to a switch in therapy to camizestrant and subsequently improved PFS.⁵³ In the NCT05826964 trial, ctDNA will be tested frequently in patients with HR+/HER2–metastatic breast cancer (MBC) who are on an aromatase inhibitor (AI) and a CDK4/6 inhibitor, with the goal of switching therapy in cases of early molecular progression.

The MONDRIAN study (NCT04720729) is evaluating the use of ctDNA measurement to determine if a treatment regimen should be changed in patients with HER2+ MBC who are receiving chemotherapy.

Although liquid NGS also has the benefits of being a minimally invasive test, able to capture tumor heterogeneity and aid in real-time disease monitoring with a quick turnaround time, it has limitations as well.⁵⁴⁻⁵⁸ Its sensitivity is lower than that of tissue-based NGS, and the false-negative rate is higher owing to low ctDNA levels. Liquid NGS may also produce results that do not agree with those of tissue-based NGS, as liquid NGS may detect variants not found in a tissue sample.⁵⁹⁻⁶² In addition, tissue-based NGS remains the standard for TMB and microsatellite instability testing. Furthermore, liquid NGS cannot be used for ancillary studies (eg, in situ hybridization, RNA-based fusion detection, molecular subtyping) that require an intact cellular structure (Table 1).^{59-61,63}

Clinical Implications of Specific Genomic Alterations

Endocrine-based therapy is the guideline-preferred first-line treatment for HR+ MBC in the absence of visceral crisis, with endocrine therapy plus a CDK4/6 inhibitor the standard in most patients.⁸⁵⁻⁸⁸ Ribociclib (Kisqali, Novartis), abemaciclib (Verzenio, Lilly), and palbociclib (Ibrance, Pfizer) have all shown substantial PFS and overall survival (OS) benefit when combined with an AI or fulvestrant and are now widely used in the first-line setting.⁸⁹⁻⁹⁴

Despite these advances, the optimal sequencing after progression in patients on CDK4/6 inhibition remains unsettled, and overcoming endocrine resistance is a major challenge. Primary endocrine resistance is relapse within the initial 2 years after adjuvant endocrine therapy or progression within the first 6 months after endocrine therapy for MBC; secondary resistance is relapse after at least 2 years of adjuvant endocrine therapy, or relapse during or within 1 year after the completion of adjuvant treatment for early breast cancer, or progression after at least 6 months following the initiation of endocrine therapy for advanced/metastatic breast cancer.⁹⁵ Multiple mechanisms drive resistance, including alterations in ER signaling and cross talk with growth factor pathways such as phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR).⁹⁶

PI3K/AKT/mTOR Signaling Pathway Activation

The *PIK3CA* mutation either can occur in early tumor development or can be an acquired mutation at the time of disease progression.⁹⁷ *PIK3CA* mutations occur in

Table 1. Data on Available NGS Testing Platforms

Vendor	Test	Throughput	Bioinformatics	Sample	Turnaround
Foundation Medicine	FoundationOne CDx ^{25,64}	High	Genomic profiling of 324 genes, TMB, MSI, and fusions	Tissue/liquid	~9 d
MSK	MSK-IMPACT Panel ^{65,66}	High	Genomic profiling of 505 genes	Tissue	~2-3 wk
	MSK-ACCESS panel ⁶⁷	High	Genomic profiling of 129-146 cancer-associated genes	Liquid	
OmniSeq	OmniSeq IN-SIGHT ⁶⁸	High	Genomic profiling of 523 genes	Tissue	~7-10 d
Guardant Health	Guardant360 Tissue ^{69,70}	High	Analyzes 74 genes	Tissue/liquid	~5-7 d
	Guardant360 Liquid ⁷¹				
BostonGene	BostonGene ^{72,73}	High	WES and genomic profiling of approximately 22,000 genes, along with whole-transcriptome sequencing and IHC analysis	Tissue	~2-3 wk
Tempus	xT assay ^{74,75}	High	DNA and RNA sequencing and genomic profiling of approximately 648 genes in tissue sample and approximately 523 for liquid NGS	Tissue/liquid	~2 wk
	xF+ assay ⁷⁶				
Caris Life Sciences	MI Cancer Seek ^{77,78}		WES and WTS	Tissue	~10 d
Caris Life Sciences	Caris Assure ⁷⁹		WES and WTS of both plasma and whole-blood cells, clinically reporting 302 genes	Peripheral blood	6-9 d
Thermo Fisher Scientific	Oncomine Breast cfDNA ^{80,81}		Liquid biopsy genomic profiling of ~10 genes and >150 hotspots	Blood	~2 d
	Oncomine Precision Assay		Analyzes 50 key genes for SNVs, indels, CNVs, and fusions	Tissue/liquid	~24 h
Illumina	TruSight Oncology 500 ^{82,83}		Pantumor genomic profiling of 523 genes	Tissue/blood	~4-5 d
	TruSight Oncology 500 ctDNA ⁸⁴				

cfDNA, cell-free DNA; CNV, copy number variation; ctDNA, circulating tumor DNA; d, days; h, hours; IHC, immunohistochemistry; MSI, microsatellite instability; NGS, next-generation sequencing; SNV, single-nucleotide variant; TMB, tumor mutational burden; WES, whole-exome sequencing; wk, weeks; WTS, whole-transcriptome sequencing.

roughly 40% of ER+/HER2– breast cancers and lead to activation of the PI3K/AKT/mTOR signaling pathway; phosphatase and tensin homologue (*PTEN*) loss of *AKT* mutations can similarly promote pathway overactivity.^{98,99} *PI3K* activation generates phosphatidylinositol 3,4,5-trisphosphate with downstream activation of *AKT* and *MTOR*, whereas *PTEN* counteracts this by dephosphorylating pathway intermediates.^{99,100}

Alpelisib (Piqray, Novartis), an orally bioavailable selective inhibitor of *PIK3CA*, improved PFS (11 vs 5.7 months; HR, 0.65; 95% CI, 0.50-0.85; $P<.001$) and response rates when combined with fulvestrant in *PIK-*

3CA-mutant HR+/HER2– MBC in the SOLAR-1 trial, although grade 3 or higher hyperglycemia (36.6%), rash, and diarrhea were common and led to a 25% discontinuation rate.⁴⁷ OS was numerically but not statistically significantly higher with alpelisib plus fulvestrant than with fulvestrant alone, at 39.3 vs 31.4 months, respectively (HR, 0.86; 95% CI, 0.64-1.15; $P=.15$).⁴⁷ A key limitation was minimal prior CDK4/6 inhibitor exposure. In the phase 2 BYLieve study, 50.4% of patients remained progression-free at 6 months on alpelisib/fulvestrant after progression on a CDK4/6 inhibitor, supporting its activity in this setting.¹⁰¹

The oral pan-AKT inhibitor capivasertib (Truqap, AstraZeneca) doubled median PFS from 3.6 to 7.2 months overall when added to fulvestrant in the CAPITello-291 trial (HR, 0.60; 95% CI, 0.51-0.71) in patients with MBC whose disease progressed on an AI with or without a CDK4/6 inhibitor.⁹⁹ OS trends favored capivasertib without reaching significance at interim analysis, and benefit in AKT pathway wild-type disease was modest (HR, 0.79; 95% CI, 0.61-1.02), consistent with prior FAKTION data suggesting maximal benefit in *AKT1/PTEN/PIK3CA*-altered tumors.^{99,102} Regulatory approval is therefore restricted to this biomarker-defined population, and the safety profile, with rates of high-grade hyperglycemia much lower than with alpelisib, is comparatively more manageable.^{47,99}

Inavolisib (Itovebi, Genentech), a potent selective inhibitor of the alpha isoform of the p110 catalytic subunit of the PI3K complex, improved median PFS from 7.3 to 15 months (HR, 0.43; 95% CI, 0.32-0.59) and the overall response rate (ORR; 58.4% vs 25%) when added to palbociclib/fulvestrant in *PIK3CA*-mutated HR+/HER2- MBC in INAVO120, with better interim OS (34 vs 27 months; HR, 0.67; 95% CI, 0.48-0.94) but increased rates of hyperglycemia, stomatitis, and diarrhea.^{103,104} Data with other CDK4/6 inhibitors and after adjuvant CDK4/6 exposure remain limited.

Gedatolisib (Gedatolisib, Celcuity), a class I pan-PI3K and dual mTORC1/2 inhibitor, improved PFS in the phase 3 VIKTORIA-1 trial in *PIK3CA* wild-type disease. VIKTORIA-1 was a global, open-label, 2-part clinical trial that included study 1 (*PIK3CA* wild-type patients) and study 2 (*PIK3CA*-mutated patients), with results available only for study 1. The researchers found that in the *PIK3CA* wild-type cohort, median PFS was 9.3 months with gedatolisib/palbociclib/fulvestrant and 7.4 months with gedatolisib/fulvestrant vs 2 months with fulvestrant alone (HRs, 0.24 and 0.33, respectively), supporting multitarget PI3K/mTOR strategies beyond *PIK3CA*-mutant tumors.¹⁰⁵

For patients without targetable *PIK3CA/AKT/PTEN* or *ESR1* alterations after an AI plus a CDK4/6 inhibitor, mTOR inhibition remains an option. Everolimus combined with endocrine therapy increased time to progression and PFS in GINECO (tamoxifen + everolimus), BOLERO-2 (everolimus + exemestane), and Pre0102 (everolimus + fulvestrant), although all preceded widespread CDK4/6 inhibitor use.¹⁰⁶⁻¹⁰⁸ A prospective study by Vasseur and colleagues supports everolimus-plus-fulvestrant activity after a CDK4/6 inhibitor.¹⁰⁹ The dual mTORC1/2 inhibitor vistusertib did not outperform everolimus in the MANTA study, likely reflecting incomplete mTORC1 inhibition or the limited clinical impact of mTORC2.¹¹⁰

ESR1 Mutations

ESR1 mutations confer ligand-independent ER activation and are a key mechanism of resistance to AIs, although selective ER degraders (SERDs) and some selective ER modulators (SERMs) can partially overcome this problem.¹¹¹ Mutation rates range from approximately 1% in patients with treatment-naïve disease to nearly 40% in heavily pretreated patients, and mutations frequently emerge in patients on endocrine therapy, prompting recommendations for testing at progression with tissue or ctDNA, with ctDNA preferred for sensitivity and dynamic monitoring.¹¹²⁻¹¹⁶

Elaeestrant (Orserdu, Stemline Therapeutics), an oral SERD, significantly improved PFS vs standard endocrine monotherapy in the phase 3 EMERALD trial, both overall (HR, 0.70; 95% CI, 0.55-0.88) and in patients with *ESR1*-mutant tumors (HR, 0.55; 95% CI, 0.39-0.77), with a 12-month PFS rate of 26.8% vs 8.2% in the *ESR1*-mutant subgroup and more pronounced benefit in patients with at least 12 months of prior CDK4/6-endocrine therapy (median PFS, 8.6 vs 1.9 months; HR, 0.41; 95% CI, 0.26-0.63).^{117,118} An ongoing umbrella trial called ELEVATE is exploring elaeestrant in combination with agents such as everolimus, abemaciclib, and alpelisib; early data on everolimus and abemaciclib show consistent PFS benefit regardless of *ESR1* mutation status after progression on endocrine therapy with or without a CDK4/6 inhibitor.¹¹⁹

In the PADA-1 trial, among patients on an AI plus palbociclib in whom an *ESR1* mutation developed on ctDNA without radiologic progression, switching from an AI to fulvestrant while continuing palbociclib significantly prolonged PFS. This finding supports a ctDNA-guided, preprogression switch strategy, although further prospective validation is required before routine adoption.¹²⁰ SERENA-6 extended this concept by randomizing patients with an emergent *ESR1* mutation on first-line treatment with an AI plus a CDK4/6 inhibitor (without clinical progression) either to continue current therapy or to switch to camizestrant, a next-generation SERD, with the same CDK4/6 inhibitor. Camizestrant nearly doubled PFS (16 vs 9.2 months; HR, 0.44; 95% CI, 0.31-0.60), with OS data pending.⁵³

Imlunestrant (Inluriyo, Lilly), another next-generation oral SERD, demonstrated PFS improvement vs standard endocrine therapy in *ESR1*-mutant disease (5.5 vs 3.8 months; $P < .001$) in the EMBER-3 trial but not in the overall population. The combination of imlunestrant plus abemaciclib improved PFS to 9.4 months vs 5.5 months with imlunestrant alone.¹²¹ Rates of central nervous system progression were lower with imlunestrant than with standard of care in patients with *ESR1*-mutant disease (1.5% vs 6.7%; HR, 0.18; 95% CI, 0.04-0.90)

and in the overall population (1.6% vs 3.0%; HR, 0.47; 95% CI, 0.16-1.38).¹²¹ Updated results showed an OS benefit for imlunestrant vs standard endocrine therapy in *ESR1*-mutant cases (34.5 vs 23.1 months; HR, 0.60; $P=.0043$), although OS data in the overall population remain immature, particularly for the imlunestrant/abemaciclib combination.¹²²

Given increasing early-line CDK4/6 inhibitor use, post-CDK4/6 strategies are essential. The evERA trial evaluated giredestrant plus everolimus vs physician's choice of endocrine therapy plus everolimus in ER+/HER2- MBC after CDK4/6 inhibitor and showed improved PFS in *ESR1*-mutant (9.99 vs 5.45 months; HR, 0.38; 95% CI, 0.27-0.54) and overall populations (8.77 vs 5.49 months; HR, 0.56; 95% CI, 0.44-0.71), with benefit irrespective of *PIK3CA/AKT1/PTEN* status and prior CDK4/6 inhibitor duration.^{123,124}

BRCA1/2 and PALB2 Mutations

Approximately 5% of patients with breast cancer harbor germline *BRCA* mutations, with a higher prevalence in HER2- subtypes. Poly(ADP-ribose) polymerase inhibitors are now standard options for germline *BRCA*-associated advanced disease.¹²⁵ In OlympiAD, olaparib (Lynparza, AstraZeneca) improved PFS vs nonplatinum chemotherapy (7 vs 4.2 months; HR, 0.58; 95% CI, 0.43-0.80), with a trend toward greater benefit in triple-negative disease and a more modest effect in HR+ tumors (HR, 0.82; 95% CI, 0.55-1.26). OS was not significantly different overall, but a prespecified subgroup without prior chemotherapy for metastatic disease derived an OS benefit (HR, 0.51; 95% CI, 0.29-0.90).^{126,127} Talazoparib (Talzenna, Pfizer) in EMBRACA similarly improved PFS (8.6 vs 5.6 months; HR, 0.54; 95% CI, 0.41-0.71) in germline *BRCA*-mutated HER2- disease, with benefit maintained in patients with HR+ disease and no significant OS difference.^{128,129} Because PARP inhibitors are generally better tolerated than many chemotherapies and lack cumulative toxicity, guidelines recommend their use after progression on endocrine therapy in germline *BRCA*-mutated, HR+ MBC, although the best sequencing relative to CDK4/6 inhibition is unclear. In addition, retrospective data suggest shorter PFS and OS with CDK4/6 inhibition in germline *BRCA*-mutated HR+ disease.^{85,130,131}

Beyond *BRCA*, phase 2 data from the TBCRC 048 study support olaparib activity in cases with other homologous recombination repair defects; high response rates and prolonged PFS were observed in germline *PALB2*-mutated MBC (ORR, ~75%-82%; PFS, ~9.6-13.3 months), with meaningful activity in somatic *BRCA1/2* mutations and minimal benefit in *ATM*- or *CHEK2*-mutated tumors.^{132,133}

HER2-Low and HER2 Mutations

HER+ disease is defined by an immunochemistry (IHC) score of 3+ or of 2+ with in situ hybridization amplification. HER2- tumors may express low levels of HER2 (HER2-low), which is now an actionable category.^{134,135} DESTINY-Breast04 established trastuzumab deruxtecan (Enhertu, Daiichi-Sankyo/AstraZeneca) as superior to chemotherapy in HER2-low MBC previously treated with endocrine therapy and 1 to 2 lines of chemotherapy, improving PFS (10.1 vs 5.4 months; HR, 0.51; 95% CI, 0.40-0.64) and OS (23.9 vs 17.5 months; HR, 0.64; 95% CI, 0.49-0.84) in the HR+ cohort.¹³⁶ DESTINY-Breast06 extended these findings to earlier-line treatment and to HER2-ultralow (IHC of 0 with faint membrane staining [0<IHC<1]) tumors. The trial showed PFS benefit in HER2-low (13.2 vs 8.1 months; HR, 0.64) and similar gains in the intention-to-treat population, suggesting extension of benefit to HER2-ultralow disease, leading to earlier-line approval in HR+ HER2-low/ultralow disease, and supporting more refined scoring of IHC 0 cases by membrane staining.^{137,138}

HER2-activating mutations, distinct from amplification, occur in approximately 3% to 5% of HER2-nonamplified breast cancers and up to 10% of lobular cancers, and they represent another targetable subset.^{139,140} In the phase 2 MutHER trial, neratinib (Nerlynx, Puma Biotechnology) plus fulvestrant achieved clinical benefit rates of 30% to 38% in HR+/HER2-mutant, HER2-nonamplified MBC, regardless of prior CDK4/6 inhibition.¹³⁹ SUMMIT showed that neratinib, trastuzumab, and fulvestrant produced a 39% ORR and median PFS of 8.3 months in HR+/HER2-mutant disease after CDK4/6 inhibition.¹⁴¹ A basket study of tucatinib (Tukysa, Seagen) plus trastuzumab ($\pm$ fulvestrant) reported an ORR of 41.9%, median duration of response 12.6 months, and PFS of 9.5 months, collectively supporting HER2-targeted combinations in this genomically defined group (Table 2).¹⁴²

Challenges and Future Implications

Despite its transformative role, NGS faces barriers that include high cost, limited access, and low patient awareness. Socioeconomic disparities, geographic constraints, and variability in the healthcare infrastructure further restrict equitable utilization.^{143,144} Tumor heterogeneity in breast cancer raises concerns about the representativeness of small biopsy samples, and the lack of standardization of the NGS technique and the variability in testing technique make profiling challenging.

Tissue sampling following progression is often difficult. The emergence of ctDNA has offered minimally invasive alternatives for detecting targetable mutations,

Table 2. Clinically Relevant Targets in HR+/HER2– Metastatic Breast Cancer

Mutation	Drug	Study	Description	Phase	Median PFS (HR, 95% CI)	Median OS (HR, 95% CI)
<i>PIK3CA</i>	Alpelisib	SOLAR-1 ⁴⁷	Alpelisib/fulvestrant vs placebo/fulvestrant	3	11 vs 5.7 mo (0.65, 0.5-0.85)	39.3 vs 31.4 (0.86, 0.64-1.15)
<i>PIK3CA/AKT/PTEN</i>	Capivasertib	CAPItello-291 ⁹⁹	Capivasertib/fulvestrant vs placebo/fulvestrant	3	7.3 vs 3.1 mo (0.50, 0.38-0.65)	18-mo OS was 73.2% vs 62.9% in the AKT pathway–altered population (0.69, 0.45-1.05)
<i>PIK3CA</i>	Inavolisib	INAVO120 ^{103,104}	Inavolisib/palbociclib/fulvestrant vs placebo/palbociclib/fulvestrant in <i>PIK3CA</i> -mutated HR+ pts who had disease recurrence during or within 12 mo of adjuvant endocrine therapy	3	15 vs 7.3 mo (0.43, 0.32-0.59)	18-mo OS was 73.7% vs 67.5% (0.64, 0.43-0.97)
<i>ESR1</i>	Elacestrant	EMERALD ^{117,118}	Elacestrant vs SOC endocrine therapy	3	-In <i>ESR</i> -mutated pts: PFS at 12 mo was 26.8% vs 8.2% -In pts with prior >12-mo exposure to CDK4/6, median PFS was 8.6 mo vs 1.9 mo	At interim analysis, in patients with <i>ESR1</i> mutation (0.59, 0.36-0.96; <i>P</i> =.03, nonsignificant)
HER2-low	Trastuzumab deruxtecan	DESTINY-Breast04 ¹³⁶	Trastuzumab deruxtecan vs physician's choice of chemotherapy	3	10.1 vs 5.4 mo (0.51, 0.4-0.64)	23.9 vs 17.5 mo (0.64, 0.48-0.86)
<i>gBRCA1/2</i>	Olaparib	OlympiAD ^{126,127}	Olaparib vs physician's choice of nonplatinum chemotherapy	3	7 vs 4.2 mo (0.58, 0.43-0.80)	19.3 vs 17.1 mo (0.90, 0.66-1.23)
<i>gBRCA1/2</i>	Talazoparib	EMBRACA ^{128,129}	Talazoparib vs physician's choice of chemotherapy	3	8.6 vs 5.6 mo (0.54, 0.41-0.71)	19.3 vs 19.5 mo (0.848, 0.670-1.073)
HER2-activating mutation	Neratinib + fulvestrant	mutHER ¹³⁹	Neratinib + fulvestrant in HR+/HER2–mutated, HER2 nonamplified pts	2	PFS 24 and 20 wk respectively for fulvestrant-treated and fulvestrant-naïve pts	NA
HER2-activating mutation	Neratinib + fulvestrant + trastuzumab	SUMMIT ¹⁴¹	Neratinib + fulvestrant + trastuzumab in HR+/HER2-mutated, HER2 nonamplified pts and prior CDK4/6 exposure	2	8.3 mo	NA

AKT, protein kinase B; *ESR1*, estrogen receptor 1; *gBRCA*, germline breast cancer gene; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; mo, months; NA, not available; OS, overall survival; PFS, progression-free survival; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *PTEN*, phosphatase and tensin homolog; wk, weeks.

monitoring resistance, and assessing prognosis. The integration of artificial intelligence remains limited, but alternative approaches to biomarker evaluation are under research, such as mass spectrometry-based proteomics, that enable the detection of proteins like CDK1, the status of *ERBB2* and the ER, and tumor grading.^{145,146}

Nanoparticles have been studied and utilized for the precise delivery of drug or contrast media to tumor cells, limiting the exposure of noncancer cells and decreasing toxicity.¹⁴⁷ A study by Pourtau and colleagues showed that trastuzumab conjugated with a nanoparticle delivery system can specifically target bone metastasis.¹⁴⁸ More recently, the use of an antibody-conjugated, tumor-targeted nanolipogel to deliver CRISPR/Cas9 plasmids into triple-negative breast cancer for genomic editing led to the knockout of lipocalin 2 and resulted in the significant suppression of tumor growth.¹⁴⁹ In ER+ breast cells, estradiol-conjugated nanogels and polymerases have demonstrated enhanced uptake with cytotoxic effects.¹⁵⁰ The concomitant delivery of fulvestrant and abemaciclib has been shown to improve drug half-life and bioavailability.¹⁵¹

Proteolysis targeting chimera (PROTAC) technology enables targeted protein degradation via the ubiquitin-proteasome system. In the VERITAC trial, vepdegestrant (Veppanu, Arvinas) improved PFS more effectively than fulvestrant in patients with *ESR1*-mutant HR+/HER2- MBC whose disease progressed on prior endocrine therapy including a CDK4/6 inhibitor (5.0 vs 3.7 months; HR, 0.58), with modest benefit in the overall population.^{152,153}

CDK2 has emerged as a key driver of resistance to CDK4/6 inhibition, primarily through CCNE1 amplification and cyclin E1 overexpression, which maintain G1/S cell cycle progression despite CDK4/6 blockade.¹⁵⁴ Several selective CDK2 inhibitors are in early clinical development. For example, tegtociclib (PF-07104091) is being evaluated as monotherapy and in combination with fulvestrant or palbociclib in post-CDK4/6 inhibitor disease (NCT04553133) and in dual CDK4+CDK2 blockade strategy with atirmociclib (NCT05262400). BLU-222 is under investigation in the VELA trial (NCT05252416) in combination with ribociclib and fulvestrant and is showing early signals of tolerability and pharmacodynamic activity, and INX-315 (NCT05735080) and INCB123667 (NCT05238922) are in phase 1/2 trials with dedicated HR+/HER2- post-CDK4/6 inhibitor cohorts. In a phase 3 trial, the pan-CDK2/4/6 inhibitor culmemiciclib in combination with fulvestrant improved PFS vs placebo plus fulvestrant in pretreated HR+/HER2- breast cancer (16.6 vs 7.5 months; HR, 0.36; 95% CI, 0.26-0.51; $P < .0001$), with an ORR of 40.2% vs 12.1%. These findings provide the

most mature data indicating that concurrent CDK2/4/6 blockade can meaningfully overcome endocrine resistance in this setting.¹⁵⁵

Disclosures

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