Review

Post–CDK4/6 Inhibitor Therapeutic Approaches in Hormone Receptor-Positive, HER2-Negative Metastatic Breast Cancer: Current Evidence and Emerging Strategies - A Narrative Review

Humaid O. Al-Shamsi *, Nadia Abdelwahed, Siddig Ibrahim Abdelwahab, Mawada Hussein, Amin Abyad, Saeed Rafii, Hassan Jaafar, Sonia Otsmane, Dima Abdul Jabbar, Hala Abdellatif, Faryal Iqbal, Mudhasir Ahmad, Hampig Kourie, Kefah Mokbel

Posted Date: 22 April 2026

doi: 10.20944/preprints202604.1575.v1

Keywords: CDK4/6 inhibitors; ESR1 mutations; ctDNA; elacestrant; inavolisib; capivasertib

 doi:10.20944/preprints202604.1575.v1

Review

Post–CDK4/6 Inhibitor Therapeutic Approaches in Hormone Receptor-Positive, HER2-Negative Metastatic Breast Cancer: Current Evidence and Emerging Strategies - A Narrative Review

Humaid O. Al-Shamsi [1,2,3,4,5], Nadia Abdelwahed [1], Siddig Ibrahim Abdelwahab [6], Mawadah Hussein [1], Amin Abyad [1], Saeed Rafii [7], Hassan Jaffar [1], Sonia Otsmane [1], Dima Abdul Jabbar [1], Hala Abdellatif [1], Faryal Iqbal [1], Mudhasir Ahmad [1], Hampig Kourie [8] and Kefah Moqbel [9]

[1] Burjeel Cancer Institute, Abu Dhabi, United Arab Emirates
[2] Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, USA
[3] Ras Al Khaimah Medical and Health Sciences University, United Arab Emirates
[4] Gulf Medical University, Ajman, United Arab Emirates
[5] Emirates Oncology Society, Dubai, United Arab Emirates
[6] Health Research Centre, Jazan University, Jazan, Saudi Arabia
[7] Department of Medical Oncology, Mediclinic City Hospital, Dubai, United Arab Emirates
[8] Hematology-Oncology Department, Faculty of Medicine, Saint Joseph University, Beirut, Lebanon
[9] London Breast Institute, The Princess Grace Hospital, London, UK
* Correspondence: humaid.al-shamsi@medportal.ca

Abstract

Background: Therapeutic resistance following cyclin-dependent kinase 4/6 inhibitor (CDK4/6i) plus endocrine therapy (ET) remains a key unmet need in hormone receptor-positive, human epidermal growth factor receptor 2-negative (HR+/HER2−) metastatic breast cancer (mBC). Treatment paradigms have advanced from non-targeted options, such as fulvestrant monotherapy or everolimus-based combinations, to precision medicine strategies, including inhibitors of the PI3K/AKT pathway, oral selective estrogen receptor degraders (SERDs), and novel ER-modulating agents, often guided by biomarkers and molecular surveillance. **Methods:** This narrative review synthesizes evidence from randomized clinical trials, real-world studies, and biomarker-driven analyses published from 2010 to 2026, with emphasis on next-generation sequencing (NGS)-guided genomic profiling, targeted pathway therapies, and circulating tumor DNA (ctDNA)-based proactive interventions in the post-CDK4/6i setting. This review was conducted and reported in accordance with SANRA recommendations for narrative reviews. **Results:** Early second-line standards, including fulvestrant and alpelisib (for PIK3CA-mutated tumors), established the basis for biomarker-guided treatment in hormone receptor–positive, HER2-negative metastatic breast cancer. With the widespread use of CDK4/6 inhibitors in the first-line setting, the optimal post-progression strategy has shifted toward combination approaches rather than single-agent endocrine therapy, as endocrine monotherapy has shown limited efficacy in the setting of acquired resistance. Multiple randomized studies have demonstrated that the addition of targeted agents to endocrine therapy results in significantly improved progression-free survival compared with hormonal therapy alone, supporting combination regimens as the preferred strategy after CDK4/6 inhibitor progression. Current evidence strongly supports that in hormone receptor–positive metastatic breast cancer progressing after CDK4/6 inhibitors, combination therapy with endocrine agents and targeted treatments should be favored over single-agent hormonal therapy, except in carefully selected patients with low disease burden, indolent biology, or frailty where treatment tolerability is a major concern. Precision-based trials have further refined this approach. Elacestrant improved progression-free survival in ESR1-mutated disease in the EMERALD trial, capivasertib demonstrated significant

 doi:10.20944/preprints202604.1575.v1

benefit when combined with fulvestrant in tumors harboring AKT/PIK3CA/PTEN pathway alterations in CAPItello-291, and inavolisib achieved both progression-free and overall survival improvement in PIK3CA-mutated patients with early relapse in INAVO120. These findings consistently reinforce that endocrine therapy combined with pathway-targeted agents provides superior clinical outcomes compared with endocrine therapy alone, particularly in patients previously treated with CDK4/6 inhibitors. Real-world analyses further confirm the effectiveness of these combination strategies across diverse clinical subgroups. Comprehensive genomic profiling has identified multiple resistance mechanisms including ESR1 mutations, PI3K/AKT/mTOR pathway activation, RB1 loss, and FGFR alterations, which frequently co occur in up to one third of tumors and contribute to reduced sensitivity to endocrine monotherapy, further emphasizing the importance of combination treatment strategies. Notably, while ESR1 and PI3K pathway alterations guide approved therapies, FGFR alterations remain early-phase targets without integrated standard-of-care options, though ongoing trials are evaluating selective FGFR inhibitors. Proactive switching approaches evaluated in SERENA-6 and PADA-1 demonstrate that serial circulating tumor DNA (ctDNA) monitoring can guide early therapeutic modification, extending endocrine-based disease control by approximately 5 to 7 months when ESR1 mutations are detected prior to radiographic progression. **Conclusions:** Post-CDK4/6i management increasingly relies on NGS-guided precision approaches, integrating pathway-specific therapies and ctDNA surveillance to tailor sequencing based on resistance profiles, prior ET response, and tumor heterogeneity. Future investigations into novel ER degraders and multi-targeted combinations hold potential to further optimize algorithms, extend non-chemotherapy options, and enhance survival in HR+/HER2− mBC.

Keywords: CDK4/6 inhibitors; ESR1 mutations; ctDNA; elacestrant; inavolisib; capivasertib

1. Introduction

Hormone receptor–positive, human epidermal growth factor receptor 2–negative metastatic breast cancer (HR+/HER2− mBC) accounts for approximately 60 to 70% of advanced breast cancers and represents the largest biologic subtype requiring chronic systemic therapy [1]. The incorporation of cyclin-dependent kinase 4/6 inhibitors (CDK4/6i)—palbociclib, ribociclib, and abemaciclib—into first-line endocrine therapy has extended median progression-free survival (PFS) to approximately 24 to 28 months in contemporary cohorts, with overall survival frequently exceeding 38 to58 months in selected populations [2–4]. These advances have transformed HR+/HER2− mBC into a molecularly stratified, long-trajectory disease.

Nevertheless, therapeutic resistance remains inevitable due to intrinsic or emerging resistance mechanisms. Resistance may be intrinsic, driven by baseline alterations such as RB1 loss or PI3K pathway activation, or acquired through clonal evolution under therapeutic pressure, most commonly via emergent ESR1 mutations and secondary pathway activation [5]. This dynamic architecture limits the durability of single-pathway strategies and supports the need for longitudinal molecular monitoring [6].

ESR1 mutations, which are an emerging or acquired resistance, arise in approximately 30 to 48% of tumors following prolonged endocrine exposure, while intrinsic PI3K/AKT/PTEN pathway alterations occur in 40 to 50% (PIK3CA ~30 to 40%, AKT1 ~4 to 5%, PTEN loss ~5%) [7–10]. Additional mechanisms, which can be both intrinsic and acquired, include RB1 loss (2 to 9%), FGFR amplifications (15 to 41%), and cyclin E amplification. Whole-exome sequencing of CDK4/6i-exposed tumors has identified resistance alterations in 66% of cases, with nearly one-third harboring multiple concurrent drivers, underscoring the polyclonal and adaptive nature of post-progression disease [11].

Progression following first-line CDK4/6i therapy necessitates biomarker-directed therapeutic sequencing. In PIK3CA-mutated disease, alpelisib plus fulvestrant remains an established standard of care based on SOLAR-1, while inavolisib-based triplet therapy has demonstrated both progression-free and overall survival benefit in endocrine-resistant populations. For tumors harboring AKT,

 doi:10.20944/preprints202604.1575.v1

PIK3CA, or PTEN alterations, capivasertib plus fulvestrant provides pathway-directed benefit [4,6,12–16]. In ESR1-mutated disease without dominant PI3K pathway activation, oral selective estrogen receptor degraders such as elacestrant represent evidence-supported options [7,16–18]. PARP inhibitors are indicated in germline BRCA1/2–mutated disease. Emerging strategies, including giredestrant-based combinations and the gedatolisib triplet evaluated in VIKTORIA-1 for PIK3CA–wild-type tumors, may further expand second-line options pending regulatory maturation.

Three critical challenges define the post-CDK4/6i era: (1) prolonging endocrine sensitivity while delaying chemotherapy exposure; (2) optimizing sequencing in tumors with co-occurring ESR1 and PI3K pathway alterations (present in 20 to 40% of cases); and (3) integrating proactive molecular surveillance strategies to intervene before radiographic progression. This review synthesizes evidence across three clinically actionable domains: (i) ctDNA guided proactive switching paradigms, (ii) real world performance of newly approved agents relative to trial benchmarks, and (iii) molecular decision frameworks for complex, co altered disease. The focus is restricted to endocrine-based strategies, PI3K/AKT/mTOR inhibitors, and next-generation estrogen receptor degraders, providing a precision-oriented roadmap for post-CDK4/6i therapeutic sequencing in HR+/HER2− metastatic breast cancer. Regulatory approvals referenced reflect U.S. FDA status as of early 2026. Table 1 summarizes the approved biomarker-directed therapies after CDK4/6 inhibitor progression.

Table 1. Approved and Emerging Biomarker-Directed Therapies After CDK4/6 Inhibitor Progression in HR+/HER2− Metastatic Breast Cancer.

Therapeutic Class	Agent	Molecular Selection	Pivotal Trial(s)	Median PFS (months)	OS Signal	Regulatory Status (as of early 2026)	Clinical Positioning / Notes
Oral SERD	Elacestrant	ESR1-mutated	EMERALD	3.8 (overall); 8.6 (≥12 mo prior CDK4/6i)	No statistically significant OS benefit yet	FDA approved (2023)	Preferred in ESR1-mut endocrine-sensitive disease; best in longer prior CDK4/6i exposure
AKT inhibitor	Capivasertib	AKT1/PIK3CA/PTEN altered	CAPItello-291	7.2 (overall); 7.3 (altered)	Not mature / no clear OS benefit yet	FDA approved	Pathway-altered (AKT/PI3K/PTEN) dominant tumors; post-CDK4/6i option
PI3Kα inhibitor (triplet)	Inavolisib (triplet: + palbociclib + fulvestrant)	PIK3CA-mutated	INAVO120	15.0	Yes (HR 0.67; mature OS benefit)	FDA approved (2024)	PIK3CA-mut endocrine-resistant tumors; triplet strategy with clear OS benefit
mTOR inhibitor	Everolimus (+ exemestane)	None required	BOLERO-2	7.8 (pre-CDK4/6i era); ~3.8–5.4	No OS benefit	Approved (earlier line)	Later-line endocrine-based strategy; reduced

 doi:10.20944/preprints202604.1575.v1

				in RWE post-CDK4/6i			efficacy post-CDK4/6i in real-world data
PI3K inhibitor	Alpelisib (+ fulvestrant)	PIK3CA-mutated	SOLAR-1	11.0	No OS (numeric 7.9 mo improvement, not stat sig)	Approved	Earlier PI3K option post-CDK4/6i in PIK3CA-mut; established but hyperglycemia common
Endocrine therapy (backbone)	Fulvestrant	ER+	Various (e.g., PALOMA-3 reference)	Limited monotherapy activity post-CDK4/6i	N/A	Approved	Backbone; limited single-agent efficacy post-CDK4/6i; used in combinations
PARP inhibitor	Olaparib	Germline BRCA1/2 mutated	OlympiAD / others	N/A (PARP context)	OS benefit in gBRCA population	Approved	Germline BRCA-mutated population; post-CDK4/6i if applicable
PARP inhibitor	Talazoparib	Germline BRCA1/2 mutated/PALB2 mutated	EMBRACA	N/A (PARP context)	OS benefit in gBRCA population	Approved	Germline BRCA-mutated population; post-CDK4/6i if applicable
Oral SERD	Camizestrant	ESR1-mutated	SERENA-6 (proactive switch)	N/A (investigational)	N/A	Phase III / investigational	Investigational; proactive ctDNA-guided in ESR1-mut
Oral SERD / PROTAC ER degrader	Imlunestrant	ESR1-mutated	EMBER-3	N/A (investigational)	N/A	Phase III / region-dependent	Investigational; emerging oral SERD
PROTAC ER degrader	Vepdegestrant	ESR1-mutated	VERITAC-2	N/A (investigational)	N/A	Regulatory review / emerging	Emerging; PROTAC-based for ESR1-mut
Oral SERD	Giredestrant	ESR1-mutated	persevERA (negative primary)	N/A (investigational)	N/A	Phase III (primary endpoint negative)	Investigational; limited promise based on trial results
PI3K/mTOR inhibitor	Gedatolisib	PIK3CA-WT / PI3K pathway	VIKTORIA-1	N/A (investigational)	N/A	Phase III	Investigational; for PIK3CA wild-type

 doi:10.20944/preprints202604.1575.v1

							pathway-driven tumors
Pan-mutant selective PI3K inhibitor	Zovegalisi (+fulvestrant)	PIK3CA-mutated	ReDiscover (Ph 1/2) ReDiscover-2(Ph3 ongoing)	11.1	Not mature	Breakthrough Therapy Designation (FDA, Feb 2026); Ph 3 ongoing	Mutant-selective (allosteric, pan-mutant) PI3Kα inhibitor.

2. Methods

This narrative review synthesizes current evidence and emerging strategies for therapeutic approaches in hormone receptor-positive/human epidermal growth factor receptor 2-negative (HR+/HER2−) metastatic breast cancer (mBC) following progression on cyclin-dependent kinase 4/6 inhibitors (CDK4/6i). This review was conducted and reported in accordance with SANRA recommendations for narrative reviews [19]. Literature was identified through targeted searches in PubMed/MEDLINE, Embase, and Cochrane Library from January 2010 to February 2026, using keywords such as "CDK4/6 inhibitors," "post-CDK4/6 resistance," "ESR1 mutations," "PI3K/AKT inhibitors," "oral SERDs," "ctDNA monitoring," and "HR+/HER2− metastatic breast cancer." Additional sources included conference abstracts from ASCO, ESMO, and San Antonio Breast Cancer Symposium, as well as ClinicalTrials.gov for ongoing trials. Two authors (HOA and NA) reviewed and selected the trials to be included in this narrative review.

Selection prioritized phase 2/3 randomized controlled trials (RCTs), real-world evidence (RWE) studies, and biomarker analyses demonstrating clinical relevance, such as pivotal trials (e.g., EMERALD, CAPItello-291, INAVO120, SERENA-6, PADA-1) and genomic profiling studies on resistance mechanisms. Inclusion focused on endocrine-based strategies, pathway inhibitors (PI3K/AKT/mTOR), and next-generation estrogen receptor degraders, excluding chemotherapy-centric or early-stage breast cancer studies. Approximately 50 main references were selected based on recency, impact factor, and alignment with review objectives, emphasizing U.S. FDA approvals as of early 2026, along with 30 additional supporting references, for a total of 80 references.

Data extraction involved summarizing study designs, patient populations, interventions, outcomes (e.g., progression-free survival [PFS], overall survival [OS], hazard ratios), resistance drivers (e.g., ESR1 mutations, PIK3CA alterations), and real world applicability. Synthesis was thematic, organized into domains: ctDNA guided switching, RWE performance, and molecular frameworks for co altered disease. No formal quality assessment or meta-analysis was performed, as is typical for narrative reviews; instead, expert interpretation integrated clinical implications, limitations, and future directions. This approach allows flexibility to highlight evolving paradigms while acknowledging potential selection bias inherent to narrative formats.

3. Genomic Profiling in Diagnostic Workup

Comprehensive genomic profiling has become an essential component of the diagnostic and therapeutic algorithm in hormone receptor–positive, HER2-negative metastatic breast cancer, particularly following progression on CDK4/6 inhibitors [41]. Next-generation sequencing (NGS), performed on tumor tissue and/or circulating tumor DNA (ctDNA), enables identification of actionable alterations including ESR1, PIK3CA, AKT1, and PTEN, which directly inform treatment selection [40]. In addition, genomic profiling reveals resistance mechanisms such as RB1 loss, FGFR amplifications, and cyclin pathway alterations, which may influence prognosis and guide clinical trial eligibility [5,11,13,44]. Importantly, ctDNA-based assays allow dynamic, minimally invasive monitoring of tumor evolution, facilitating early detection of emergent mutations—most notably

 doi:10.20944/preprints202604.1575.v1

ESR1—months prior to radiographic progression [9,17,71]. This enables timely therapeutic adaptation, as demonstrated in recent trials of ctDNA-guided switching strategies [16,72,74]. Given the high prevalence of polyclonal resistance, with up to one-third of tumors harboring multiple concurrent genomic drivers, repeat molecular profiling at progression is strongly recommended to refine treatment sequencing and support a precision oncology approach [5,11].

4. Real-World Evidence and Pathway-Directed Therapeutic Strategies

Unlike controlled trials, real-world cohorts reflect broader clinical heterogeneity including elderly, frail, and heavily pretreated patients, making real-world evidence essential for evaluating treatment generalizability and tolerability. Real-world evidence remains essential for assessing treatment generalizability, identifying specific subgroups deriving meaningful clinical benefit, and evaluating practical tolerability profiles [20,21].

4.1. Alpelisib

Alpelisib, a selective PI3Kα inhibitor, represents an established biomarker-directed option for PIK3CA-mutated HR+/HER2− mBC, particularly following CDK4/6i progression. Approved by the FDA in 2019 based on the SOLAR-1 trial, where it extended median PFS to 11.0 months (vs 5.7 months with fulvestrant alone; HR 0.65; 95% CI 0.50–0.85) in PIK3CA-mutated patients[22], alpelisib's role in the post-CDK4/6i era has been further validated by dedicated studies and emerging RWE [23]. The phase 2 BYLieve trial specifically addressed this setting, enrolling patients post-CDK4/6i plus aromatase inhibitor, and reported a median PFS of 7.3 months with alpelisib plus fulvestrant in cohort A, with an ORR of 17% and clinical benefit rate of 46% [23]. Recent phase 3 data from EPIK-B5 (NCT05038735) reinforced this, showing a median PFS of 7.4 months (vs 2.8 months with fulvestrant; HR 0.52; 95% CI 0.37–0.72) in CDK4/6i-pretreated patients, highlighting consistent benefit [24].

Real-world analyses confirm alpelisib's effectiveness beyond clinical trials, often in more heterogeneous populations. A comparative study using the Flatiron Health database matched post-CDK4/6i patients receiving alpelisib plus fulvestrant against standard therapies (e.g., everolimus plus exemestane, chemotherapy), demonstrating a median PFS of 7.3 months vs 3.6 months (weighted HR 0.48; 95% CI 0.31–0.73), underscoring superior outcomes [84]. In a single-institution retrospective review of 27 patients (median 3 prior lines), the ORR was 12.5% with a median duration of response of 5.8 months, though treatment duration was limited by toxicity in half the cohort [26]. Broader RWE from multinational registries reports median time-to-treatment discontinuation of 4–6 months, with effectiveness maintained across subgroups including visceral disease and older patients, albeit with frequent dose adjustments [27].

Safety in real-world practice mirrors trial data, with hyperglycemia (50–60%), rash (20–30%), and gastrointestinal effects (30–40%) as the predominant AEs, often leading to dose interruptions (50–60%) or discontinuations (20–30%) [27]. Proactive management, including metformin for hyperglycemia and dermatologic prophylaxis, has improved tolerability in community settings.

4.2. Elacestrant

Elacestrant received FDA approval in January 2023 for ESR1-mutated HR+/HER2− metastatic breast cancer based on the EMERALD trial, which demonstrated median progression-free survival of 3.8 versus 1.9 months with standard endocrine therapy (HR 0.55; P=0.0005) in the ESR1-mutated population. In post-hoc analysis results, patients with ≥12 months of prior CDK4/6i benefit achieved median progression-free survival of 8.6 versus 1.9 months (HR 0.41), establishing duration of prior endocrine response as a predictive biomarker [28,29].

Multiple 2023-2026 real-world cohorts (cumulative n~300-750) report median time-to-next-treatment of 7.9 months overall (95% CI 7.1-9.8), frequently exceeding trial expectations.36 Subgroup analyses identify particularly favorable outcomes in earlier treatment lines (8.2-10.8 months), patients with ≥12 months prior CDK4/6i benefit (8.4 months), those without prior fulvestrant exposure (12.9

 doi:10.20944/preprints202604.1575.v1

months), and individuals without prior chemotherapy (8.4 months). Patients with visceral metastases achieved 7.9 months and those with hepatic metastases achieved 7.2 months [18]. Critically, co-occurring ESR1 and PI3K pathway mutations retained clinically meaningful benefit (median time-to-next-treatment 6.3 months), supporting elacestrant as an initial endocrine-directed approach even in molecularly complex tumors [18,30]. Treatment discontinuation due to adverse events remained low, consistent with elacestrant's manageable oral profile characterized primarily by mild-to-moderate nausea, fatigue, and arthralgia.

Table 2 presents comprehensive real-world evidence data across multiple cohorts, while Table 1 provides a comparative analysis of trial versus real-world performance metrics. While elacestrant marks a key advance in ESR1-mutated endocrine resistance, pathway-directed strategies targeting PIK3CA and AKT alterations further expand treatment options, especially post-CDK4/6i.

Table 2. Key Real-World Evidence Studies Post-CDK4/6 Inhibitor.

Study/Agent	Study Type	Population (n)	Key Subgroups	Median TTNT/PFS (95% CI)	Median OS (95% CI)	Key Findings
Elacestrant RWE (2023-2026 cohorts)	Multicenter retrospective	ESR1-mutated HR+/HER2− mBC (n~300-750)	Overall 1-2 prior ET lines No prior fulvestrant ≥12mo prior CDK4/6i ESR1+PIK3CA co-mut	Overall: 7.9 mo (7.1-9.8) 1-2 prior: 8.2-10.8 mo No fulv: 12.9 mo ≥12mo: 8.4 mo Co-mut: 6.3 mo	Not reported	RWE exceeds trial PFS Retained benefit in co-mutated Low discontinuation
Everolimus + ET Post-CDK4/6i RWE	Multicenter retrospective	HR+/HER2− mBC with prior CDK4/6i (n~200-400)	Post-CDK4/6i CDK4/6i-naïve	Post-CDK4/6i: 5.3 mo (4.6-7.1) Naïve: 6.7 mo (5.8-7.6) P=0.046	Post-CDK4/6i: 21.8 mo (18.5-25.5) Naïve: 27.3 mo (23.2-30.2) P=0.01	Attenuated vs pre-CDK4/6i era Toxicity limits use (20-30% D/C)
Capivasertib RWE (emerging 2025)	Early real-world cohorts	Pathway-altered HR+/HER2− mBC post-CDK4/6i	AKT/PIK3CA/PTEN-altered	5-7 mo (limited data)	Not reported	Modest benefit Manageable toxicity with dose modifications

4.3. Inavolisib

Inavolisib received accelerated FDA approval in October 2024 based on the INAVO120 trial, evaluating a triplet combination of inavolisib (selective PI3Kα inhibitor with dual mechanism of kinase inhibition and mutant p110α degradation) plus palbociclib plus fulvestrant in PIK3CA-mutated, endocrine-resistant disease. The triplet achieved median progression-free survival of 15.0 months at primary analysis versus 7.3 months with placebo plus palbociclib plus fulvestrant (HR 0.43; P<0.0001) [31]. Updated data at 34.2 months median follow-up demonstrated sustained benefit with median progression-free survival of 17.2 months [85].

Final overall survival analysis revealed the first survival benefit for a PI3K pathway inhibitor: median overall survival 34.0 months (95% CI 29.3-39.1) versus 27.0 months (95% CI 23.1-31.9; HR 0.67; P=0.019) [32]. Time to chemotherapy initiation was substantially prolonged: 35.6 versus 12.6

 doi:10.20944/preprints202604.1575.v1

months (HR 0.43), representing nearly two years' delay in cytotoxic exposure [38]. Inavolisib's safety profile demonstrates meaningful improvements over earlier-generation PI3K inhibitors, with treatment discontinuation due to adverse events occurring in only 6.8% versus historically 25% with alpelisib [8]. Grade 3-4 hyperglycemia occurred in approximately 20% versus 36-65% with alpelisib, attributed to inavolisib's enhanced selectivity and intermittent dosing schedule (21 days on, 7 days off) [31,32]. These data establish inavolisib as the preferred PI3K pathway inhibitor for the 30-40% of patients harboring PIK3CA mutations.

Complete INAVO120 trial design, efficacy endpoints including overall survival data, and comparative safety profile versus alpelisib are detailed in Tables 1 and 6.

4.4. Capivasertib

Capivasertib, a pan-AKT inhibitor, received FDA approval in November 2023 based on the CAPItello-291 trial. In the overall intention-to-treat population, capivasertib plus fulvestrant achieved median progression-free survival of 7.2 versus 3.6 months (HR 0.60; P <0.001). In the biomarker-altered subgroup harboring AKT1, PIK3CA, or PTEN aberrations (41% of the trial population), median progression-free survival was 7.3 versus 3.1 months (HR 0.50; P <0.001) [34]. Approximately 70% of patients had received prior CDK4/6i exposure, validating activity in heavily pretreated populations [33,35]. Capivasertib's biomarker-inclusive design collectively addresses 40-50% of patients—representing broader applicability than PIK3CA-selective agents. The safety profile demonstrates substantially lower rates of severe hyperglycemia compared with PI3K inhibitors (all grades hyperglycemia was 16.3% vs ~64% with alpelisib), though characteristic toxicities include rash (21.2% grade ≥3) and diarrhea (9.3% grade ≥3) [41,43]. Emerging real-world evidence suggests modest progression-free survival of 5-7 months in pathway-altered disease with manageable toxicity [36]. Emerging real-world findings from large database analysis of US patients (n = 412 patients with MBC) demonstrate the effectiveness of capivasertib + fulvestrant in real-world practice. Clinical outcomes in 2L and 3L closely match those observed in the CAPItello-291 Phase 3 randomized controlled trial, which supported FDA approval of the capivasertib + fulvestrant regimen. Numerically improved outcomes were observed in patients who used capivasertib in earlier vs later line settings (2L and 3L median rwTTNT was 7.1 mos (IQR 5.8, NE) and 6.9 mos (IQR 6.1, 7.8), respectively; 2L and 3L median rwTTD was 6.9 mos (IQR 5.3, NE) and 6.6 mos (IQR 5.2, 7.1), respectively) [37].

In exploratory ctDNA analyses from the Phase 3 CAPItello-291, clinical benefit (PFS) of capivasertib + fulvestrant in the ctDNA-altered group was consistent with the primary tissue-based analysis and irrespective of ESR1m. Notably, patients receiving capivasertib + fulvestrant in 2L post AI+CDK4/6i who had ESR1 and PIK3CA/AKT/PTEN alterations achieved a 7-month mPFS benefit, regardless of the duration of prior CDK4/6i+ET [25].

Tables 3 and 5 present complete CAPItello-291 data including biomarker-stratified analyses and comparative positioning relative to other pathway inhibitors.

Table 3. Key Genomic Profiling and Resistance Mechanism Studies.

Study	Study Type	Population (n)	Key Findings	Resistance Mechanisms Identified	Clinical Implications
Wander et al. (Cancer Discovery 2020)	Whole-exome sequencing	CDK4/6i-exposed tumors (n=59)	8 distinct resistance mechanisms in 66% of cases 29.3% harbor ≥2 concurrent	RB1 loss (9.8%) AKT1 mutations (9.8%) RAS pathway (9.8%) AURKA amp CCNE2 amp ERBB2 mutations	Multi-targeted strategies needed Single-pathway approaches inadequate in 30% due to polyclonal resistance

 doi:10.20944/preprints202604.1575.v1

			drivers (polyclonal)	FGFR2 alterations ER loss	
PALOMA-3 ctDNA Analysis	Serial liquid biopsy	Palbociclib-treated patients	Acquired RB1 mutations during treatment ESR1 mutations emerge on therapy	RB1 mutations (~5%) ESR1 mutations (increasing VAF)	Direct CDK4/6i target inactivation Serial monitoring detects resistance early
BYLieve ctDNA Substudy	Prospective ctDNA profiling	PIK3CA-mutated mBC post-CDK4/6i	ctDNA fraction strongly prognostic Low ctDNA (<10%) predicts superior PFS	High ctDNA burden associated with worse outcomes	Low ctDNA: PFS 16.7 mo High ctDNA: PFS 5.4 mo HR 0.31 ctDNA quantification is prognostic biomarker
AURORA Molecular Screening Program	Large-scale genomic profiling	HR+/HER2− mBC	TP53 and ESR1 mutations independently predict worse outcomes	TP53 mutations: HR 1.59 for PFS ESR1 mutations: HR 3.10 for PFS	Prognostic stratification TP53-mutant may benefit from alternative strategies
Multiple ctDNA Cohorts (FGFR Analysis)	Retrospective ctDNA profiling	Post-CDK4/6i populations	FGFR amplifications highly prevalent in resistant disease	FGFR1/2 amplifications (15-41% by ctDNA)	Potentially actionable target FGFR inhibitors under investigation

4.5. Everolimus

Everolimus plus exemestane was established in the pre-CDK4/6 inhibitor era by the BOLERO-2 trial, which demonstrated a median progression-free survival (PFS) of 7.8 versus 3.2 months (HR 0.45) in aromatase inhibitor–resistant HR+/HER2− metastatic breast cancer [38]. However, its activity appears attenuated in contemporary post-CDK4/6i populations. Recent real-world cohorts (2024–2025) report a median PFS of 5.3 months (95% CI 4.6–7.1) in patients previously exposed to CDK4/6 inhibitors compared with 6.7 months in CDK4/6i-naïve patients (P =0.046), with corresponding median overall survival of 21.8 versus 27.3 months (P =0.01).

In current practice, everolimus retains a role in patients without actionable ESR1 or PI3K pathway alterations and in later treatment lines following exhaustion of oral SERDs and pathway-targeted agents. Nevertheless, its clinical utility is limited by a characteristic toxicity profile (including stomatitis, fatigue, hyperglycemia, and pneumonitis) which results in treatment discontinuation in approximately 20 to 30% of patients and dose reductions in 30 to 40% [38,39].

 doi:10.20944/preprints202604.1575.v1

Accordingly, everolimus is generally positioned as a later-line endocrine-based option rather than a preferred early post-CDK4/6i strategy.

Table 1 provides a systematic comparison of approved agents, summarizing regulatory status, biomarker selection, efficacy benchmarks from clinical trials and real-world studies, toxicity profiles, and evidence-based positioning. Collectively, these data support a biomarker-driven treatment framework in the post-CDK4/6i setting, integrating molecular stratification with real-world performance to guide individualized therapeutic sequencing in HR+/HER2− metastatic breast cancer.

5. Genomic Resistance Architecture and Clinical Decision Frameworks

Co-occurrence of ESR1 mutations with PI3K/AKT/PTEN pathway alterations in second line affects approximately 8.2% of patients following first line progression [40], substantially complicating therapeutic decision-making in the absence of head-to-head sequencing trials [41,42]. These genomic patterns reflect both intrinsic resistance present before therapy and acquired clonal evolution under CDK4/6i selective pressure. Clinical management therefore requires integration of molecular context with disease kinetics, prior endocrine sensitivity, presence of pathway-dominant alterations, visceral crisis, and anticipated treatment tolerability.

Comprehensive whole-exome sequencing of 59 CDK4/6i-exposed tumors identified eight distinct resistance mechanisms in 66% of cases, with approximately 29% harboring multiple concurrent drivers—highlighting substantial polyclonal complexity [11]. Identified alterations included RB1 biallelic loss, AKT1 mutations, RAS pathway activation (each ~10%), AURKA and CCNE2 amplification, ERBB2 mutations, FGFR2 alterations, and complete estrogen receptor loss. Additional studies have demonstrated acquired RB1 mutations emerging during CDK4/6i therapy (~5%) [9], FAT1 loss driving CDK6 upregulation via Hippo pathway dysregulation [43], and CCNE1/CCNE2 amplification enabling CDK2-mediated cell cycle bypass independent of CDK4/6 signaling (estimated 5–10%) [44]. FGFR amplifications, particularly FGFR1, have been detected in a substantial proportion of post-CDK4/6i tumors (15–41% across ctDNA cohorts), representing investigational but potentially actionable targets [13,45]. FGFR alterations remain early-phase targets without integrated standard-of-care options, though ongoing trials (e.g., NCT04546009) are evaluating selective FGFR inhibitors [46].

Importantly, resistance alterations such as ESR1 and PIK3CA mutations function primarily as predictive biomarkers guiding targeted therapy selection, whereas other genomic features—including TP53 mutations and elevated circulating tumor DNA (ctDNA) fraction—serve predominantly prognostic roles reflecting tumor burden and genomic instability. In the BYLieve ctDNA substudy, low ctDNA fraction (<10%) at progression was associated with markedly superior PFS compared with high ctDNA burden (16.7 vs 5.4 months; HR 0.31) [47]. Similarly, in the AURORA molecular screening program, TP53 mutations (HR 1.59) and acquired ESR1 mutations (HR 3.10) independently predicted inferior progression-free survival [86]. These findings underscore the dual importance of identifying actionable resistance drivers and quantifying global genomic risk.

From a clinical implementation perspective, these data strongly support repeat next-generation sequencing—preferably incorporating ctDNA analysis—at radiologic progression. Molecular tumor board review should prioritize dominant resistance drivers while acknowledging polyclonal architecture that may limit single-pathway strategies. In selected cases, combination or sequential multi-pathway approaches may be more appropriate than isolated targeted therapy. Table 3 synthesizes key genomic resistance studies, including whole-exome sequencing analyses, serial ctDNA investigations, and large-scale molecular screening programs that collectively define the contemporary resistance landscape. Figure 1 summarizes the proposed biomarker-directed sequencing framework.

 doi:10.20944/preprints202604.1575.v1

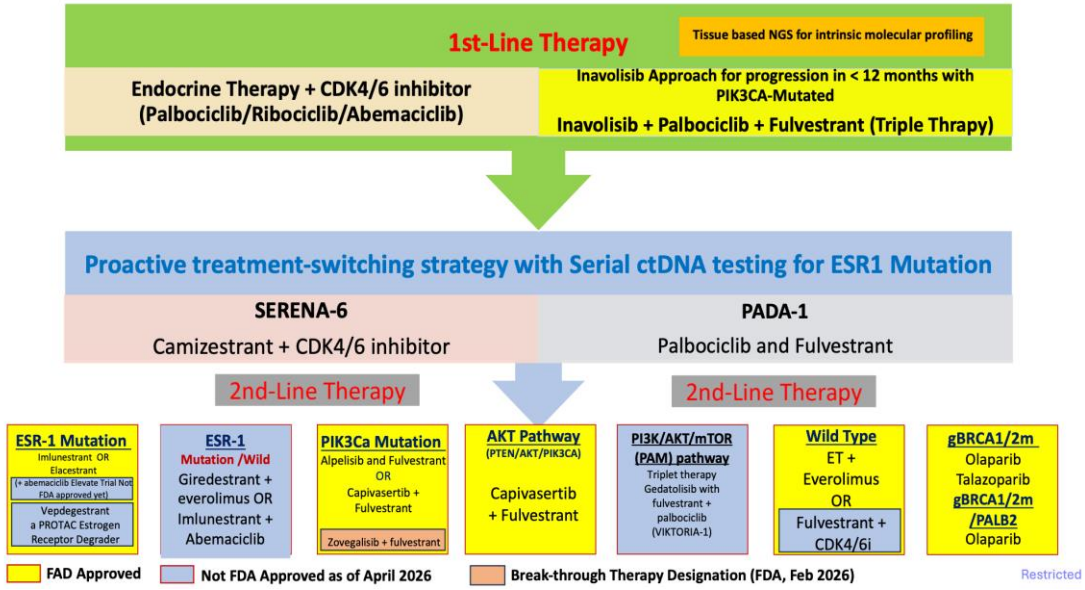

Figure 1. Proposed biomarker-directed treatment algorithm for HR+/HER2− metastatic breast cancer following CDK4/6 inhibitor progression. Figure 1. First-line therapy consists of endocrine therapy plus a CDK4/6 inhibitor. Comprehensive molecular profiling using tissue-based next-generation sequencing (NGS) and/or circulating tumor DNA (ctDNA) is recommended at baseline and progression. In patients with PIK3CA-mutated tumors and early progression (and early recurrence while on/or within 12 month of completing adjuvant treatment), inavolisib-based triplet therapy represents an evidence-supported strategy. Serial ctDNA monitoring for emergent ESR1 mutations enables proactive switching to next-generation oral selective estrogen receptor degrader (SERD) (Camizestant), as evaluated in SERENA-6, or fulvestrant as in PADA-1. Second-line therapy selection is guided by dominant molecular alterations, including ESR1 mutations, PI3K/AKT/PTEN pathway alterations, homologous recombination deficiency (gBRCA1/2), and wild-type disease. Investigational strategies are indicated where applicable.

5.1. Decision Framework for Co-Altered Populations

In patients demonstrating endocrine-sensitive disease characteristics—defined by ≥12 months of clinical benefit on the most recent prior endocrine therapy plus CDK4/6i regimen, absence of visceral crisis presentations, and manageable disease kinetics—elacestrant monotherapy represents the preferred initial strategy. Real-world evidence supports durable benefit even in co-mutated ESR1 and PI3K pathway-altered tumors (median time-to-next-treatment 6.3 months), comparable to pathway wild-type outcomes [18]. This approach capitalizes on elacestrant's excellent tolerability profile, oral administration, and broad activity across ESR1 mutation variants, reserving pathway-directed therapies for subsequent lines following elacestrant progression. In exploratory ctDNA analyses from the Phase 3 CAPItello-291, clinical benefit (PFS) of capivasertib + fulvestrant in the ctDNA-altered group was consistent with the primary tissue-based analysis and irrespective of ESR1m. Notably, patients receiving capivasertib + fulvestrant in 2L post AI+CDK4/6i who had ESR1 and PIK3CA/AKT/PTEN alterations achieved a 7-month mPFS benefit, regardless of the duration of prior CDK4/6i+ET [25].

Conversely, patients exhibiting pathway-dominant features—including specific high-impact hotspot mutations (PIK3CA H1047R, E545K), rapid disease progression kinetics, liver-predominant metastatic burden, or acceptable hyperglycemia tolerance—may derive superior benefit from upfront pathway targeting with inavolisib triplet therapy (in PIK3CA-mutated disease) or capivasertib plus fulvestrant (in broader AKT/PIK3CA/PTEN-altered populations). CAPItello-291 demonstrated particularly robust efficacy in pathway-altered subgroups (median progression-free survival 7.3 vs 3.1 months; HR 0.50) [34]. An illustrative clinical case exemplifies this framework: a patient harboring

 doi:10.20944/preprints202604.1575.v1

co-occurring ESR1 Y537S and PIK3CA E545K mutations following 18 months of palbociclib plus letrozole benefit would typically receive elacestrant initially, with anticipated time-to-next-treatment of 6-8 months, reserving inavolisib or capivasertib for subsequent progression. If rapid progression occurs on elacestrant (<4-6 months), switching to pathway inhibition addresses pathway-dominant resistance.

The clinical decision algorithm for co-altered tumor populations (Figure 1B) integrates biomarker-driven sequencing principles, prioritizing oral selective estrogen receptor degraders (elacestrant) in ESR1-mutated, endocrine-sensitive cases while directing PI3K/AKT pathway-altered tumors toward capivasertib plus endocrine therapy based on pathway dominance features. This framework synthesizes evidence from Tables 2-5, operationalizing molecular profiling results into evidence-based treatment selection.

5.2. Therapeutic Strategies for Patients Lacking Actionable Genomic Alterations in Post-CDK4/6i HR+/HER2− mBC

In patients without actionable ESR1, PI3K/AKT/PTEN, germline BRCA1/2, or other targetable alterations, the decision framework prioritizes maintaining endocrine-based therapy when feasible, particularly in those with indolent disease or low burden. Everolimus combined with fulvestrant or exemestane represents a standard option, supported by BOLERO-2 data showing improved PFS (7.8 months vs. 3.2 months with exemestane alone; HR 0.45) in endocrine-resistant settings [49], though real-world efficacy post-CDK4/6i is modest (mPFS ~4-5 months) [50]. For select patients with prolonged prior CDK4/6i benefit (>12 months), continuing CDK4/6 inhibition (e.g., abemaciclib or ribociclib) with an endocrine switch may extend control, as suggested by MAINTAIN and postMONARCH trials [51,52].

In cases of rapid progression, visceral crisis, or endocrine resistance, transition to chemotherapy (e.g., capecitabine, taxanes, or eribulin) or antibody-drug conjugates is advised. Sacituzumab govitecan offers superior OS (HR 0.79 vs. chemotherapy) in later lines, while trastuzumab deruxtecan is preferred for HER2-low tumors [53].

6. Emerging Molecularly Targeted Therapies

Vepdegestrant (ARV-471), a proteolysis-targeting chimera (PROTAC) estrogen receptor degrader, represents the first phase 3 validation of targeted ER degradation technology in solid tumors [54,55]. In the VERITAC-2 trial, patients with ESR1-mutated HR+/HER2− advanced breast cancer progressing on endocrine therapy plus CDK4/6 inhibition achieved a median progression-free survival (PFS) of 5.0 months (95% CI 3.7–7.4) with vepdegestrant compared with 2.1 months (95% CI 1.9–3.5) with fulvestrant (HR 0.57–0.58; P <0.001), confirming activity in molecularly selected disease (Table 4) [87]]. Treatment discontinuation due to adverse events occurred in 2.9%, with fatigue and nausea as the predominant toxicities [87]. Regulatory review is ongoing [56].

Imlunestrant demonstrated clinically meaningful efficacy in the phase 3 EMBER-3 trial [56]. In ESR1-mutated tumors, monotherapy achieved a median PFS of 5.5 versus 3.8 months (HR 0.62; P = 0.0007), with an overall survival signal favoring treatment (34.5 vs 23.1 months; HR 0.60; P = 0.0043) (Table 4) [57–59]. Notably, combination therapy with abemaciclib—representing continued CDK4/6 inhibition beyond prior exposure—extended median PFS to 9.4 months compared with 5.5 months for monotherapy (HR 0.57; P <0.001), demonstrating activity irrespective of ESR1 status and supporting the concept of rational CDK4/6 re-challenge in selected patients (Table 4) [57].

Giredestrant, evaluated in phase 3 persevERA [60], did not meet its primary PFS endpoint in the overall post-CDK4/6 population (Table 5) [61–63]. Although a numerical improvement was observed in the ESR1-mutated subgroup, this did not reach statistical significance. These findings underscore both the biological promise and clinical challenges of oral SERDs in heavily pretreated disease [12,64].

Gedatolisib, a highly potent multitarget inhibitor of the PI3K/AKT/mTOR (PAM) signaling pathway, has demonstrated significant clinical activity in the phase 3 VIKTORIA-1 trial [65], validating PAM pathway inhibition as a therapeutic strategy in hormone receptor–positive, HER2-

 doi:10.20944/preprints202604.1575.v1

negative advanced breast cancer. In this study, patients with disease progression after endocrine therapy plus CDK4/6 inhibition received gedatolisib with fulvestrant with or without palbociclib versus fulvestrant alone. In patients with PIK3CA-wild-type tumors, median progression-free survival (PFS) was 9.3 months with the gedatolisib triplet (HR 0.24) and 7.4 months with the gedatolisib doublet (HR 0.33;), compared with 2.0 months with fulvestrant alone, with consistent benefit regardless of the prior CDK4/6 inhibitor used, including in patients who experienced progression on first-line therapy within less than 6 months [66].

Treatment discontinuation due to treatment-related adverse events occurred in 2.3% and 3.1% of patients in the triplet and doublet arms, respectively, indicating good tolerability [66]. These results confirm the PAM pathway as a key molecular driver in PIK3CA-wild-type disease, and gedatolisib plus fulvestrant, with or without palbociclib, may represent a new standard of care after CDK4/6 inhibitor progression.

Combination strategies are increasingly being explored to enhance endocrine backbone efficacy and delay resistance. The results from phase 2 ELEVATE trial, an open-label, umbrella study [67] reported median PFS of 8.3 months with elacestrant plus everolimus and 14.3 months with elacestrant plus abemaciclib in the post-CDK4/6 setting (Table 4) [18,20]. The ongoing phase 3 ADELA trial is evaluating elacestrant plus everolimus versus elacestrant monotherapy in ESR1-mutated disease and may clarify the role of oral SERD doublet combinations [68] (Table 5). Additionally, INAVO121 is directly comparing inavolisib with alpelisib in PIK3CA-mutated tumors, addressing comparative pathway inhibition strategies [69] (Table 5). The CAPItello-292 Phase III study is evaluating Capivasertib + CDK4/6i + Fulvestrant in 1L patients with and without PIK3CA/AKT1/PTEN alterations, and Victoria-2 Phase III trial evaluates Gedatolisib+CDK4/6+fulvestrant in 1L patients with HR+/HER2- advanced breast cancer (ABC) who are endocrine therapy resistant [65].

Zovegalisib (RLY-2608) plus fulvestrant represents a next-generation advancement in PI3K pathway inhibition. The U.S. Food and Drug Administration granted Breakthrough Therapy designation in February 2026 to zovegalisib (an allosteric, pan-mutant-selective PI3Kα inhibitor) in combination with fulvestrant for adults with PIK3CA-mutated, hormone receptor-positive, HER2-negative locally advanced or metastatic breast cancer following progression on or after a CDK4/6 inhibitor [70]. This designation, supported by data from the phase 1/2 ReDiscover trial, underscores the evolving role of precision PI3Kα inhibition in endocrine-resistant HR+/HER2− disease. In heavily pretreated post-CDK4/6i patients receiving the recommended phase 3 dose (400 mg BID with food) plus fulvestrant, the regimen achieved a median progression-free survival of 11.1 months (95% CI: 7.3–13.0), with highly consistent benefit across kinase-domain and non-kinase-domain PIK3CA mutations and encouraging objective response rates (43% overall; 52% in second-line)[71]. The safety profile was notable for markedly reduced wild-type–sparing effects, resulting in predominantly low-grade, manageable hyperglycemia and limited treatment discontinuations. A global phase 3 trial (ReDiscover-2) is actively enrolling and directly compares zovegalisib plus fulvestrant versus capivasertib plus fulvestrant in this exact setting, offering the potential to further refine second-line options for the approximately 30–40% of patients with PIK3CA-mutated tumors [70,72].

Collectively, next-generation ER degraders and rational combination regimens aim to achieve deeper estrogen receptor suppression and multi-pathway blockade. Their mechanistic rationale is supported by prior evidence from EMERALD (elacestrant), CAPItello-291 (capivasertib) [73], and INAVO120 (inavolisib) [48], which demonstrated that molecularly selected strategies improve outcomes when matched to dominant resistance biology (Table 4). Together, these data reinforce the importance of comprehensive genomic profiling and dynamic resistance monitoring to guide therapy selection following CDK4/6 inhibitor progression.

Tables 4-5 illustrate this heterogeneity through systematic compilation of variable inclusion criteria, biomarker selection methodologies, and endpoint definitions across pivotal trials.

Table 4. Approved Biomarker-Directed Therapies After CDK4/6 Inhibitor Progression: Comparative Efficacy and Positioning After CDK4/6 Inhibitor Progression.

 doi:10.20944/preprints202604.1575.v1

Trial (NCT)	Phase	Molecular Selection	Intervention vs Control	Primary Endpoint	Key Results	HR (95% CI)	Regulatory Status
EMERALD (NCT03778931)	3	ESR1-mutated ER+/HER2− mBC post-ET	Elacestrant vs SOC ET	PFS (ESR1-mut)	Median PFS 3.8 vs 1.9 mo; ≥12 mo prior ET+CDK4/6i: 8.6 vs 1.9 mo	0.55 (0.39–0.77) (Exploratory)	FDA approved (Jan 2023)
INAVO120 (NCT04191499)	3	PIK3CA-mutated HR+/HER2− LA/mBC	Inavolisib + palbociclib + fulvestrant vs placebo + palbociclib + fulvestrant	PFS	Median PFS 17.2 vs 7.3 mo; OS 34.0 vs 27.0 mo; delayed chemotherapy	PFS: 0.43 (0.32–0.59); OS: 0.67 (0.49–0.91)	FDA approved (Oct 2024)
CAPItello-291 (NCT04305496)	3	HR+/HER2− advanced BC; AKT pathway-altered subgroup	Capivasertib + fulvestrant vs placebo + fulvestrant	PFS	Overall: 7.2 vs 3.6 mo; Altered: 7.3 vs 3.1 mo	Overall: 0.60 (0.51–0.71); Altered: 0.50 (0.38–0.65)	FDA approved (Nov 2023)
BOLERO-2	3	AI-resistant HR+/HER2− BC (pre-CDK4/6 era)	Everolimus + exemestane vs placebo + exemestane	PFS	7.8 vs 3.2 mo; post-CDK4/6 RWE ~5.3 mo	0.45 (0.38–0.54)	Established therapy

Table 5. Emerging ER Degraders and Rational Combination Strategies.

Trial (NCT)	Phase	Therapeutic Class	Population	Intervention	Primary Endpoint	Key Findings	Status
VERITAC-2 (NCT05654623)	3	PROTAC ER degrader	ESR1-mut HR+/HER2− post-ET + CDK4/6i	Vepdegestrant vs fulvestrant	PFS (ESR1-mut)	5.0 vs 2.1 mo; HR 0.57–0.58; low AE discontinuation	Regulatory review ongoing
EMBER-3 (NCT04975308)	3	Oral SERD ± CDK4/6i	HR+/HER2− advanced BC	Imlunestrant vs SOC; Imlunestrant + abemaciclib	PFS	ESR1-mut: 5.5 vs 3.8 mo (HR 0.62); Combo: 9.4 vs 5.5 mo (HR 0.57)	Regulatory status evolving
PERSEVERE (NCT04546009)	3	Oral SERD	HR+/HER2− post-CDK4/6i	Giredestrant vs physician's choice ET	PFS	Primary endpoint not met; numerical ESR1-mut benefit	Development strategy ongoing
ELEVATE	2	SERD-based combinations	HR+/HER2− post-CDK4/6i	Elacestrant + everolimus / abemaciclib	PFS	8.3 mo (everolimus); 14.3 mo (abemaciclib)	Proof-of-concept

 doi:10.20944/preprints202604.1575.v1

ADELA (NCT06382948)	3	SERD + mTOR inhibitor	ESR1-mut HR+/HER2−	Elacestrant + everolimus vs monotherapy	PFS	Ongoing	Recruiting
INAVO121	3	PI3K inhibitor comparison	PIK3CA-mut HR+/HER2−	Inavolisib vs alpelisib	PFS	Direct comparative efficacy study	Active enrollment
ReDiscover (NCT05216432) ReDiscover-2 (NCT06982521)	1/2 (Ph 3 ongoing)	PIK3CA-mutated HR+/HER2− mBC post-CDK4/6i	Zovegalisib + fulvestrant (Ph 3: vs capivasertib + fulvestrant)	Zovegalisib + fulvestrant (Ph 3: vs capivasertib + fulvestrant)	ReDiscover = Safety, ReDiscover-2 = PFS	Median PFS 11.1 mo (95% CI 7.3–13.0) at recommended Phase 3 dose 400 mg BID with food	Breakthrough Therapy Designation (FDA, Feb 2026); Phase 3 ongoing

7. Circulating Tumor DNA-Guided Proactive Switching for ESR1 Mutations: Evidence and Implementation

Traditional therapeutic paradigms have relied upon radiographic evidence of disease progression per RECIST criteria to trigger treatment modifications, an approach that permits continued tumor expansion and progressive accumulation of resistance mechanisms under ongoing selective pressure. Circulating tumor DNA enables minimally invasive, real-time molecular surveillance, detecting emergent resistance alterations—particularly ESR1 mutations—months prior to radiographic manifestations [16,74]. Two landmark phase 3 trials have established that proactive, ctDNA-guided therapeutic switching meaningfully extends progression-free survival and quality of life.

7.1. SERENA-6 Trial

The SERENA-6 trial (NCT04964934) prospectively enrolled patients with ER+/HER2− advanced breast cancer receiving first-line aromatase inhibitor plus CDK4/6i for at least 6 months, implementing serial ctDNA monitoring via Guardant360 CDx every 2-3 months. Among 3,256 patients screened, 315 developed emergent ESR1 mutations without radiographic progression (Of the 3,325 patients screened for inclusion, ctDNA from patient blood samples were tested for ESR1m using Guardant360CDx (Guardant Health, Redwood City, CA, US). A crude estimate of the proportion of patients with emergent ESR1m during the study period is 42%, calculated from the 548 patients with a positive test/ (the number of patients tested for ESR1m [n=3,256] minus the number of patients that were still ongoing in surveillance when screening closed [n=1,949]). These patients did not undergo further ESR1m testing and could have had ESR1m arise later on). Then, 315 patients were randomized 1:1 to switch to camizestrant (next-generation oral SERD) while continuing CDK4/6i, or maintain aromatase inhibitor plus CDK4/6i [75,76].

At median follow-up of 12.6 months, median progression-free survival was 16.0 months (95% CI 12.7-18.2) with camizestrant versus 9.2 months (95% CI 7.2-9.5) with continued aromatase inhibitor (HR 0.44; 95% CI 0.31-0.60; P < 0.0001), representing a 56% risk reduction [24]. One-year progression-free survival rates were 60.7% versus 33.4%. Benefit was consistent across CDK4/6i type, age, visceral disease status, and ESR1 variant. Median time to deterioration in global health status was 21.0 versus 6.4 months (HR 0.54; P = 0.001) [76,77]. Treatment discontinuation due to adverse events remained low (1.3% vs 1.9%). Molecular analyses demonstrated a 100% median reduction in ESR1 mutant allele frequency at 8 weeks with camizestrant versus a 66.7% increase with continued aromatase inhibitor, validating the biological rationale for early intervention [17,76]. Table 6 summarizes complete trial design, population characteristics, primary endpoints, and key efficacy outcomes for SERENA-6 and PADA-1 ctDNA-guided switching trials.

 doi:10.20944/preprints202604.1575.v1

7.2. PADA-1 Trial

The PADA-1 trial provided complementary validation through a distinct design, randomizing patients with rising ESR1 mutations (detected via droplet digital PCR every 2 months during palbociclib plus aromatase inhibitor) to switch to fulvestrant plus palbociclib or continue aromatase inhibitor plus palbociclib. Among 1,017 patients screened, 172 (16.9%) developed ESR1 mutations prior to conventional progression [17,78]. Fulvestrant switching doubled median progression-free survival: 11.9 months (95% CI 9.4-13.5) versus 5.7 months (95% CI 3.9-7.5; HR 0.61; P = 0.0040) [27]. Median time to ESR1 detection was 18 months, providing substantial lead time for intervention. Molecular characterization revealed polyclonal ESR1 mutations increased from 26.3% at initial detection to 69.2% at eventual progression, underscoring rapid clonal diversification and narrowing therapeutic windows [17].

Table 6. ctDNA-Guided Switching Trials.

Trial	Strategy	Population	Median PFS (mo)	HR	Key Finding
SERENA-6	Camizestrant + CDK4/6i vs AI + CDK4/6i	Emergent ESR1	16.0 vs 9.2	0.44	Proactive switch prolongs PFS
PADA-1	Fulvestrant + palbo vs AI + palbo	Rising ESR1	11.9 vs 5.7	0.61	Early intervention beneficial

7.3. Implementation Considerations

These trials collectively examined the serial ctDNA monitoring during first-line CDK4/6i plus endocrine therapy, with active switching upon ESR1 emergence, which extends endocrine-based treatment duration by 5-7 months. Optimal implementation requires monitoring every 2-3 months during stable disease, utilizing platforms with analytical sensitivity <0.1-0.5% variant allele fraction. Both Guardant360 CDx and targeted droplet digital PCR demonstrate clinical validity, though comprehensive next-generation sequencing panels offer additional detection of concurrent pathway alterations (PIK3CA, AKT1, PTEN) [79,80]. Turnaround times of 7-14 days are generally acceptable for asymptomatic patients. False-negative results occur more frequently in low-shedding disease (bone-only metastases), necessitating integration with conventional imaging surveillance [80]. Clinical implementation requires integration with imaging surveillance, particularly in low-shedding disease such as bone-only metastases. Cost-effectiveness modeling suggests potential economic favorability when considering prolonged progression-free survival, delayed transition to expensive therapies, and maintained quality of life, though definitive health economic evaluations across diverse healthcare systems remain needed [15]. As of February 2026, ctDNA-guided switching has not yet been incorporated into major international guidelines but is positioned to influence imminent updates given the strength of phase 3 evidence [59,81–83]. Together, these trials provide compelling evidence to reconsider current monitoring guidelines and pave the way for integrating molecular response surveillance as a standard component of care in HR+/HER2− mBC. Beyond molecularly guided switching strategies, the therapeutic landscape has further evolved through pathway-directed agents that target dominant resistance biology following CDK4/6 inhibitor progression.

8. Limitations

This narrative review must be interpreted in light of several methodological and translational limitations. First, the evidence base comprises heterogeneous clinical trials that vary considerably in design, patient selection criteria, biomarker stratification methods, control arms, and endpoint definitions. These differences preclude direct cross-trial comparisons and limit the feasibility of formal meta-analysis. Nonetheless, synthesizing this data was essential to inform real-world clinical

 doi:10.20944/preprints202604.1575.v1

decision-making in a landscape where head-to-head sequencing trials remain absent. Second, the absence of prospective randomized trials comparing post-CDK4/6i treatment sequences in molecularly defined subgroups (e.g., ESR1-mutated vs PI3K-altered tumors) necessitates reliance on indirect comparisons and expert consensus rather than Level 1 evidence. The treatment decision framework developed in this review is thus proposed as a provisional model grounded in available clinical, molecular, and real-world data.

Third, several of the key insights regarding agent efficacy, particularly for elacestrant and pathway-directed therapies, derive from real-world evidence (RWE). While RWE enhances external validity, it is subject to well-documented limitations including retrospective data collection, non-standardized biomarker testing, selection bias, and variability in outcome reporting. Fourth, long-term outcome data—especially overall survival and quality-of-life metrics—remain immature for many recently approved agents, such as, imlunestrant and inavolisib. Nevertheless, their inclusion is justified based on their regulatory approval, promising surrogate endpoints, and substantial clinical relevance in current practice. Fifth, this review was deliberately scoped to focus on oral selective estrogen receptor degraders (SERDs), PI3K/AKT/mTOR pathway inhibitors, and PROTAC degraders. Cytotoxic chemotherapy, antibody-drug conjugates, and emerging immunotherapeutic options were not included, and therefore the review does not provide exhaustive coverage of the entire post-CDK4/6i treatment landscape. Limitations include absence of direct head-to-head comparisons and evolving regulatory landscapes. Finally, cost-effectiveness data remain limited for most novel endocrine-based therapies, and disparities in access across health systems globally may constrain the implementation of biomarker-driven paradigms. Future studies should prioritize comprehensive economic evaluations and implementation science approaches to support equitable adoption. Importantly, each analysis included in this review—ranging from genomic resistance mapping and ctDNA monitoring paradigms to treatment decision algorithms and comparative trial synthesis—was selected to directly address the review's central aim: to operationalize biomarker-informed therapeutic sequencing strategies in HR+/HER2− metastatic breast cancer following CDK4/6 inhibitor progression. These analytical choices reflect clinical practice gaps and align with the translational urgency to refine treatment personalization amid increasing molecular complexity.

9. Conclusions and Future Directions

The therapeutic landscape following CDK4/6 inhibitor progression in HR+/HER2− metastatic breast cancer has evolved substantially, driven by biomarker-directed therapies and improved understanding of resistance biology. Recent trials, including SERENA-6 and PADA-1, demonstrate that serial circulating tumor DNA (ctDNA) monitoring with preemptive therapeutic modification upon emergent ESR1 mutation detection can extend progression-free survival by approximately 5–7 months and delay quality-of-life deterioration. Although these data support a shift toward earlier molecularly informed adaptation, ctDNA-guided proactive switching remains investigational and has not yet been incorporated into major international guidelines.

In routine practice, comprehensive next-generation sequencing (NGS) testing at radiologic progression has become central to therapeutic individualization. Molecular profiling identifies actionable alterations—including ESR1, PIK3CA, AKT1, PTEN, and FGFR—as well as prognostic markers such as TP53 mutations and elevated ctDNA fraction. Integrating these findings with clinical parameters, particularly duration of endocrine sensitivity and disease kinetics, enables rational sequencing decisions that move beyond empiric approaches.

Endocrine-directed therapy continues to provide meaningful benefit in selected patients. Real-world data support the durability of elacestrant in ESR1-mutated, endocrine-sensitive disease. Pathway-directed strategies have further expanded options: inavolisib has demonstrated the first overall survival advantage for a PI3K pathway inhibitor in PIK3CA-mutated disease, and capivasertib provides activity across broader AKT/PIK3CA/PTEN-altered populations. These advances underscore the importance of aligning treatment selection with dominant resistance pathways.

 doi:10.20944/preprints202604.1575.v1

Emerging evidence suggests that combination strategies may be more effective than single-agent approaches in overcoming resistance. Triplet regimens incorporating pathway inhibition, optimized endocrine partners, and continued CDK4/6 blockade exemplify this strategy. Notably, CDK4/6 inhibition beyond prior exposure has shown clinical benefit in selected patients, particularly when combined with alternative endocrine backbones, indicating that resistance to one CDK4/6-based regimen does not universally preclude subsequent benefit.

Resistance following CDK4/6 inhibitors is frequently heterogeneous and polyclonal, supporting the need for longitudinal molecular reassessment. Approximately two-thirds of resistant tumors harbor identifiable genomic drivers, and a substantial proportion exhibit concurrent pathway alterations. These findings reinforce the rationale for early molecular profiling and repeated testing at progression to detect evolving resistance mechanisms and adapt therapy accordingly. Future priorities include prospective sequencing trials within molecularly defined subgroups, validation of ctDNA-guided strategies beyond ESR1 mutations, optimization of rational combination regimens, and robust cost-effectiveness analyses to support equitable implementation. Continued development of next-generation estrogen receptor degraders, mutant-selective PI3Kα inhibitors, and CDK2-targeted strategies may further refine treatment algorithms. In summary, management after CDK4/6 inhibitor progression is transitioning toward biomarker-informed precision care. While proactive ctDNA-guided switching remains investigational, comprehensive genomic profiling at progression—and increasingly earlier in the disease course—has become integral to personalized treatment selection. Integrating serial molecular assessment with rational combination strategies offers the opportunity to extend endocrine-based disease control, delay chemotherapy, and improve patient outcomes in HR+/HER2− metastatic breast cancer.

Author Contributions: Conceptualization: HOA, NA; Literature review and data curation: All authors; Manuscript drafting: HOA, NA; Critical revision: All authors approved the final version of this article.

Funding: The APC was funded by Burjeel Cancer Institute, Burjeel Medical City, Abu Dhabi, United Arab Emirates.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: The authors acknowledge the patients and families affected by metastatic breast cancer whose participation in clinical trials and real-world data registries has enabled the therapeutic advances synthesized in this review.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ADC	**antibody–drug conjugate**
AE	adverse event
AI	aromatase inhibitor
AKT	protein kinase B
ASCO	American Society of Clinical Oncology
AURKA	aurora kinase A
BC	breast cancer
CDK	cyclin-dependent kinase

 doi:10.20944/preprints202604.1575.v1

CDK2	cyclin-dependent kinase 2
CDK4/6	cyclin-dependent kinase 4 and 6
CDK4/6i	cyclin-dependent kinase 4/6 inhibitor
CCNE	cyclin E
CI	confidence interval
CNS	central nervous system
CT	chemotherapy
ctDNA	circulating tumor DNA
DCR	disease control rate
DNA	deoxyribonucleic acid
D/C	discontinuation
ddPCR	droplet digital polymerase chain reaction
ER	estrogen receptor
ERBB2,	Erb-B2 receptor tyrosine kinase 2
ESMO	European Society for Medical Oncology
ESR1	estrogen receptor 1 gene
ET	endocrine therapy
FDA	Food and Drug Administration
FGFR	fibroblast growth factor receptor
HR	hazard ratio
HR+	hormone receptor–positive
HER2	human epidermal growth factor receptor 2
HER2−	human epidermal growth factor receptor 2–negative
ITT	intention-to-treat
mBC	metastatic breast cancer
mPFS	median progression-free survival
mTOR	mechanistic target of rapamycin
NCCN	National Comprehensive Cancer Network
NCT	National Clinical Trial identifier
NDA	new drug application
NGS	next-generation sequencing
OS	overall survival
PD	progressive disease
PDUFA	Prescription Drug User Fee Act
PFS	progression-free survival
PI3K	phosphoinositide 3-kinase

 doi:10.20944/preprints202604.1575.v1

PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PRO	patient-reported outcome
PROTAC	proteolysis-targeting chimera
QoL	quality of life
RAS	rat sarcoma viral oncogene
RB1	retinoblastoma 1 gene
RECIST	Response Evaluation Criteria in Solid Tumors
RWE	real-world evidence
SERD	selective estrogen receptor degrader
SOC	standard of care
TP53	tumor protein 53
TTNT	time to next treatment
VAF	variant allele frequency

References

1. Siegel RL, Giaquinto AN, Jemal A: Cancer statistics, 2024. CA Cancer J Clin 2024, 74(1):12-49.
2. Harbeck N, Brufsky A, Rose CG, Korytowsky B, Chen C, Tantakoun K, Jazexhi E, Nguyen DHV, Bartlett M, Samjoo IA et al.: Real-world effectiveness of CDK4/6i in first-line treatment of HR+/HER2- advanced/metastatic breast cancer: updated systematic review. Front Oncol 2025, 15:1530391.
3. Goyal RK, Nagar SP, Kabir ER, al. e: Progression-free survival and overall survival with CDK 4/6 inhibitors combination therapy versus endocrine monotherapy in hormone receptor-positive, HER2-negative metastatic breast cancer: a systematic literature review and meta-analysis. Breast Cancer 2023, 30(3):387-402.
4. Piezzo M, Cocco S, Caputo R, al. e: Targeting cell cycle in breast cancer: CDK4/6 inhibitors. International Journal of Molecular Sciences 2020, 21(18):6479.
5. Wander SA, Cohen O, Gong X, Johnson GN, Buendia-Buendia JE, Lloyd MR, Kim D, Luo F, Mao P, Helvie K et al.: The Genomic Landscape of Intrinsic and Acquired Resistance to Cyclin-Dependent Kinase 4/6 Inhibitors in Patients with Hormone Receptor-Positive Metastatic Breast Cancer. Cancer Discov 2020, 10(8):1174-1193.
6. da Silva JL, Oliveira LJC, de Resende CAA, Reinert T, de Albuquerque LZ, Leite da Silva LF, Mano MS: Understanding and overcoming CDK4/6 inhibitor resistance in HR+/HER2- metastatic breast cancer: clinical and molecular perspectives. Ther Adv Med Oncol 2025, 17:17588359251353623.
7. Spoerke JM, Gendreau S, Walter K, al. e: Heterogeneity and clinical significance of ESR1 mutations in ER-positive metastatic breast cancer patients receiving fulvestrant. Nature Communications 2016, 7:11579.
8. André F, Ciruelos E, Rubovszky G, al. e: Alpelisib for PIK3CA-mutated, hormone receptor-positive advanced breast cancer. New England Journal of Medicine 2019, 380(20):1929-1940.
9. O'Leary B, Cutts RJ, Liu Y, al. e: The genetic landscape and clonal evolution of breast cancer resistance to palbociclib plus fulvestrant in the PALOMA-3 trial. Cancer Discovery 2018, 8(11):1390-1403.
10. Razavi P, Chang MT, Xu G, al. e: The genomic landscape of endocrine-resistant advanced breast cancers. Cancer Cell 2018, 34(3):427-438.
11. Wander SA, Cohen O, Gong X, al. e: The genomic landscape of intrinsic and acquired resistance to cyclin-dependent kinase 4/6 inhibitors in patients with hormone receptor-positive metastatic breast cancer. Cancer Discovery 2020, 10(8):1174-1193.

 doi:10.20944/preprints202604.1575.v1

12. Apostolidou K, Zografos E, Papatheodoridi MA, Fiste O, Dimopoulos MA, Zagouri F: Oral SERDs alone or in combination with CDK 4/6 inhibitors in breast cancer: Current perspectives and clinical trials. Breast 2024, 75:103729.
13. Formisano L, Lu Y, Servetto A, al. e: Aberrant FGFR signaling mediates resistance to CDK4/6 inhibitors in ER+ breast cancer. Nature Communications 2019, 10(1):1373.
14. Li Z, Zhang Y, Zhang Z, al. e: The role of CDK4/6 inhibitors in breast cancer treatment: a systematic review and meta-analysis. Oncology Letters 2020, 19(6):3555-3566.
15. Lynce F, Shajahan-Haq AN, Swain SM: CDK4/6 inhibitors in breast cancer therapy: current practice and future opportunities.
16. Turner NC, Oliveira M, Howell SJ, al. e: Camizestrant + CDK4/6 inhibitor for the treatment of emergent ESR1 mutations during first-line endocrine-based therapy ahead of disease progression in patients with HR+/HER2− advanced breast cancer: phase 3 SERENA-6 trial. Journal of Clinical Oncology 2025, 43(17 suppl):LBA4.
17. Chandarlapaty S, Chen D, He W, al. e: Prevalence of ESR1 mutations in cell-free DNA and outcomes in metastatic breast cancer: a secondary analysis of the BOLERO-2 clinical trial. JAMA Oncology 2016, 2(10):1310-1315.
18. Rugo HS, Lerebours F, Ciruelos E, al. e: Elacestrant in ER-positive, HER2-negative metastatic breast cancer with ESR1-mutated tumors: subgroup analyses from the EMERALD trial by prior duration of CDK4/6i and other clinical and biomarker factors. Clinical Cancer Research 2024, 30(19):4299-4309.
19. Baethge C, Goldbeck-Wood S, Mertens S: SANRA-a scale for the quality assessment of narrative review articles. Res Integr Peer Rev 2019, 4:5.
20. Rugo HS, Di Palma JA, Cortes J, al. e: Real-world outcomes of elacestrant in patients with estrogen receptor-positive, HER2-negative, ESR1-mutant metastatic breast cancer. Clinical Cancer Research 2026, 32(1):179-187.
21. Waks AG, Winer EP: Breast cancer treatment: a review. JAMA 2019, 321(3):288-300.
22. André F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, Iwata H, Conte P, Mayer IA, Kaufman B et al.: Alpelisib for PIK3CA-Mutated, Hormone Receptor–Positive Advanced Breast Cancer. New England Journal of Medicine 2019, 380(20):1929-1940.
23. Rugo HS, Lerebours F, Ciruelos E, Drullinsky P, Ruiz-Borrego M, Neven P, Park YH, Prat A, Bachelot T, Juric D et al.: Alpelisib plus fulvestrant in PIK3CA-mutated, hormone receptor-positive advanced breast cancer after a CDK4/6 inhibitor (BYLieve): one cohort of a phase 2, multicentre, open-label, non-comparative study. The Lancet Oncology 2024, 25(12):e629-e638.
24. Rugo HS, et al. Alpelisib plus fulvestrant for PIK3CA-mutated, HR+, HER2− advanced breast cancer after progression on CDK4/6 inhibitor: primary results from EPIK-B5. Abstract RF7-02. Clin Cancer Res. 2026;32(4_Suppl):RF7-02. (EPIK-B5 trial).
25. Turner NC, Oliveira M, Howell SJ, et al. Capivasertib plus fulvestrant in hormone receptor-positive (HR+) advanced breast cancer (ABC): exploratory ctDNA analyses from the Phase 3 CAPItello-291 trial. Abstract RF7-05. Presented at: San Antonio Breast Cancer Symposium; December 9-12, 2025; San Antonio, TX.
26. Alaklabi A, et al. Real world outcomes with alpelisib in metastatic hormone receptor positive breast cancer: a single institution experience. Front Oncol. 2022;12:1012391. doi:10.3389/fonc.2022.1012391.
27. Sultanbaev AK, Kolyadina IV, et al. Results of a retrospective study on the efficacy and safety of alpelisib in patients with HR+/HER2− metastatic breast cancer in real-world clinical practice. J Clin Oncol. 2024;42(16_suppl):e13058.
28. Bardia A, Aftimos P, Bihani T, al. e: EMERALD: phase III trial of elacestrant (RAD1901) vs endocrine therapy for previously treated ER+ advanced breast cancer. Future Oncology 2019, 15(28):3209-3218.
29. Bidard FC, Kaklamani VG, Neven P, al. e: Elacestrant versus standard endocrine therapy for estrogen receptor-positive, human epidermal growth factor receptor 2-negative advanced breast cancer: results from the randomized phase III EMERALD trial. Journal of Clinical Oncology 2022, 40(26):3246-3256.
30. Juric D, Ciruelos Garcia EM, Loibl S, al. e: Inavolisib plus palbociclib and fulvestrant in PIK3CA-mutated, hormone receptor-positive, HER2-negative locally advanced or metastatic breast cancer: updated efficacy and safety results from INAVO120. Annals of Oncology 2024, 35(suppl 2):S339.

 doi:10.20944/preprints202604.1575.v1

31. Juric D, Kalinsky K, Turner NC, al. e: Inavolisib or placebo in combination with palbociclib and fulvestrant in patients with PIK3CA-mutated, hormone receptor-positive, HER2-negative locally advanced or metastatic breast cancer: primary results from the phase 3 INAVO120 trial. Journal of Clinical Oncology 2024, 42(suppl 17):LBA1000.
32. Turner NC, et al. INAVO120: Phase III trial final overall survival (OS) analysis of first-line inavolisib/placebo + palbociclib + fulvestrant in patients with PIK3CA-mutated, HR+, HER2– endocrine-resistant advanced breast cancer. J Clin Oncol 2025;43(16_suppl):1003 (ASCO 2025 abstract).
33. Turner NC, Oliveira M, Howell SJ, al. e: Capivasertib in hormone receptor-positive advanced breast cancer. New England Journal of Medicine 2023, 388(22):2058-2070.
34. Oliveira M, Pominchuk D, Nowecki Z, al. e: Capivasertib and fulvestrant for patients with aromatase inhibitor-resistant hormone receptor-positive, HER2-negative advanced breast cancer (CAPItello-291): overall survival results from a randomized, double-blind, placebo-controlled, phase 3 trial. Lancet Oncology 2024, 25(11):1461-1471.
35. Schmid P, Zaiss M, Harper-Wynne C, al. e: Capivasertib plus paclitaxel versus placebo plus paclitaxel as first-line therapy for metastatic triple-negative breast cancer: the phase 3 CAPItello-290 trial. Nature Medicine 2024, 30(5):1408-1416.
36. Jerusalem G, Rorive A, Collignon J: Chemotherapy options for patients with triple-negative breast cancer in the post-anthracycline and taxane era: a systematic review. Current Oncology Reports 2016, 18(4):24.
37. Natsuhara KH, Park L, Udayachalerm S, Mireia R, Murphy B, Nordstrom B, Huppert LA: Abstract PS5-05-23: Real-world Treatment Patterns of Capivasertib in Metastatic Breast Cancer in the US. Clinical Cancer Research 2026, 32(4_Supplement):PS5-05-23-PS05-05-23.
38. Yardley DA, Noguchi S, Pritchard KI, al. e: Everolimus plus exemestane in postmenopausal patients with HR+ breast cancer: BOLERO-2 final progression-free survival analysis. Advances in Therapy 2013, 30(10):870-884.
39. Lee JS, Yost SE, Li SM, al. e: Real-world clinical outcomes of patients with metastatic breast cancer treated with everolimus plus exemestane after progression on a cyclin-dependent kinase 4/6 inhibitor. Clinical Breast Cancer 2021, 21(6):e675-e682.
40. Bhave MA, Quintanilha JCF, Tukachinsky H, Li G, Scott T, Ross JS, Pasquina L, Huang RSP, McArthur H, Levy MA et al.: Comprehensive genomic profiling of ESR1, PIK3CA, AKT1, and PTEN in HR(+)HER2(-) metastatic breast cancer: prevalence along treatment course and predictive value for endocrine therapy resistance in real-world practice. Breast Cancer Res Treat 2024, 207(3):599-609.
41. Mosele F, Remon J, Mateo J, al. e: Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. Annals of Oncology 2020, 31(11):1491-1505.
42. Nayar U, Cohen O, Kapstad C, al. e: Acquired HER2 mutations in ER+ metastatic breast cancer confer resistance to estrogen receptor-directed therapies. Nature Genetics 2019, 51(2):207-216.
43. Li Z, Razavi P, Li Q, al. e: Loss of the FAT1 tumor suppressor promotes resistance to CDK4/6 inhibitors via the Hippo pathway. Cancer Cell 2018, 34(6):893-905.
44. Turner NC, Liu Y, Zhu Z, al. e: Cyclin E1 expression and palbociclib efficacy in previously treated hormone receptor-positive metastatic breast cancer. Journal of Clinical Oncology 2019, 37(14):1169-1178.
45. Hanker AB, Sudhan DR, Arteaga CL: Overcoming endocrine resistance in breast cancer. Cancer Cell 2020, 37(4):496-513.
46. A Study Evaluating the Efficacy and Safety of Giredestrant Combined With Palbociclib Compared With Letrozole Combined With Palbociclib in Participants With Estrogen Receptor-Positive, HER2-Negative Locally Advanced or Metastatic Breast Cancer (persevERA Breast Cancer). ClinicalTrials.gov Identifier: NCT04546009. Hoffmann-La Roche; 2020 [updated February 20, 2026; accessed March 3, 2026].
47. Jacobson A. Alpelisib Plus Fulvestrant or Letrozole Demonstrates Sustained Benefits Across Subgroups of Patients with PIK3CA-Mutated HR+/HER2- Advanced Breast Cancer. Oncologist. 2022 Mar 28;27(Suppl 1):S13-S14. doi: 10.1093/oncolo/oyac011. PMID: 35348782; PMCID: PMC8963159.

 doi:10.20944/preprints202604.1575.v1

48. Rugo HS, Lerebours F, Ciruelos E, al. e: Alpelisib plus fulvestrant in PIK3CA-mutated, hormone receptor-positive advanced breast cancer after a CDK4/6 inhibitor (BYLieve): one cohort of a phase 2, multicentre, open-label, non-comparative study. Lancet Oncology 2021, 22(4):489-498.
49. Piccart M, Hortobagyi GN, Campone M, Pritchard KI, Lebrun F, Ito Y, Noguchi S, Perez A, Rugo HS, Deleu I et al.: Everolimus plus exemestane for hormone-receptor-positive, human epidermal growth factor receptor-2-negative advanced breast cancer: overall survival results from BOLERO-2†. Ann Oncol 2014, 25(12):2357-2362.
50. Mo H, Renna CE, Moore HCF, Abraham J, Kruse ML, Montero AJ, LeGrand SB, Wang L, Budd GT: Real-World Outcomes of Everolimus and Exemestane for the Treatment of Metastatic Hormone Receptor-Positive Breast Cancer in Patients Previously Treated With CDK4/6 Inhibitors. Clin Breast Cancer 2022, 22(2):143-148.
51. Kalinsky K, Accordino MK, Chiuzan C, Mundi PS, Sakach E, Sathe C, Ahn H, Trivedi MS, Novik Y, Tiersten A et al.: Randomized Phase II Trial of Endocrine Therapy With or Without Ribociclib After Progression on Cyclin-Dependent Kinase 4/6 Inhibition in Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Metastatic Breast Cancer: MAINTAIN Trial. J Clin Oncol 2023, 41(24):4004-4013.
52. Kalinsky K, Bianchini G, Hamilton E, Graff SL, Park KH, Jeselsohn R, Demirci U, Martin M, Layman RM, Hurvitz SA et al.: Abemaciclib Plus Fulvestrant in Advanced Breast Cancer After Progression on CDK4/6 Inhibition: Results From the Phase III postMONARCH Trial. J Clin Oncol 2025, 43(9):1101-1112.
53. Singareeka Raghavendra A, Damodaran S, Barcenas CH, Fuqua SA, Layman RM, Tripathy D: Personalizing therapies over the course of hormone receptor-positive/HER2-negative metastatic breast cancer. CA: A Cancer Journal for Clinicians 2026, 76(1):e70055.
54. Cutts RJ, Ijaz H, Huang X, al. e: Patterns of genomic instability and clonal evolution in breast cancer from AURORA, the Breast International Group Molecular Screening Initiative. Journal of Clinical Oncology 2023, 41(16 suppl):1009.
55. Damodaran S, Plourde PV, Moore HCF, al. e: Vepdegestrant versus fulvestrant in patients with oestrogen receptor-positive, HER2-negative, locally advanced or metastatic breast cancer (VERITAC-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncology 2024, 25(11):1441-1454.
56. Hamilton EP, Schott AF, Nanda R, al. e: ARV-471, a PROTAC estrogen receptor degrader, combined with palbociclib in advanced ER+/HER2− breast cancer: phase 1b cohort of a phase 1/2 study. Journal of Clinical Oncology 2024, 42(16 suppl):1050.
57. Food U, Administration D: FDA grants accelerated approval to erdafitinib for metastatic urothelial carcinoma. FDA https://www fda gov/drugs/resources-information-approved-drugs/fda-grants-accelerated-approval-erdafitinib-metastatic-urothelial-carcinoma (updated 12 Apr 2019) 2019.
58. Jhaveri K, Shapiro GI, Krop IE, al. e: **The oral selective estrogen receptor degrader imlunestrant versus investigator choice of endocrine monotherapy in patients with ER+, HER2− advanced breast cancer following progression on endocrine therapy: primary results from EMBER-3**. Journal of Clinical Oncology 2024, **42**(16 suppl):LBA1000.
59. Jhaveri KL, Neven P, Casalnuovo ML, Kim S-B, Tokunaga E, Aftimos P, Saura C, O'Shaughnessy J, Harbeck N, Carey LA et al.: **Imlunestrant with or without Abemaciclib in Advanced Breast Cancer**. New England Journal of Medicine 2025, **392**(12):1189-1202.
60. **A Study Evaluating the Efficacy and Safety of Giredestrant Combined With Palbociclib Compared With Letrozole Combined With Palbociclib in Participants With Estrogen Receptor-Positive, HER2-Negative Locally Advanced or Metastatic Breast Cancer (persevERA Breast Cancer). ClinicalTrials.gov identifier: NCT04546009. Updated February 20, 2026.**
61. Basu S, Rosas Diaz AN, Narvaez-Paliza JM, Curtin C, Kurella S, Urias M, More Verde L, Patel JM, Asnani A: **Selective Estrogen Receptor Degraders Induce Bradycardia by Modulating Nuclear Estrogen Signaling**. JACC Basic Transl Sci 2026, **11**(2):101454.
62. Brooks L, Dolton M, Langenhorst J, Yoshida K, Lien YTK, Malhi V, Li C, Perez-Moreno P, Bond J, Chen YC et al.: **Concentration QTc analysis of giredestrant: Overcoming QT/heart rate confounding in the presence of drug-induced heart rate changes**. Clin Transl Sci 2023, **16**(5):823-834.

 doi:10.20944/preprints202604.1575.v1

63. NCT04546009: **A Phase III Randomized, Double-Blind, Placebo-Controlled, Multicenter Study Evaluating the Efficacy and Safety of GDC-9545 Combined With Palbociclib Compared With Letrozole Combined With Palbociclib in Patients With Estrogen Receptor-Positive, HER2-Negative Locally Advanced or Metastatic Breast Cancer**. In.; 2020.
64. **Turner NC, et al. persevERA Breast Cancer (BC): Phase III study evaluating the efficacy and safety of giredestrant (GDC-9545) + palbociclib compared with letrozole + palbociclib for patients with ER-positive, HER2-negative locally advanced or metastatic BC. J Clin Oncol. 2021;39(15_suppl):TPS1103. doi:10.1200/JCO.2021.39.15_suppl.TPS1103**.
65. Hurvitz S, Pistilli B, Kaklamani V, Layman RM, Cortés J, Park KH, Cristofanilli M, Sullivan B, Moser EC, Gorbatchevsky I et al.: **Abstract CT258: A randomized, open-label, phase 3 study of fulvestrant and CDK4/6 inhibitors with or without gedatolisib as first-line treatment in patients with HR+/HER2- advanced breast cancer (VIKTORIA-2)**. Cancer Research 2025, **85**(8_Supplement_2):CT258-CT258.
66. Hurvitz SA, Layman RM, Curigliano G, André F, Cristofanilli M, Kim SB, Rodriguez JLM, Nadal JC, Kim GM, Emile G et al.: **LBA17 Gedatolisib (geda) + fulvestrant ± palbociclib (palbo) vs fulvestrant in patients (pts) with HR+/ HER2-/PIK3CA wild-type (WT) advanced breast cancer (ABC): First results from VIKTORIA-1**. Annals of Oncology 2025, **36**:S1562-S1563.
67. Rugo H, Tolaney SM, Chan N, Borges G, Yerushalmi R, Sharifi M, McHayleh W, Beck T, Vidula N, Hamilton E et al.: **Abstract RF7-01: Elacestrant in combination with everolimus or abemaciclib in patients with ER+/HER2- locally advanced or metastatic breast cancer (mBC): phase 2 results from ELEVATE, an open-label, umbrella study**. Clinical Cancer Research 2026, **32**(4_Supplement):RF7-01-RF07-01.
68. **ClinicalTrials.gov ID NCT06382948 details a Phase 3 study on Elacestrant plus Everolimus for ER+/HER2- advanced breast cancer, active but not recruiting as of December 2025.**
69. Juric D, Kalinsky K, Im S-A, Ciruelos E, Bianchini G, Barrios CH, Jacot W, Schmid P, Loi S, Rugo HS et al.: **INAVO121: Phase III study of inavolisib (INAVO) + fulvestrant (FUL) vs. alpelisib (ALP) + FUL in patients (pts) with hormone receptor-positive, HER2-negative (HR+, HER2–), PIK3CA-mutated (mut) locally advanced or metastatic breast cancer (LA/mBC)**. Journal of Clinical Oncology 2024, **42**(16_suppl):TPS1136-TPS1136.
70. **Relay Therapeutics. Zovegalisib granted Breakthrough Therapy designation by the FDA for PIK3CA-mutated HR-positive, HER2-negative advanced breast cancer [Internet]. 2026 Feb 3. Available from: https://ir.relaytx.com/news-releases/news-release-details/relay-therapeutics-announces-zovegalisib-granted-breakthrough**.
71. **Varkaris A, Italiano A, Sammons S, Felip Falgas E, Curigliano G, et al. Dose optimization of zovegalisib, a novel PI3Kα inhibitor, in patients (pts) with PIK3CA-mutant (m) HR+/HER2– advanced breast cancer (BC): Results from the first-in-human (FIH) study to support the recommended phase III dose [abstract 90O]. ESMO Open. 2026;11(Suppl 2):106232. doi:10.1016/j.esmoop.2026.106232. Available from: https://oncologypro.esmo.org/congress-resources/esmo-targeted-anticancer-therapies-congress-2026?presentation=dose_optimization_of_zovegalisib__a_novel_pi3ka_in**.
72. **ClinicalTrials.gov . Bethesda (MD): National Library of Medicine (US). Identifier NCT06982521, A Phase 3 Open-Label Randomized Study Assessing the Efficacy and Safety of RLY-2608 + Fulvestrant Versus Capivasertib + Fulvestrant as Treatment for PIK3CA-mutant Hormone Receptor Positive, Human Epidermal Growth Factor Receptor 2 Negative (HR+/HER2-) Locally Advanced or Metastatic Breast Cancer Following Recurrence or Progression On or After Treatment With a CDK4/6 Inhibitor; 2025 May 21 [updated 2026 Apr 2; cited 2026 Apr 19]. Available from: https://clinicaltrials.gov/study/NCT06982521**.
73. Lloyd MR, Wander SA, Hamilton E, Razavi P, Bardia A: **Next-generation selective estrogen receptor degraders and other novel endocrine therapies for management of metastatic hormone receptor-positive breast cancer: current and emerging role**. Ther Adv Med Oncol 2022, **14**:17588359221113694.
74. Turner NC, Oliveira M, Howell SJ, al. e: **Camizestrant, a next-generation oral SERD, versus continued aromatase inhibitor, for treatment of ER-positive, HER2-negative, advanced breast cancer patients with acquired ESR1 mutation on first-line aromatase inhibitor-based therapy ahead of disease progression:**

 doi:10.20944/preprints202604.1575.v1

primary results from the randomised, phase 3 SERENA-6 study. New England Journal of Medicine 2025, **393**(6):569-580.

75. Bidard FC, Callens C, Dalenc F, al. e: **Circulating tumor DNA analysis in the randomized PADA-1 trial: clinical implications for hormone receptor-positive metastatic breast cancer**. Cancer Discovery 2023, **13**(10):2128-2143.
76. Lux MP, Becnel WE, Loibl S, al. e: **Health-related quality of life results from SERENA-6**. Annals of Oncology 2025, **36**(suppl 4):S1234.
77. Bertrand F, Rochigneux P, Vicier C, al. e: **Circulating tumour DNA-guided switch from aromatase inhibitor plus palbociclib to fulvestrant plus palbociclib in women with oestrogen receptor-positive, HER2-negative advanced breast cancer developing an ESR1 mutation (PADA-1): a randomised, open-label, phase 3 trial**. Lancet Oncology 2022, **23**(11):1367-1377.
78. Bidard F, Hardy-Bessard A, Dalenc F et al. Switch to fulvestrant and palbociclib versus no switch in advanced breast cancer with rising ESR1 mutation during aromatase inhibitor and palbociclib therapy (PADA-1): a randomised, open-label, multicentre, phase 3 trial. The Lancet Oncology, 2022; 23, 1367-1377
79. Keup C, Storbeck M, Hauch S, al. e: **Multimodal targeted deep sequencing of circulating tumor DNA in breast cancer patients**. Genome Medicine 2021, **13**(1):4.
80. Tolaney SM, Garrett-Mayer E, White J, al. e: **Updated standardized definitions for efficacy end points in adjuvant breast cancer clinical trials: STEEP version 2.0**. Journal of Clinical Oncology 2021, **39**(24):2720-2731.
81. Collin SM, Hillman S, Shah CH, Tank R, Bhave M, Traina TA: **Real-World Treatment Patterns and Outcomes for Patients With Metastatic Triple-Negative Breast Cancer in the United States: An Observational Study**. JCO Oncology Practice 2025:OP-25-00822.
82. Gennari A, André F, Barrios CH, al. e: **ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer**. Annals of Oncology 2021, **32**(12):1475-1495.
83. Kamli H, Khan NU: **Emerging Role of ctDNA Fragmentomics and Epigenetic Signatures in the Early Detection, Minimal Residual Disease Assessment, and Precision Monitoring of Renal Cell Carcinoma**. Journal of Cellular and Molecular Medicine 2026, **30**(3):e71019.
84. Turner S, Chia S, Kanakamedala H, Hsu WC, Park J, Chandiwana D, Ridolfi A, Yu CL, Zarate JP, Rugo HS. Effectiveness of Alpelisib + Fulvestrant Compared with Real-World Standard Treatment Among Patients with HR+, HER2-, PIK3CA-Mutated Breast Cancer. Oncologist. 2021 Jul;26(7):e1133-e1142. doi: 10.1002/onco.13804. Epub 2021 May 13. PMID: 33909934; PMCID: PMC8265362.
85. Nicholas C. Turner et al. INAVO120: Phase III trial final overall survival (OS) analysis of first-line inavolisib (INAVO)/placebo (PBO) + palbociclib (PALBO) + fulvestrant (FULV) in patients (pts) with PIK3CA-mutated, hormone receptor-positive (HR+), HER2-negative (HER2–), endocrine-resistant advanced breast cancer (aBC).. J Clin Oncol 43, 1003-1003(2025). DOI:10.1200/JCO.2025.43.16_suppl.1003
86. Elisa Agostinetto et al. Clinico-molecular characteristics associated with outcomes in breast cancer patients treated with CDK4/6 inhibitors: Results from the AURORA Molecular Screening Initiative.. J Clin Oncol 41, 1019-1019(2023). DOI:10.1200/JCO.2023.41.16_suppl.1019
87. Hamilton EP, et al. Vepdegestrant versus fulvestrant in ER-positive/HER2-negative advanced breast cancer: Results of the global, randomized, phase 3 VERITAC-2 study. J Clin Oncol 2025;43(17_suppl):LBA1000.