The Breast 79 (2025) 103859

Contents lists available at ScienceDirect

The Breast

journal homepage: www.journals.elsevier.com/the-breast

Beyond Hormone Receptors: liquid biopsy tools to unveil new clinical meanings and empower therapeutic decision-making in Luminal-like metastatic breast cancer

Brenno Pastò [a,b,1], Riccardo Vida [a,b,1], Arianna Dri [a,b], Lorenzo Foffano [a,b], Serena Della Rossa [a,b], Lorenzo Gerratana [a,b,*], Fabio Puglisi [a,b]

[a] Department of Medicine (DMED), University of Udine, 33100, Udine, Italy
[b] Department of Medical Oncology, Centro di Riferimento Oncologico di Aviano (CRO), IRCCS, 33081, Aviano, Italy

ARTICLE INFO

Keywords:
Metastatic breast cancer
Hormone receptors
Immunohistochemistry
Liquid biopsy
Biomarkers
Endocrine resistance
Therapeutic decision-making

ABSTRACT

Immunohistochemical (IHC) tissue profiling is a standard practice in the management of metastatic breast cancer (mBC), that enables the identification of distinct biological phenotypes based on hormone receptors' expression. Luminal-like tumors primarily benefit from a first line treatment strategy combining endocrine therapy and cyclin-dependent kinase 4/6 inhibitors. However, IHC analyses necessitate invasive procedures and may encounter technical and interpretational challenges. In the current era of precision medicine, liquid biopsy holds potential to provide clinicians with additional insights into disease biology, including mechanisms underlying endocrine resistance and disease progression. Several liquid-based biomarkers are entering clinical practice and hold prognostic and predictive values in Luminal-like mBC, while many others are currently being investigated. The present work aims to summarize the current evidence regarding the clinical meanings of hormone receptors and their downstream molecular pathways, alongside their implications for therapeutic decision-making in Luminal-like mBC.

1. Introduction: current practice and limitations of hormone receptors' determination

Immunohistochemical (IHC) tissue profiling is routinely used in metastatic breast cancer (mBC) to define distinct biological subtypes based on the expression of hormone receptors (HRs), including estrogen (ER) and progesterone (PR) receptors, and the human epidermal growth factor receptor 2 (HER2) [1]. According to the American Society of Clinical Oncology and College of American Pathologists (ASCO/CAP) guidelines, a tumor sample is categorized as ER-negative if $< 1\%$ of cell nuclei exhibit immunoreactivity, while ER positivity is defined by staining in 1–100% of cells [2].

However, technical limitations and interpretation issues may hinder the application and interpretation of this approach. First, IHC is performed on tissue samples and results strongly depend on their origin and quality. In addition, the obtained profiles may not be representative of the overall tumor burden, due to intratumor heterogeneity [3]. Furthermore, multiple ER isoforms exist, but commonly used sample staining may not effectively detect all of them, with potential false negatives [4,5]. Lastly, from a biological perspective, IHC profiling should be considered a surrogate for a more complex molecular landscape, which strongly impacts on both prognosis and response to treatment; besides, discordances between IHC and gene expression analyses have been reported [6–9].

Liquid biopsy is a feasible option to overcome these issues: not only the detection of specific alterations can influence therapeutic decision-making, but also circulating tumor DNA (ctDNA)-based genomic signatures can identify complex biological features, such as tumor proliferation and ER signaling, opening new opportunities for the discovery of additional genomic predictors [10–12].

This review will summarize the current evidence regarding the clinical role of HRs beyond their classical IHC determination, focusing on how liquid biopsy tools could uncover hidden clinical features of the downstream molecular pathways, as well as their possible impact on

* Corresponding author. Department of Medical Oncology, Centro di Riferimento Oncologico di Aviano (CRO), IRCCS, Via Franco Gallini 2, 33081, Aviano (PN), Italy.
E-mail address: lorenzo.gerratana@cro.it (L. Gerratana).
[1] These authors contributed equally.

https://doi.org/10.1016/j.breast.2024.103859
Received 7 September 2024; Received in revised form 29 October 2024; Accepted 11 December 2024
Available online 12 December 2024

therapeutic decision-making in Luminal-like mBC.

2. Molecular landscape of hormone receptors

2.1. Estrogen receptor downstream pathways and crosstalk

The natural history of Luminal-like mBC intimately depends on ER signaling, which is triggered by the binding of estradiol (E2) to specific receptors expressed on mammary epithelial cells. The ER family is composed of a G protein-coupled receptor (GPER1) and two isoforms of nuclear ER. ERα is the main responsible of carcinogenesis and is encoded by the *ESR1* gene located within chromosome 6, while ERβ is encoded by the *ESR2* gene on chromosome 14 and negatively controls cell proliferation [13–16] (Fig. 1).

ERα, initially located in the cytoplasm, binds to E2 and the resulting complex translocates into the nucleus and binds to specific DNA sequences, namely estrogen response elements (EREs), which promote the transcription of genes involved in cell proliferation, mitogenic signaling and survival. ERα can also interact with genes lacking EREs via alternative transcription factors in the cell nucleus [13,14,17]. Concomitantly, the E2-ERα complex exerts a "non-genomic" effect, by activating cytosolic kinase cascades, mainly the Ras-Raf-mitogen-activated protein kinase kinase (MEK)-mitogen-activated protein kinase (MAPK) and the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathways. GPER1 can also control these signaling cascades, as well as downregulating pro-apoptotic gene sets [13,14,18,19] (Fig. 1).

Several molecules play a key role in ER signaling, serving as coactivators (e.g., AIB1, BCAS3, CIZ1, DBC1, MTA1, PELP1, SRCA1/2/3) or corepressors (e.g., NCOR1, BRCA1, DACH1) of ER-mediated gene expression via different mechanisms. In fact, they can control chromatin remodeling, modulate ER binding to cytosolic kinases or retain it within the cytoplasm [13,20]. Transcription factors may also contribute to regulate ER signaling, with a predominant role played by Forkhead box protein A1 (FOXA1) [21]. Moreover, functional crosstalk between ER and other steroid HRs (e.g., PR, androgen receptor [AR]) and tyrosine kinase receptors like HER2 exist, whose interactions may crucially influence cellular processes [22,23].

2.2. Molecular mechanisms of endocrine resistance

ER signaling represents a composite system in which all involved molecular factors potentially contribute to the development of endocrine therapy (ET) resistance. While the clinical implications of certain molecular alterations are well-defined, others remain to be elucidated.

ESR1 point mutations are among the most common mechanisms of ET resistance [24]. These activating alterations typically affect the ligand-binding domain region of ERα, leading to a constitutively active ER that promotes endocrine-independent cell growth [25]. Although being potentially detectable in primary lesions, *ESR1* variants are more frequently acquired under treatment pressure. Indeed, they have been identified in around 20–40% of patients with Luminal-like mBC after disease progression (PD) on aromatase inhibitors (AIs) [11,26]. Besides, recent evidence suggests that novel *ESR1* non-hotspot mutations may also contribute to the emergence of ET resistance. In fact, their variable binding affinity to selective ER modulators (SERMs) and degraders

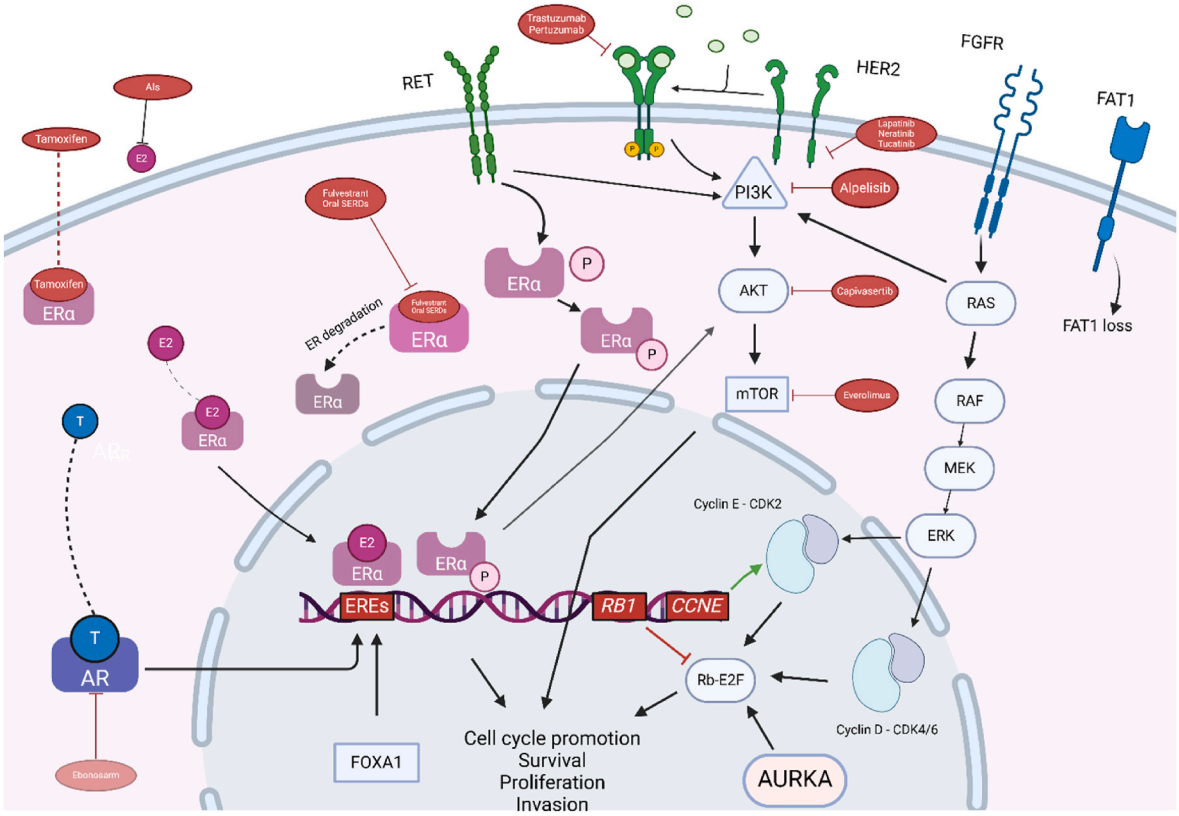

Fig. 1. A summary of estrogen receptor downstream signaling and relevant crosstalk with other molecular pathways; the main therapeutic options available are also displayed (Created with BioRender.com).

Abbreviations: AIs, aromatase inhibitors; AR, androgen receptor; AKT, protein kinase B; AURKA, aurora kinase A; *CCNE1*, cyclin E1 gene; CDK, cyclin-dependent kinases; E2, estradiol; ERα, estrogen receptor alpha; EREs, estrogen responsive elements; ERK, extracellular signal-regulated kinase; FGFR, fibroblast growth factor receptor; FOXA1, forkhead box A1; HER2, human epidermal growth factor receptor 2; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; P, phosphate group; PI3K, phosphatidylinositol 3-kinase; Rb, retinoblastoma protein; *RB1*, retinoblastoma 1 gene; RET, rearranged during transfection receptor; SERDs, selective estrogen receptor degraders; T, testosterone.

(SERDs) could influence the pharmacodynamic profile and antitumor effectiveness of these drugs [27,28].

Alterations in the PI3K/AKT/mTOR pathway are another well-established mechanism of ET resistance; its overactivation is mainly driven by *PIK3CA* somatic mutations, which account for about 40% of all detected mutations in Luminal-like mBC. Moreover, they can confer resistance to cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) by constitutively activating the p110α subunit of PI3K [29]. *AKT* activating and *PTEN* inhibiting variants can also be found, in smaller proportions [30–33]. The detection of these gene alterations seems to be equally reliable in either tissue specimens or ctDNA samples, with concordance rates for *PIK3CA* status reported to be about 70% for primary tumors and 80% for metastatic sites. A high degree of reliability was also observed with other cancer-derived circulating biomarkers (e.g., circulating tumor cells [CTCs]) [34–37].

ERBB2 somatic activating mutations, affecting the kinase domain of HER2, can concur to the development of ET resistance, via a crosstalk between HER2 and ER signaling pathways [22,23] (Fig. 1). These alterations account for approximately 2% of all mBC cases and, despite their lower frequency, they increase to 3–5% in the Luminal-like biological subtype and may be observed independently of HER2 amplification or overexpression [38–40]. Notably, *ERBB2* mutations appear to be mutually exclusive with *ESR1* variants, so they may represent a possible independent prognostic and predictive marker in Luminal-like mBC [41]. Their mutational status could be effectively defined by liquid biopsy analyses conducted on both ctDNA and CTCs [42,43].

Aurora kinase A (*AURKA*) amplification enables increased phosphorylation of ERα transactivation domain, inducing its down-regulation, and has therefore been associated with resistance to tamoxifen and increased likelihood of disease relapse [44]. Targeted drugs, like alisertib, are currently being investigated as a potential mechanism to restore ET sensitivity [45] (Fig. 1).

The receptor tyrosine kinase (RTK)/RAS pathway is also involved in ET resistance. *FGFR* amplification works as a promoter of anchorage-independent proliferation and ET resistance, leading to increased tumor proliferation and aggressiveness; it can be found in about 16–27% of Luminal-like mBC, mostly with lower HRs expression [46]. ctDNA analyses performed in patients treated with ET plus CDK4/6i have identified *FGFR* alterations in about 5% of cases; investigations are ongoing to better elucidate their potential prognostic and predictive role, as well as possible targeted treatment strategies [47–50] (Fig. 1).

2.3. Estrogen receptor expression and its dynamic evolution

ER expression's reduction has been recognized as a possible mechanism of ET resistance. The most recent update of the ASCO/CAP guidelines has introduced the ER-low category [51]. Tumors falling into this category have been associated with poorer prognosis compared to the ER-positive counterparts, exhibiting annual recurrence patterns more akin to the triple negative (TNBC) biological subtype [52,53]. Additionally, they typically manifest more aggressive biological features, such as larger tumor volumes, poorer differentiation and higher proliferation indexes, and have consistently proven reduced sensitivity to ET and higher response rates to chemotherapy [54–59]. Interestingly, higher levels of *CCNE1* mRNA were found mostly in ER-low mBC and were associated with lower sensitivity to ET plus CDK4/6i [60]. Furthermore, a molecular analysis of 173 HER2-negative breast cancer patients revealed that stromal tumor-infiltrating lymphocytes, $CD8^+$ T cells, PD-L1 protein expression and immune-related gene signatures were more pronounced in the ER-low subgroup, suggesting an enhanced immune sensitivity of these diseases, likewise TNBC [61].

Substantial evidence have pointed out that ER expression can evolve over time and space, between the primary lesion and metastatic sites, with significant consequences in prognosis and prediction [62,63]. Dynamic monitoring would be warranted, although performing repeated tissue biopsies does not seem to be clinically feasible. Prat et al. performed a ctDNA 0.5X shallow whole-genome sequencing analysis on 459 patients with mBC, looking for possible associations between 150 ctDNA-based signatures and tissue ER and HER2 status. Signatures related to Luminal biological processes were significantly enriched in the ER-positive subgroup, as expected. Moreover, after applying an unsupervised hierarchical cluster analysis to the ctDNA-based signatures, four distinct clusters were identified, with a high degree of correspondence to tissue-based RNA intrinsic subtyping. Interestingly, Cluster 2, which was found to be more associated with Luminal B features, could be further subdivided into two sub-clusters based on the different enrichment in proliferation hallmarks and Luminal A signatures. Furthermore, in an exploratory analysis of the PACE trial, blood samples were evaluated for CTCs enumeration and ER expression, with the help of a digital software: interestingly, a decrease in ER-positive CTCs was observed (26.3% at baseline vs 5.5% at PD), with concomitant increase in the rate of ER-negative samples with high CTCs count (i.e., $\geq$ 5 CTCs/7.5 mL [64]) [65]. Overall, these results underline the technical and analytical feasibility of liquid biopsy methods to identify and dynamically monitor complex biological features, including variations in ER expression, similar to what tissue-based profiling could accomplish [12].

3. Emerging and complementary biomarkers

3.1. Androgen receptor

AR is another member of the steroid HRs superfamily, whose overexpression is found in up to 70–90% of Luminal-like mBC. AR exerts its downstream effects by acting through both "genomic" and "non-genomic" pathways, similar to ER, and can contribute to the imbalance between androgen and estrogen levels [66–71] (Fig. 1).

So far, research efforts concerning AR have primarily focused on TNBC, aiming to identify new potential therapeutic targets. However, a crosstalk between AR and ER has been described, as they can bind to similar EREs. Moreover, preclinical evidence has shown that AR is capable of modulating estrogen-dependent signaling, thereby influencing ERα expression in Luminal-like tumors [72–75].

The prognostic role of AR appears to be context-dependent, strongly determined by concomitant ER expression. In Luminal-like breast cancer, AR has been associated with favorable clinicopathologic features (e.g., small tumor size, low grade, and node-negative status), as well as reduced mortality. Conversely, these findings have not been observed in ER-negative tumors [68,76]. Interestingly, the AR/ER crosstalk may be involved in the development of ET resistance: indeed, a high AR/ER ratio has been associated with poor survival outcomes and increasing ET resistance [77–79]. Instead, the predictive role of AR is yet to be fully elucidated. A phase I/II study reported a certain activity of abiraterone acetate in post-menopausal patients with both ER- and AR-positive mBC who had previously received two or more ETs, while another trial did not yield positive results [80,81]. Data are also emerging on the activity of selective AR modulators, which have proven capable of inhibiting tumor cell growth in preclinical models of ET-resistant mBC. Enobosarm has demonstrated a 29–32% clinical benefit rate (CBR) at 24 weeks of treatment, with limited toxicities, in post-menopausal women previously treated with $\geq$ 1 ET line and having previously shown ET sensitivity [82] (Fig. 1).

3.2. FOXA1

FOXA1 is a pioneer transcription factor that regulates chromatin remodeling, facilitating the access of steroid HRs to their EREs [83]. IHC analyses and large-scale gene expression studies have demonstrated a positive correlation between FOXA1 and ER expression in primary breast cancer samples [84,85] (Fig. 1).

Evidence on the role of FOXA1 are conflicting. Indeed, early analyses have associated higher FOXA1 levels with longer disease-free survival

and late recurrence in Luminal-like breast cancer patients [21,86]. Conversely, FOXA1 methylation silencing was found more frequently in *BRCA*-deficient, basal-like tumors [87]. As such, FOXA1 was first recognized as a determinant of ET responsiveness, in light of its role in chromatin remodeling [88].

However, *FOXA1* gene amplification or overexpression, resulting in high FOXA1 levels, has been reported to be primarily involved in the promotion of oncogenic signatures highly associated with ET resistance, cell growth and invasiveness. Possible mechanisms involve a reprogramming of ER chromatin targets and an induction of interleukin-8 signaling [33,89–91]. Specific *FOXA1* mutations have also been considered, with neomorphic alterations inducing the transcription of alternative gene sets, while hypermorphic variants entail an overexpression of ER-dependent transcripts [92]. Thus, FOXA1 likely exerts different roles in ET-sensitive and ET-resistant breast cancers, according to the differential activation of ER-related genes [91].

Interestingly, recent reports have described *FOXA1* alterations as recurrent structural variants frequently observed after ET exposure, although they appear to be mostly nonconcurrent with *ESR1* variants. This observation suggest that both factors are strongly involved in ET resistance, with different underlying mechanisms [93,94]. In a biomarker analysis conducted within the phase III CONFIRM trial, increased FOXA1 transcriptional signaling appeared to be associated with decreased response to fulvestrant [95]. However, a comprehensive explanation is lacking and further studies will be necessary to clarify the meanings of FOXA1 signaling in Luminal-like mBC.

3.3. *RET* mutations

RET is a proto-oncogene that codes for a single-pass transmembrane RTK. Gene fusions between *RET* and other partners can occur, leading to the creation of a constantly active chimeric protein. These mutations have been widely described in different tumor types, mostly medullary thyroid, non-small cell lung and colon cancers [96–98] (Fig. 1).

In Luminal-like breast cancer, a crosstalk between RET and ER signaling has been reported. Estrogens can upregulate *RET*, promoting cell survival, proliferation, distant migration and invasion, while *RET* contributes to ET resistance through the activation of the PI3K/AKT/mTOR or MAPK pathways. Moreover, the chimeric RET protein is able to phosphorylate ER, keeping it in a ligand-independent, constantly active status [99–101].

The interplay between *RET* alterations and ER signaling in Luminal-like mBC has been underexplored so far; nevertheless, it looks like a promising actionable target to be investigated in the near future [102,103].

3.4. *ESR1* epigenetic signatures

ESR1 can be affected by several molecular mechanisms, other than point mutations. In particular, epigenetic signatures are being paid increasing attention: *ESR1* methylation has been associated to reduced tumor dependency on ER signaling, suggesting it as a possible escape strategy, while its loss or amplification could drive ET resistance [104]. Their detection via non-invasive techniques, such as liquid biopsy, is becoming more and more available and could be a reliable tool to assess ET sensitivity [105,106]. Other mechanisms are also being investigated, including DNA methylation of specific ER CpG islands, histone deacetylation and repression of ER transcription mediated by the transcription factor Twist [107].

4. Clinical evidence supporting decision-making

The expansion of knowledge on the biology underlying tumor development, together with a constant progress of biotechnology, is transforming oncology practice towards a “precision medicine” model, allowing more and more patients to undergo a comprehensive molecular profile, which may subsequently guide tailored therapeutic choices [108–110]. In the field of Luminal-like mBC, a first line approach consisting of a combination of ET with a CDK4/6i is the current standard of care [111–113]. However, resistance mechanisms can develop and PD will inevitably present; in this context, identifying actionable molecular targets is a priority to assist clinicians in tackling therapeutic decision-making, possibly delaying the need to use chemotherapy [114,115].

4.1. *ESR1* mutations

Preclinical and clinical data are pointing towards a relatively higher sensitivity of *ESR1* mutant (*ESR1*mut) tumors to agents directly targeting ER, particularly SERDs. In a combined analysis of two phase III trials, detecting *ESR1* mutations in baseline blood samples was associated with worse survival outcomes in patients treated with exemestane than in those who received fulvestrant [11,116,117]. Accordingly, fulvestrant is recommended as a subsequent option in patients who have experienced PD under previous first line ET with AIs or relapse within 12 months of its conclusion in the adjuvant setting [118].

Due to the suboptimal pharmacokinetic profile of fulvestrant, which requires an intramuscular injection, novel orally available SERDs are in development [119–122]. The first compound to provide evidence of clinical efficacy in a phase III study is elacestrant, which led to a statistically significant improvement in PFS with respect to standard second line ET in the *ESR1*mut cohort [119]. An individual patient data metanalysis was conducted across the EMERALD, AMEERA-3, acelERA and SERENA-2 trials. A total of 1290 patients were considered: overall results suggested a general PFS benefit with oral SERDs mainly driven by *ESR1*mut cohorts, which preserved statistical significance in the subgroup analysis as opposed to *ESR1* wild type patients [123]. On the basis of the EMERALD results, elacestrant has been approved for use by both the US and the European regulatory agencies in patients with Luminal-like, *ESR1*mut mBC experiencing PD after $\geq$ 1 ET line. However, performances of single agent ET still seem limited to few months of PFS benefit and, although encouraging, the first part of the Kaplan-Meier curves of the EMERALD trial suggests that there is still room for improving treatment strategy. Recently, the ADELA trial (NCT06382948) is opening to enrollment, testing the addition of everolimus to elacestrant in patients with Luminal-like, *ESR1*mut mBC (Table 1).

ESR1 mutations have also proven valuable for refining disease monitoring by anticipating the identification of PD. The PADA-1 trial showed that an early switch of the ET backbone, prompted by the detection of *ESR1* mutations in the absence of clinical PD, while continuing with the same CDK4/6i (i.e., palbociclib), can significantly improve PFS, defined as the time elapsed from randomization to clinical PD or death [124]. Moreover, a recently presented biomarker analysis highlighted a higher rate of *ESR1* mutations’ ctDNA clearance at 2 months in patients switching to fulvestrant. Conversely, the type of *ESR1* mutation and polyclonality did not appear to play any prognostic role [125]. The strategy of continuing CDK4/6i beyond PD has also been explored, both in a phase II trial and in a real-world context, suggesting a favorable impact on prognosis, with a potential added benefit specifically in the *ESR1*mut population [126,127]. Based on these results, further exploration is ongoing in the SERENA-6 phase III trial, which has been designed similarly to PADA-1, with main differences represented by the use of a newer oral SERD (i.e., camizestrant) in combination with any CDK4/6i and by the implementation of a double blind strategy [128,129] (Table 1).

4.2. *ERBB2* mutations

Neratinib, a second-generation oral pan-HER tyrosine kinase inhibitor, has previously demonstrated a certain capability to overcome ET resistance in Luminal-like, *ERBB2* mutant (*ERBB2*mut) breast cancer cell

Table 1
List of the main ongoing trials employing ctDNA as either an inclusion criteria or decision factor for early treatment switch in Luminal-like metastatic breast cancer.

Trial	Phase	Study treatments	Disease setting	ctDNA utility and mutation assessed	Study status
PADA-1 (NCT03079011)	II	- Observational phase: palbociclib + AI - Randomized phase: o palbociclib + AI o palbociclib + fulvestrant	I/II line	Early switch *ESR1* mutations	Active, not recruiting Published results
SERENA-6 (NCT04964934)	III	- Observational phase: investigator's choice CDK4/6i + AI - Randomized phase: o investigator's choice CDK4/6i + AI o investigator's choice CDK4/6i + fulvestrant	I/II line	Early switch *ESR1* mutations	Active, not recruiting Results pending
SAFIR-03 (NCT05625087)	II	- SCREENING: investigator's choice CDK4/6i + fulvestrant - ARRIBA: o alpelisib + fulvestrant o ribociclib + fulvestrant	I/II line	Early switch *PIK3CA* mutations	Recruiting
INTERACT (NCT04256941)	II	- Investigator's choice CDK4/6i + fulvestrant - Investigator's choice CDK4/6i + AI	I line	Inclusion criteria *ESR1* mutations	Completed
ADELA (NCT06382948)	III	- Elacestrant + everolimus - Elacestrant + placebo	II/III line	Inclusion criteria *ESR1* mutations	Not yet recruiting
ELAINE-3 (NCT05696626)	III	- Lasofoxifene + abemaciclib - Fulvestrant + abemaciclib	II/III line	Inclusion criteria *ESR1* mutations	Recruiting
INAVO120 (NCT04191499)	III	- Inavolisib + palbociclib + fulvestrant - Placebo + palbociclib + fulvestrant	I line	Inclusion criteria *PIK3CA* mutations	Active, not recruiting Published results
INAVO121 (NCT05646862)	III	- Inavolisib + fulvestrant - Alpelisib + fulvestrant	I/II/III line	Inclusion criteria *PIK3CA* mutations	Recruiting
CAPItello-292 (NCT04862663)	Ib/III	- Capivasertib + palbociclib + fulvestrant - Placebo + palbociclib + fulvestrant	> II line	Inclusion criteria for phase III AKT alterations	Recruiting

Abbreviations: AI, aromatase inhibitor; AKT, protein kinase B; CDK4/6i, cyclin-dependent kinase 4/6 inhibtors; ctDNA, circulating tumor DNA; *ESR1*, estrogen receptor 1 gene; *PIK3CA*, phosphatidylinositol 3-kinase catalytic subunit alpha gene.

lines and xenograft models. Its association with fulvestrant may lead to a concomitant suppression of both HER2 and ER pathways [23,130]. The clinical utility of neratinib is currently being investigated in pre-treated patients with mBC, either as a single agent or in combination with other drugs [131]. In the MutHER phase II trial, a 30–38% CBR was documented in patients with Luminal-like, *ERBB2*mut mBC who either received a previous treatment with fulvestrant or were naïve to it, respectively [132]. In the SUMMIT basket trial, the CBR was reported to be as high as 30.4% for neratinib monotherapy and 46.8% for neratinib plus fulvestrant, respectively. Furthermore, the median PFS and duration of response (DoR) were numerically longer within the combination arm, although the trial was not designed to compare them [133].

ctDNA analyses conducted within these studies identified the occurrence of secondary *ERBB2* activating variants upon PD as putative mechanisms of resistance to neratinib. This observation prompted the exploration of dual HER2 targeting to achieve a more comprehensive inhibition of the pathway, potentially maximizing treatment response [132,133]. It was postulated that a triplet strategy with neratinib, fulvestrant and trastuzumab would at least delay the emergence of further *ERBB2* alterations and prolong treatment responses. To test this hypothesis, a cohort of Luminal-like, HER2 non-amplified, *ERBB2*mut patients was incorporated in the SUMMIT trial and a comparison between fulvestrant, alone or in combination with trastuzumab, and the triplet was conducted in a small, randomized subcohort of 21 individuals. The additional role of neratinib eventually proved to be substantial: indeed, DoR, CBR and PFS were all superior in patients receiving the triplet compared with those undergoing fulvestrant ± trastuzumab. Moreover, as patients were allowed to cross over to the triplet arm after PD on fulvestrant ± trastuzumab, subsequent more profound responses were observed [134].

Significantly, these results were obtained in a cohort of patients with Luminal-like, *ERBB2*mut mBC classified as HER2 negative (i.e., IHC score 0, 1+ or 2+ without in situ hybridization [ISH] amplification). A careful interpretation is required, as the trial population was limited; however, the triplet regimen has provided promising clinical efficacy in this specific subgroup. Interestingly, the identification of *ERBB2* mutations in DNA samples from archival biopsies suggests the potential for early detection, enhancing the understanding of tumor biology and treatment customization [42,135].

4.3. Mutations in the PI3K/AKT/mTOR axis

Several post-hoc biomarker analyses conducted within pivotal phase III trials agreed that *PIK3CA* mutations have no predictive role for CDK4/6i efficacy, both in the first line and in the post-ET progression setting. However, a *PIK3CA* mutant (*PIK3CA*mut) status was associated with numerically shorter PFS and OS [48,112,136–139].

The first compound targeting the PI3K/AKT/mTOR axis to be introduced was everolimus, an mTOR inhibitor: when associated to exemestane, it provided a significant PFS benefit compared with single agent AI, with only a numerically longer OS; however, patients were not selected for molecular alterations. A biomarker analysis reported no differences in PFS benefit according to *PIK3CA* status [140,141]. Later, alpelisib, an α-specific PI3K inhibitor, demonstrated a statistically significant PFS improvement when combined with fulvestrant in the SOLAR-1 phase III trial. Patients were enrolled based on tissue-detected *PIK3CA*mut; still, a ctDNA subgroup analysis was performed, confirming a PFS benefit regardless of the tissue status. Again, OS was only numerically longer, both in the overall population and in ctDNA-detected patients [142–144]. The activity of alpelisib plus fulvestrant after CDK4/6i failure was also explored in the phase II BYLieve trial; a biomarker analysis reported that a low ctDNA fraction (i.e., < 10%) was associated with improved PFS in all study cohorts [145,146]. Ongoing phase III trials are testing the efficacy of PI3K inhibitors (e.g., alpelisib or inavolisib), focusing specifically on selected *PIK3CA*mut patient populations [147,148].

More recently, a new class of selective AKT inhibitors is being considered. Capivasertib, a first-in-class compound, has already proved able to improve both PFS and OS, in combination with fulvestrant, in the FAKTION phase II trial, regardless of any alteration in the PI3K/AKT/PTEN pathway; however, the analytical plan was only limited to tissue or blood testing for *PIK3CA* exons 9 or 20 variants and tissue IHC for

PTEN alterations [149]. Lately, an expanded biomarker analysis, performed with more accurate tissue and blood next generation sequencing (NGS) assays, suggested a greater advantage in patients harboring PI3K/AKT/PTEN defects [150].

Similar data emerged from the phase III CAPItello-291 trial, in which capivasertib or placebo were added to fulvestrant in a molecularly-unselected population. A PFS benefit was documented both in the overall population and in patients harboring PI3K/AKT/PTEN pathway alterations, regardless of previous CDK4/6i exposure. However, a significant proportion of patients deemed to be "PI3K/AKT/PTEN non-altered" were later found to carry "NGS unknown" variants. Further exploratory analyses on "non-altered" patients showed that PFS benefit was not confirmed after excluding "NGS unknown" variant carriers. Moreover, an "NGS unknown" status has been associated with sustained PFS advantage, leading to the assumption that it may encompass a large proportion of molecular alterations [32]. Accordingly, the US and European regulatory agencies have approved the use of capivasertib, in combination with fulvestrant, only for patients with Luminal-like mBC experiencing PD after $\geq$ 1 ET line and harboring at least one PI3K/AKT/PTEN alteration. Further advances in the detection of these variants might help refining patient selection in the near future.

Finally, an evolution of the early-switch strategy is being tested specifically in the context of ET-resistant disease in the phase II SAFIR-03 study (NCT05625087). In the SCREENING part of this trial, patients treated with fulvestrant plus CDK4/6i are longitudinally followed with seriated ctDNA monitoring; those with a persistently detectable *PIK3CA* mutation for at least 4 weeks are then randomized in the ARRIBA section, to receive either alpelisib or ribociclib, while continuing treatment with fulvestrant (Table 1).

4.4. BRCA1/2 and beyond

Poly(ADP-ribose) polymerase inhibitors (PARPi) are gaining increasingly prominence as a targeted therapeutic option in patients with breast cancer harboring germline *BRCA1/2* mutations (g*BRCA1/2*mut), after the demonstration of their capability to interfere with aberrant DNA damage repair mechanisms by inducing the so-called "synthetic lethality" [151,152]. g*BRCA1/2*mut are estimated to occur in about 5–6% of unselected cases, with enrichment according to variables like age, biological subtype and ancestry; they are mostly detected via liquid-based assays, analyzing germline DNA extracted from leucocytes [153].

Currently, olaparib and talazoparib have provided the most advanced evidence of clinical activity in HER2-negative mBC: both drugs have been compared with physicians' choice non-platinum single-agent chemotherapy, showing statistically significant benefits in terms of PFS and overall quality of life, with only numerical OS improvements [154–157]. Both trials included a subgroup of patients with Luminal-like mBC; in these cohorts, both olaparib and talazoparib seemed to produce similar PFS benefits, as compared to the overall trial population, without significant differences [156–158]. As a result, these drugs have been endorsed for clinical use in patients with Luminal-like mBC carrying g*BRCA1/2*mut, who had been previously exposed to anthracycline- and taxane-based chemotherapy, after PD on $\geq$ 1 prior ET line. Therefore, g*BRCA1/2*mut are currently classified as Tier I actionable alterations in breast cancer, according to the European Society of Medical Oncology Scale for Clinical Actionability of molecular Targets (ESCAT) [159], as well as an extremely important predictive biomarker in Luminal-like mBC.

Nonetheless, there are open questions that still need to be answered. First, increased sensitivity to PARPi has been described in patients carrying other defects in the homologous recombination repair molecular machinery (i.e., homologous recombination deficient, HRD), including other tumor suppressor genes, such as *PALB2, RAD51C/D, ATR, ATM, CHEK2, FANCA* and *FANCC* [160]. The predictive role of HRD alterations is well-recognized in other cancers, with most evidence deriving from ovarian cancer, while their value currently seems to be more attenuated in mBC [161]. Besides, real-world evidence suggests smaller survival benefits for patients with Luminal-like mBC and g*BRCA1/2*mut treated with ET [162]. The efficacy in g*BRCA1/2*mut carriers has not been addressed in the pivotal trials of first line CDK4/6i, but data from the PADA-1 trial are similarly pointing towards less benefit from first line palbociclib and AI, possibly due to an increasing emergence of *ESR1*mut [163]. PARPi are currently being tested in association with ET and even immune checkpoint inhibitors in patients with Luminal-like mBC and either g*BRCA1/2*mut or other HRD abnormalities: preliminary data have shown encouraging efficacy and antitumor activity, which seem at least comparable to standard second- and third line options, and manageable safety profiles [164,165]. Front-line use of PARPi is not warranted at present, as no randomized trials have directly compared these compounds to CDK4/6i; however, some real-world cases have reported interestingly good responses [166].

4.5. Understanding sensitivity and resistance to CDK4/6 inhibitors

The introduction of CDK4/6i has redefined the first line treatment landscape of Luminal-like mBC, yet the emergence of resistance to these compounds represent a pervasive challenge [167]. Extensive research has been conducted to explore the mechanisms of resistance to CDK4/6i using liquid biopsy biomarkers, such as ctDNA and CTCs [168]. A recent, comprehensive pooled analysis of ctDNA samples from the MONALEESA trials identified distinct alterations. In this study, variants involving *ANO1*, *CDKN2A/B/C* and *RB1* have been associated with decreased sensitivity or relative resistance to ribociclib, while an enhanced response was linked to alterations in *ERBB2*, *FAT3*, *FRS2*, *MDM2*, *SFRP1* and *ZNF217* genes. These results suggest that ctDNA can potentially identify patients with Luminal-like mBC likely to derive more benefit from CDK4/6i treatment, both in the first and in later lines [137].

Other molecular mechanisms driving resistance to ET and CDK4/6i are emerging and may be detected via ctDNA testing. An exploratory analysis within the PALOMA-3 trial has raised particular interest in alterations to genes involved in cell cycle regulation: *CCNE1* amplification and *RB1* loss were detected in about 17% of baseline ctDNA samples and were associated with worse prognosis and smaller benefit from palbociclib and fulvestrant [169]. Data are also emerging on the role of *FAT1* loss: in a large NGS analysis, *FAT1* and *RB1* loss were more frequently observed in patients experiencing rapid PD after CDK4/6i rechallenge and were associated with shorter time to treatment failure, suggesting a potential negative predictive value for this strategy [170] (Fig. 1).

Finally, liquid-based biomarkers are emerging as promising indicators of CDK4/6i activity. Serum thymidine kinase (sTKa) has raised particular interest in many phase II trials [171–173], especially the BioItaLee study, which enrolled postmenopausal patients with Luminal-like mBC treated with first line ribociclib plus letrozole. A sustained sTKa inhibition has been associated with more favorable PFS, whereas a lack of inhibition by day 15 of the first treatment cycle correlated with worse outcome [174].

4.6. The role of CTCs

Quantitative analysis of CTCs has proven valuable in supporting clinical decision-making in Luminal-like mBC. CTCs enumeration, stratified according to the validated cutoff of 5 CTCs/7.5 mL [64], was suggested as a potential stand-alone driver of first line treatment choices in the French STIC-CTC trial. The primary endpoint of PFS was met, showing that this liquid biopsy-based approach is at least noninferior to a clinician-driven strategy; OS data, although not significant, also showed a numerical improvement in the CTC-driven arm. Importantly, CTCs enumeration appeared to be most useful in case of discordance between the two estimates: indeed, patients with high CTCs count and a low clinical risk profile seemed to derive greater benefit by the CTC-driven choice for chemotherapy [175,176]. The external validity is

limited by the fact that the trial was run before the introduction of CDK4/6i; nonetheless, it shows the feasibility of this approach and warrants further exploration in the current treatment landscape.

Similar data are emerging in the second line setting, underlining the potential role of CTCs in predicting benefit from a CDK4/6i beyond progression strategy. In the PACE trial, patients with high CTCs count exhibited a substantial benefit from the combination of fulvestrant plus palbociclib, with or without avelumab, as compared to fulvestrant alone, after a first line CDK4/6i-based regimen [177].

Further hints are coming from more detailed CTCs analyses focused on their molecular background. Intriguingly, preliminary findings from an ongoing study evaluating the transcriptional profiles of CTCs at baseline and during palbociclib treatment have suggested a more common overexpression of *CDK2*, *WWTR1* and *YAP1* among non-responding patients, while *MLH1* and *NFKB1* seemed to be more prevalent in responders [178].

Finally, several reports have described a certain heterogeneity in HER2 expression among metastatic lesions [179]. In parallel, the concept of HER2-low expression is gaining consideration also in Luminal-like mBC, after the positive results obtained by trastuzumab deruxtecan in patients previously exposed to $\geq$ 2 ET lines [180,181]. In this context, CTCs phenotyping is emerging as a tool to further identify HER2 expression, either complete or HER2-low, in patients that would otherwise be classified as HER2-negative according to IHC [182–184]. Clinical explorations are also ongoing to evaluate the potential benefit of antiHER2 therapies in patients with HER2-expressing CTCs, with promising results [185].

5. Future perspectives

A multitude of unexplored liquid-based biomarkers exist, with potential clinical applications yet to be elucidated. The variant allele fraction (VAF) serves as a metric to assess the mutational load of a specific gene alteration, playing a pivotal role in distinguishing driver and passenger mutations [186,187]. While tissue analysis provides enhanced sensitivity in detecting low-frequency variants, calculating VAF in ctDNA samples not only offers a non-invasive approach, but also allows for a more comprehensive evaluation of the spatial and temporal heterogeneity within tumor biology [35,188]. Moreover, VAF dynamics have been associated with PFS and changes in tumor size in retrospective analyses of plasma samples, conducted both in a subset of patients with *ERBB2*mut mBC treated with neratinib and in the larger PALOMA-3 cohort [135,189]. Besides, in a longitudinal on-treatment monitoring of somatic mutations, ctDNA clearance after 30 days has been related to PFS improvements, while increasing VAF and number of alterations seemed to indicate a higher PD risk [190,191].

Concurrently, efforts are being made towards integrating ctDNA analysis to the classical radiological assessments, in order to predict and possibly anticipate PD. In the phase Ib MONALEESASIA trial, the median lead time between ctDNA increase and clinical or radiological PD was 83 days (range, 14–309 days) [192]. Despite the sensitivity of the analysis and the substantial variability in lead times, these findings suggest possible improvements in disease monitoring by the integration of liquid biopsy to radiological imaging.

Although liquid biopsy is gaining momentum, some potential caveats should be kept in mind. First, blood samples may yield lower DNA rates, as compared to tissue; moreover, when analyzing cell-free DNA, a careful distinction should be made between ctDNA and features associated with clonal hematopoiesis, that is a para-physiological process related to cell aging, consisting in the accumulation of somatic mutations and the clonal expansion of hematopoietic stem cells [193]. Finally, standardized criteria to define the somatic or germline origin of mutations are still needed, as a certain discordance exists in the available literature [194].

6. Conclusions

IHC tissue analysis currently remains the standard procedure to identify Luminal-like mBC; however, this technique is unable to fully capture the complexity underlying ER signaling. In this context, liquid biopsy enables a more in-depth evaluation of tumor heterogeneity and better understanding of mechanisms of ET resistance and PD and offers a feasible, non-invasive tool to guide treatment decision-making.

Several biomarkers have already been evaluated in phase II-III clinical trials: *ESR1* mutations have been associated with better survival outcomes for new oral SERDs, while *PIK3CA* variants have predictive significance only for PI3K inhibitors. Further studies will soon elucidate their role regarding new AKT and PTEN inhibitors. Although *ERBB2* mutations are less common, they may offer additional treatment guidance in the future, even in the absence of HER2 amplification or overexpression. Prior knowledge of g*BRCA1/2* status is now mandatory to better plan treatment choices for patients carrying these variants.

Furthermore, CTCs analysis may still play a role in supporting treatment decisions: although not formally proven, evidence are pointing towards possible clinical utility by CTCs enumeration in Luminal-like mBC. Non-invasive assessment of HER2 expression via CTCs phenotyping and the evaluation of transcriptional profiles of CTCs hold promising in current research.

Other molecular biomarkers are emerging, their potential use being currently in the early phases of investigation. Their integration into the therapeutic algorithm will be crucial for improving treatment tailoring in the near future.

Authors' disclosure of potential conflicts of interest

Lorenzo Gerratana reports consulting or advisory role for AstraZeneca, Daiichi Sankyo, Eli Lilly, GlaxoSmithKline, Incyte, Novartis, Pfizer, MSD, Menarini Stemline and Abbvie; research funding from Menarini Silicon Biosystems; travel expenses from Menarini Stemline.

Fabio Puglisi reports grants/research support from AstraZeneca, Eisai and Roche; honoraria or consultation fees from Amgen, AstraZeneca, Daichii Sankyo, Celgene, Eisai, Eli Lilly, Gilead, GSK, Ipsen, MSD, Novartis, Pierre-Fabre, Pfizer, Roche, Seagen, Takeda, Menarini and Viatris.

All reported potential conflicts of interest are outside the submitted work.

All other authors have declared no potential conflicts of interests.

Funding

This work was funded by the Italian Ministry of Health, Ricerca Corrente.

Author contributions

Conceptualization: Arianna Dri, Lorenzo Foffano, Brenno Pastò, Riccardo Vida, Lorenzo Gerratana, Fabio Puglisi. **Methodology:** Lorenzo Gerratana, Fabio Puglisi. **Data curation:** Lorenzo Foffano, Brenno Pastò. **Visualization:** Lorenzo Foffano, Brenno Pastò, Riccardo Vida. **Manuscript writing – original draft preparation:** Serena Della Rossa, Arianna Dri, Lorenzo Foffano, Brenno Pastò, Riccardo Vida. **Manuscript writing – review and editing:** all authors. **Supervision:** Lorenzo Gerratana, Fabio Puglisi. **Final approval of manuscript:** all authors.

References

[1] Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol 2013;24:2206–23. https://doi.org/10.1093/annonc/mdt303.

[2] Hammond MEH, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol 2010;28:2784–95. https://doi.org/10.1200/JCO.2009.25.6529.

[3] Ahlborn LB, Rohrberg KS, Gabrielaite M, Tuxen IV, Yde CW, Spanggaard I, et al. Application of cell-free DNA for genomic tumor profiling: a feasibility study. Oncotarget 2019;10:1388–98. https://doi.org/10.18632/oncotarget.26642.

[4] Lee LMJ, Cao J, Deng H, Chen P, Gatalica Z, Wang Z-Y. A novel variant of ER-α, is expressed in ER-positive and -negative human breast carcinomas. Anticancer Res 2008;28:479–83. ER-α36.

[5] Pagano MT, Ortona E, Dupuis ML. A role for estrogen receptor alpha36 in cancer progression. Front Endocrinol 2020;11.

[6] Prat A, Chaudhury A, Solovieff N, Paré L, Martinez D, Chic N, et al. Correlative biomarker analysis of intrinsic subtypes and efficacy across the MONALEESA phase III studies. J Clin Oncol 2021;39:1458–67. https://doi.org/10.1200/JCO.20.02977.

[7] Cheang MCU, Martin M, Nielsen TO, Prat A, Voduc D, Rodriguez-Lescure A, et al. Defining breast cancer intrinsic subtypes by quantitative receptor expression. Oncologist 2015;20:474–82. https://doi.org/10.1634/theoncologist.2014-0372.

[8] Kim HK, Park KH, Kim Y, Park SE, Lee HS, Lim SW, et al. Discordance of the PAM50 intrinsic subtypes compared with immunohistochemistry-based surrogate in breast cancer patients: potential implication of genomic alterations of discordance. Cancer Res Treat 2018;51:737–47. https://doi.org/10.4143/crt.2018.342.

[9] Holm J, Yu NY-L, Johansson A, Ploner A, Hall P, Lindström LS, et al. Concordance of immunohistochemistry-based and gene expression-based subtyping in breast cancer. JNCI Cancer Spectr 2020;5:pkaa087. https://doi.org/10.1093/jncics/pkaa087.

[10] Burstein HJ, DeMichele A, Somerfield MR, Henry NL. Testing for ESR1 Mutations to Guide Therapy for Hormone Receptor–Positive, Human Epidermal Growth Factor Receptor 2–Negative Metastatic Breast Cancer: ASCO Guideline Rapid Recommendation Update. J Clin Oncol 2023;41:3423–5. https://doi.org/10.1200/JCO.23.00638.

[11] Brett JO, Spring LM, Bardia A, Wander SA. ESR1 mutation as an emerging clinical biomarker in metastatic hormone receptor-positive breast cancer. Breast Cancer Res 2021;23:85. https://doi.org/10.1186/s13058-021-01462-3.

[12] Prat A, Brasó-Maristany F, Martínez-Sáez O, Sanfeliu E, Xia Y, Bellet M, et al. Circulating tumor DNA reveals complex biological features with clinical relevance in metastatic breast cancer. Nat Commun 2023;14:1157. https://doi.org/10.1038/s41467-023-36801-9.

[13] Miziak P, Baran M, Błaszczak E, Przybyszewska-Podstawka A, Kałafut J, Smok-Kalwat J, et al. Estrogen receptor signaling in breast cancer. Cancers 2023;15:4689. https://doi.org/10.3390/cancers15194689.

[14] Clusan L, Ferrière F, Flouriot G, Pakdel F. A basic review on estrogen receptor signaling pathways in breast cancer. Int J Mol Sci 2023;24:6834. https://doi.org/10.3390/ijms24076834.

[15] Zhou Y, Liu X. The role of estrogen receptor beta in breast cancer. Biomark Res 2020;8:39. https://doi.org/10.1186/s40364-020-00223-2.

[16] Tirado-Garibay AC, Falcón-Ruiz EA, Ochoa-Zarzosa A, López-Meza JE. GPER: an estrogen receptor key in metastasis and tumoral microenvironments. Int J Mol Sci 2023;24:14993. https://doi.org/10.3390/ijms241914993.

[17] Frasor J, Danes JM, Komm B, Chang KCN, Lyttle CR, Katzenellenbogen BS. Profiling of estrogen up- and down-regulated gene expression in human breast cancer cells: insights into gene networks and pathways underlying estrogenic control of proliferation and cell phenotype. Endocrinology 2003;144:4562–74. https://doi.org/10.1210/en.2003-0567.

[18] Castoria G, Migliaccio A, Bilancio A, Di Domenico M, de Falco A, Lombardi M, et al. PI3-kinase in concert with Src promotes the S-phase entry of oestradiol-stimulated MCF-7 cells. EMBO J 2001;20:6050–9. https://doi.org/10.1093/emboj/20.21.6050.

[19] Cabodi S, Moro L, Baj G, Smeriglio M, Di Stefano P, Gippone S, et al. p130Cas interacts with estrogen receptor α and modulates non-genomic estrogen signaling in breast cancer cells. J Cell Sci 2004;117:1603–11. https://doi.org/10.1242/jcs.01025.

[20] Altwegg KA, Vadlamudi RK. Role of estrogen receptor coregulators in endocrine resistant breast cancer. Explor Target Antitumor Ther 2021;2:385–400. https://doi.org/10.37349/etat.2021.00052.

[21] Horimoto Y, Sasahara N, Sasaki R, Hlaing MT, Sakaguchi A, Saeki H, et al. High FOXA1 protein expression might predict late recurrence in patients with estrogen-positive and HER2-negative breast cancer. Breast Cancer Res Treat 2020;183:41–8. https://doi.org/10.1007/s10549-020-05751-x.

[22] Nayar U, Cohen O, Kapstad C, Cuoco MS, Waks AG, Wander SA, et al. Acquired HER2 mutations in ER+ metastatic breast cancer confer resistance to estrogen receptor-directed therapies. Nat Genet 2019;51:207–16. https://doi.org/10.1038/S41588-018-0287-5.

[23] Croessmann S, Formisano L, Kinch LN, Gonzalez-Ericsson PI, Sudhan DR, Nagy RJ, et al. Combined blockade of activating ERBB2 mutations and ER results in synthetic lethality of ER+/HER2 mutant breast cancer. Clin Cancer Res 2019;25:277–89. https://doi.org/10.1158/1078-0432.CCR-18-1544.

[24] Dustin D, Gu G, Fuqua SAW. ESR1 mutations in breast cancer. Cancer 2019;125:3714–28. https://doi.org/10.1002/cncr.32345.

[25] Rani A, Stebbing J, Giamas G, Murphy J. Endocrine resistance in hormone receptor positive breast cancer–from mechanism to therapy. Front Endocrinol 2019;10.

[26] Zundelevich A, Dadiani M, Kahana-Edwin S, Itay A, Sella T, Gadot M, et al. ESR1 mutations are frequent in newly diagnosed metastatic and loco-regional recurrence of endocrine-treated breast cancer and carry worse prognosis. Breast Cancer Res 2020;22:16. https://doi.org/10.1186/s13058-020-1246-5.

[27] Gerratana L, Roncato R, Sturlese M, Davis AA, Velimirovic M, Reduzzi C, et al. Abstract PD10-01: PD10-01 impact of ESR1 mutations on selective estrogen receptor degraders and modulators: an integrated liquid-biopsy and pharmacodynamics approach. Cancer Res 2023;83. https://doi.org/10.1158/1538-7445.SABCS22-PD10-01. PD10-01.

[28] Kingston B, Pearson A, Herrera-Abreu MT, Sim L-X, Cutts RJ, Shah H, et al. ESR1 F404 mutations and acquired resistance to fulvestrant in ESR1-mutant breast cancer. Cancer Discov 2024;OF1–16. https://doi.org/10.1158/2159-8290.CD-22-1387.

[29] Del Re M, Crucitta S, Lorenzini G, De Angelis C, Diodati L, Cavallero D, et al. PI3K mutations detected in liquid biopsy are associated to reduced sensitivity to CDK4/6 inhibitors in metastatic breast cancer patients. Pharmacol Res 2021;163:105241. https://doi.org/10.1016/j.phrs.2020.105241.

[30] Shao YL, Rong M, Grieu F, Iacopetta B. PIK3CA mutations in breast cancer are associated with poor outcome. Breast Cancer Res Treat 2006;96:91–5. https://doi.org/10.1007/s10549-005-9048-0.

[31] Pereira B, Chin SF, Rueda OM, Vollan HKM, Provenzano E, Bardwell HA, et al. The somatic mutation profiles of 2,433 breast cancers refines their genomic and transcriptomic landscapes. Nat Commun 2016;7. https://doi.org/10.1038/ncomms11479.

[32] Turner NC, Oliveira M, Howell SJ, Dalenc F, Cortes J, Gomez Moreno HL, et al. Capivasertib in hormone receptor–positive advanced breast cancer. N Engl J Med 2023;388:2058–70. https://doi.org/10.1056/nejmoa2214131.

[33] Razavi P, Chang MT, Xu G, Bandlamudi C, Dara S, Vasan N, et al. The genomic landscape of endocrine-resistant advanced breast cancers. Cancer Cell 2019;34:427–38. https://doi.org/10.1016/j.ccell.2018.08.008.

[34] Moynahan M, Chen D, He W, et al. Correlation between PIK3CA mutations in cell-free DNA and everolimus efficacy in HR+, HER2- advanced breast cancer: results from BOLERO-2. Br J Cancer 2017;116(6):726–30. https://doi.org/10.1038/bjc.2017.25.

[35] Higgins MJ, Jelovac D, Barnathan E, Blair B, Slater S, Powers P, et al. Detection of tumor PIK3CA status in metastatic breast cancer using peripheral blood. Clin Cancer Res 2012;18:3462–9. https://doi.org/10.1158/1078-0432.CCR-11-2696.

[36] Nakai M, Yamada T, Sekiya K, Sato A, Hankyo M, Kuriyama S, et al. Use of liquid biopsy to detect PIK3CA mutation in metastatic breast cancer. J Nippon Med Sch 2022;89:66–71. https://doi.org/10.1272/jnms.JNMS.2022_89-107.

[37] Cardinali B, De Luca G, Tasso R, Coco S, Garuti A, Buzzatti G, et al. Targeting PIK3CA actionable mutations in the circulome: a proof of concept in metastatic breast cancer. Int J Mol Sci 2022;23:6320. https://doi.org/10.3390/ijms23116320.

[38] Cocco E, Carmona FJ, Razavi P, Won HH, Cai Y, Rossi V, et al. Neratinib is effective in breast tumors bearing both amplification and mutation of ERBB2 (HER2). Sci Signal 2018;11. https://doi.org/10.1126/SCISIGNAL.AAT9773.

[39] Deniziaut G, Tille JC, Bidard FC, Vacher S, Schnitzler A, Chemlali W, et al. ERBB2 mutations associated with solid variant of high-grade invasive lobular breast carcinomas. Oncotarget 2016;7:73337–46. https://doi.org/10.18632/ONCOTARGET.11819.

[40] Desmedt C, Zoppoli G, Gundem G, Pruneri G, Larsimont D, Fornili M, et al. Genomic characterization of primary invasive lobular breast cancer. J Clin Oncol : Official Journal of the American Society of Clinical Oncology 2016;34:1872–80. https://doi.org/10.1200/JCO.2015.64.0334.

[41] Eli LD, Kavuri SM. Mechanisms of neratinib resistance in HER2-mutant metastatic breast cancer. Cancer Drug Resistance 2022;5. https://doi.org/10.20517/CDR.2022.48. 873–873.

[42] Shishido SN, Masson R, Xu L, Welter L, Prabakar RK, D' Souza A, et al. Disease characterization in liquid biopsy from HER2-mutated, non-amplified metastatic breast cancer patients treated with neratinib. NPJ Breast Cancer 2022;8:22. https://doi.org/10.1038/s41523-022-00390-5.

[43] Turner NC, Kingston B, Kilburn LS, Kernaghan S, Wardley AM, Macpherson IR, et al. Circulating tumour DNA analysis to direct therapy in advanced breast cancer (plasmaMATCH): a multicentre, multicohort, phase 2a, platform trial. Lancet Oncol 2020;21:1296–308. https://doi.org/10.1016/S1470-2045(20)30444-7.

[44] Zheng D, Li J, Yan H, Zhang G, Li W, Chu E, et al. Emerging roles of Aurora-A kinase in cancer therapy resistance. Acta Pharm Sin B 2023;13:2826–43. https://doi.org/10.1016/j.apsb.2023.03.013.

[45] Haddad TC, Suman VJ, D'Assoro AB, Carter JM, Giridhar KV, McMenomy BP, et al. Evaluation of alisertib alone or combined with fulvestrant in patients with endocrine-resistant advanced breast cancer: the phase 2 TBCRC041 randomized clinical trial. JAMA Oncol 2023;9:815–24. https://doi.org/10.1001/jamaoncol.2022.7949.

[46] Turner N, Pearson A, Sharpe R, Lambros M, Geyer F, Lopez-Garcia MA, et al. FGFR1 amplification drives endocrine therapy resistance and is a therapeutic target in breast cancer. Cancer Res 2010;70:2085–94. https://doi.org/10.1158/0008-5472.CAN-09-3746.

[47] Formisano L, Lu Y, Servetto A, Hanker AB, Jansen VM, Bauer JA, et al. Aberrant FGFR signaling mediates resistance to CDK4/6 inhibitors in ER+ breast cancer. Nat Commun 2019;10:1373. https://doi.org/10.1038/s41467-019-09068-2.

[48] Neven P, Petrakova K, Val Bianchi G, et al. Abstract PD2-05: biomarker analysis by baseline circulating tumor DNA alterations in the MONALEESA-3 study. Cancer Res 2019;79:PD2–5.

[49] Hortobagyi G, Stemmer S, Campone M, Sonke G, Arteaga C, Paluch-Shimon S, et al. Abstract PD4-06: first-line ribociclib + letrozole in hormone receptor-positive, HER2-negative advanced breast cancer: efficacy by baseline circulating tumor DNA alterations in MONALEESA-2. Cancer Res 2018;78:PD4–6. https://doi.org/10.1158/1538-7445.SABCS17-PD4-06.

[50] Musolino A, Campone M, Neven P, Denduluri N, Barrios CH, Cortes J, et al. Phase II, randomized, placebo-controlled study of dovitinib in combination with fulvestrant in postmenopausal patients with HR+, HER2− breast cancer that had progressed during or after prior endocrine therapy. Breast Cancer Res 2017;19:18. https://doi.org/10.1186/s13058-017-0807-8.

[51] Allison KH, Hammond MEH, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, et al. Estrogen and Progesterone Receptor Testing in Breast Cancer: ASCO/CAP Guideline Update. J Clin Oncol 2020;38:1346–66. https://doi.org/10.1200/JCO.19.02309.

[52] Villegas SL, Nekljudova V, Pfarr N, Engel J, Untch M, Schrodi S, et al. Therapy response and prognosis of patients with early breast cancer with low positivity for hormone receptors – an analysis of 2765 patients from neoadjuvant clinical trials. Eur J Cancer 2021;148:159–70. https://doi.org/10.1016/j.ejca.2021.02.020.

[53] Paakkola N-M, Karakatsanis A, Mauri D, Foukakis T, Valachis A. The prognostic and predictive impact of low estrogen receptor expression in early breast cancer: a systematic review and meta-analysis. ESMO Open 2021;6:100289. https://doi.org/10.1016/j.esmoop.2021.100289.

[54] Chen T, Zhang N, Moran MS, Su P, Haffty BG, Yang Q. Borderline ER-positive primary breast cancer gains no significant survival benefit from endocrine therapy: a systematic review and meta-analysis. Clin Breast Cancer 2018;18:1–8. https://doi.org/10.1016/j.clbc.2017.06.005.

[55] Kim MC, Park MH, Choi JE, Kang SH, Bae YK. Characteristics and prognosis of estrogen receptor low-positive breast cancer. J Breast Cancer 2022;25:318–26. https://doi.org/10.4048/jbc.2022.25.e31.

[56] Dieci MV, Griguolo G, Bottosso M, Tsvetkova V, Giorgi CA, Vernaci G, et al. Impact of estrogen receptor levels on outcome in non-metastatic triple negative breast cancer patients treated with neoadjuvant/adjuvant chemotherapy. Npj Breast Cancer 2021;7:101. https://doi.org/10.1038/s41523-021-00308-7.

[57] Schuster EF, Lopez-Knowles E, Alataki A, Zabaglo L, Folkerd E, Evans D, et al. Molecular profiling of aromatase inhibitor sensitive and resistant ER+HER2- postmenopausal breast cancers. Nat Commun 2023;14:4017. https://doi.org/10.1038/s41467-023-39613-z.

[58] Dixon JM, Cameron DA, Arthur LM, Axelrod DM, Renshaw L, Thomas JS, et al. Accurate estrogen receptor quantification in patients with negative and low-positive estrogen-receptor-expressing breast tumors: sub-analyses of data from two clinical studies. Adv Ther 2019;36:828–41. https://doi.org/10.1007/s12325-019-0896-0.

[59] Miglietta F, Fabi A, Generali D, Dieci MV, Arpino G, Bianchini G, et al. Optimizing choices and sequences in the diagnostic-therapeutic landscape of advanced triple-negative breast cancer: an Italian consensus paper and critical review. Cancer Treat Rev 2023;114:102511. https://doi.org/10.1016/j.ctrv.2023.102511.

[60] Licata L, Barreca M, Galbardi B, Dugo M, Viale G, Győrffy B, et al. Breast cancers with high proliferation and low ER-related signalling have poor prognosis and unique molecular features with implications for therapy. Br J Cancer 2023;129:2025–33. https://doi.org/10.1038/s41416-023-02477-7.

[61] Voorwerk L, Sanders J, Keusters MS, Balduzzi S, Cornelissen S, Duijst M, et al. Immune landscape of breast tumors with low and intermediate estrogen receptor expression. Npj Breast Cancer 2023;9:1–10. https://doi.org/10.1038/s41523-023-00543-0.

[62] Cejalvo JM, Martínez de Dueñas E, Galván P, García-Recio S, Burgués Gasión O, Paré L, et al. Intrinsic subtypes and gene expression profiles in primary and metastatic breast cancer. Cancer Res 2017;77:2213–21. https://doi.org/10.1158/0008-5472.CAN-16-2717.

[63] Morganti S, Marra A, Gandini S, Ascione L, Ivanova M, Venetis K, et al. Clinicopathological features and survival outcomes of luminal-like breast tumors with estrogen receptor loss at metastatic recurrence: a case-control study. Eur J Cancer 2023;195:113397. https://doi.org/10.1016/j.ejca.2023.113397.

[64] Cristofanilli M, Budd GT, Ellis MJ, Stopeck A, Matera J, Miller MC, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 2004;351:781–91. https://doi.org/10.1056/NEJMoa040766.

[65] Serafini MS, Reduzzi C, Gerratana L, Jeselsohn R, Mahtani RL, Ma CX, et al. Abstract 3707: characterization of estrogen receptor expression on CTCs during CDK4/6i treatment in HR+/HER2- metastatic breast cancer: results from the PACE phase II study. Cancer Res 2024;84:3707. https://doi.org/10.1158/1538-7445.AM2024-3707.

[66] Basile D, Cinausero M, Iacono D, Pelizzari G, Bonotto M, Vitale MG, et al. Androgen receptor in estrogen receptor positive breast cancer: beyond expression. Cancer Treat Rev 2017;61:15–22. https://doi.org/10.1016/j.ctrv.2017.09.006.

[67] Li W, O'Shaughnessy J, Hayes D, Campone M, Bondarenko I, Zbarskaya I, et al. Biomarker associations with efficacy of abiraterone acetate and exemestane in postmenopausal patients with estrogen receptor–positive metastatic breast cancer. Clin Cancer Res 2016;22:6002–9. https://doi.org/10.1158/1078-0432.CCR-15-2452.

[68] Dai C, Ellisen LW. Revisiting androgen receptor signaling in breast cancer. Oncologist 2023;28:383–91. https://doi.org/10.1093/oncolo/oyad049.

[69] Hickey TE, Robinson JLL, Carroll JS, Tilley WD. Minireview: the androgen receptor in breast tissues: growth inhibitor, tumor suppressor, oncogene? Mol Endocrinol 2012;26:1252–67. https://doi.org/10.1210/me.2012-1107.

[70] Unni E, Sun S, Nan B, McPhaul MJ, Cheskis B, Mancini MA, et al. Changes in androgen receptor nongenotropic signaling correlate with transition of LNCaP cells to androgen independence. Cancer Res 2004;64:7156–68. https://doi.org/10.1158/0008-5472.CAN-04-1121.

[71] Anbarasu S, Anbarasu A. Cancer-biomarkers associated with sex hormone receptors and recent therapeutic advancements: a comprehensive review. Med Oncol 2023;40:171. https://doi.org/10.1007/s12032-023-02044-3.

[72] Gerratana L, Basile D, Buono G, De Placido S, Giuliano M, Minichillo S, et al. Androgen receptor in triple negative breast cancer: a potential target for the targetless subtype. Cancer Treat Rev 2018;68:102–10. https://doi.org/10.1016/j.ctrv.2018.06.005.

[73] Stella S, Martorana F, Massimino M, Vitale SR, Manzella L, Vigneri P. Potential therapeutic targets for luminal androgen receptor breast cancer: what we know so far. Onco Targets Ther 2023;16:235–47. https://doi.org/10.2147/OTT.S379867.

[74] Ravaioli S, Maltoni R, Pasculli B, Parrella P, Giudetti AM, Vergara D, et al. Androgen receptor in breast cancer: the "5W" questions. Front Endocrinol 2022;13:977331. https://doi.org/10.3389/fendo.2022.977331.

[75] Stender JD, Kim K, Charn TH, Komm B, Chang KCN, Kraus WL, et al. Genome-wide analysis of estrogen receptor α DNA binding and tethering mechanisms identifies Runx1 as a novel tethering factor in receptor-mediated transcriptional activation. Mol Cell Biol 2010;30:3943–55. https://doi.org/10.1128/MCB.00118-10.

[76] Hu R, Dawood S, Holmes MD, Collins LC, Schnitt SJ, Cole K, et al. Androgen receptor expression and breast cancer survival in postmenopausal women. Clin Cancer Res 2011;17:1867–74. https://doi.org/10.1158/1078-0432.CCR-10-2021.

[77] Rechoum Y, Rovito D, Iacopetta D, Barone I, Andò S, Weigel NL, et al. AR collaborates with ERα in aromatase inhibitor-resistant breast cancer. Breast Cancer Res Treat 2014;147:473–85. https://doi.org/10.1007/s10549-014-3082-8.

[78] Cao L, Xiang G, Liu F, Xu C, Liu J, Meng Q, et al. A high AR:ERα or PDEF:ERα ratio predicts a sub-optimal response to tamoxifen therapy in ERα-positive breast cancer. Cancer Chemother Pharmacol 2019;84:609–20. https://doi.org/10.1007/s00280-019-03891-6.

[79] Rangel N, Rondon-Lagos M, Annaratone L, Osella-Abate S, Metovic J, Mano MP, et al. The role of the AR/ER ratio in ER-positive breast cancer patients. Endocr Relat Cancer 2018;25:163–72. https://doi.org/10.1530/ERC-17-0417.

[80] Ng CHM, Macpherson I, Rea D, Spicer J, Bowman A, Jones A, et al. Phase I/II trial of abiraterone acetate (AA) in estrogen receptor (ERα) or androgen receptor (AR) positive metastatic breast cancer (MBC). Ann Oncol 2012;23:ix119. https://doi.org/10.1016/S0923-7534(20)32964-1.

[81] O'Shaughnessy J, Campone M, Brain E, Neven P, Hayes D, Bondarenko I, et al. Abiraterone acetate, exemestane or the combination in postmenopausal patients with estrogen receptor-positive metastatic breast cancer. Ann Oncol 2016;27:106–13. https://doi.org/10.1093/annonc/mdv487.

[82] Palmieri C, Linden H, Birrell SN, Wheelwright S, Lim E, Schwartzberg LS, et al. Activity and safety of enobosarm, a novel, oral, selective androgen receptor modulator, in androgen receptor-positive, oestrogen receptor-positive, and HER2-negative advanced breast cancer (Study G200802): a randomised, open-label, multicentre, multinational, parallel design, phase 2 trial. Lancet Oncol 2024;25:317–25. https://doi.org/10.1016/S1470-2045(24)00004-4.

[83] Bernardo GM, Keri RA. FOXA1: a transcription factor with parallel functions in development and cancer. Biosci Rep 2011;32:113–30. https://doi.org/10.1042/BSR20110046.

[84] Lacroix M, Leclercq G. About GATA3, HNF3A, and XBP1, three genes co-expressed with the oestrogen receptor-α gene (ESR1) in breast cancer. Mol Cell Endocrinol 2004;219:1–7. https://doi.org/10.1016/j.mce.2004.02.021.

[85] Badve S, Turbin D, Thorat MA, Morimiya A, Nielsen TO, Perou CM, et al. FOXA1 expression in breast cancer—correlation with luminal subtype A and survival. Clin Cancer Res 2007;13:4415–21. https://doi.org/10.1158/1078-0432.CCR-07-0122.

[86] Mehta RJ, Jain RK, Leung S, Choo J, Nielsen T, Huntsman D, et al. FOXA1 is an independent prognostic marker for ER-positive breast cancer. Breast Cancer Res Treat 2012;131:881–90. https://doi.org/10.1007/s10549-011-1482-6.

[87] Gong C, Fujino K, Monteiro LJ, Gomes AR, Drost R, Davidson-Smith H, et al. FOXA1 repression is associated with loss of BRCA1 and increased promoter methylation and chromatin silencing in breast cancer. Oncogene 2015;34:5012–24. https://doi.org/10.1038/onc.2014.421.

[88] Hurtado A, Holmes KA, Ross-Innes CS, Schmidt D, Carroll JS. FOXA1 is a key determinant of estrogen receptor function and endocrine response. Nat Genet 2011;43:27–33. https://doi.org/10.1038/ng.730.

[89] Fu X, Jeselsohn R, Pereira R, Hollingsworth EF, Creighton CJ, Li F, et al. FOXA1 overexpression mediates endocrine resistance by altering the ER transcriptome and IL-8 expression in ER-positive breast cancer. Proc Natl Acad Sci USA 2016;113:E6600–9. https://doi.org/10.1073/pnas.1612835113.

[90] Fu X, Pereira R, Liu C-C, De Angelis C, Shea MJ, Nanda S, et al. High FOXA1 levels induce ER transcriptional reprogramming, a pro-metastatic secretome, and metastasis in endocrine-resistant breast cancer. Cell Rep 2023;42:112821. https://doi.org/10.1016/j.celrep.2023.112821.

[91] Ross-Innes CS, Stark R, Teschendorff AE, Holmes KA, Ali HR, Dunning MJ, et al. Differential oestrogen receptor binding is associated with clinical outcome in breast cancer. Nature 2012;481:389–93. https://doi.org/10.1038/nature10730.

[92] Arruabarrena-Aristorena A, Toska E. FOXA1 mutations influence the therapeutic response of breast cancer by altering chromatin state. Mol Cell Oncol 2021;8(3):1891831. https://doi.org/10.1080/23723556.2021.1891831.

[93] Fu X, Pereira R, De Angelis C, Veeraraghavan J, Nanda S, Qin L, et al. FOXA1 upregulation promotes enhancer and transcriptional reprogramming in endocrine-resistant breast cancer. Proc Natl Acad Sci U S A 2019;116(52): 26823–34. https://doi.org/10.1073/pnas.1911584116.
[94] Lee JJ-K, Jung YL, Cheong T-C, Espejo Valle-Inclan J, Chu C, Gulhan DC, et al. ERα-associated translocations underlie oncogene amplifications in breast cancer. Nature 2023;618:1024–32. https://doi.org/10.1038/s41586-023-06057-w.
[95] Jeselsohn R, Barry WT, Migliaccio I, Biagioni C, Zhao J, De Tribolet-Hardy J, et al. TransCONFIRM: identification of a genetic signature of response to fulvestrant in advanced hormone receptor–positive breast cancer. Clin Cancer Res 2016;22:5755–64. https://doi.org/10.1158/1078-0432.CCR-16-0148.
[96] Hofstra RMW, Landsvater RM, Ceccherini I, Stulp RP, Stelwagen T, Luo Y, et al. A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. Nature 1994;367: 375–6. https://doi.org/10.1038/367375a0.
[97] Wang R, Hu H, Pan Y, Li Y, Ye T, Li C, et al. RET fusions define a unique molecular and clinicopathologic subtype of non–small-cell lung cancer. J Clin Oncol 2012; 30:4352–9. https://doi.org/10.1200/JCO.2012.44.1477.
[98] Pietrantonio F, Di Nicolantonio F, Schrock AB, Lee J, Morano F, Fucà G, et al. RET fusions in a small subset of advanced colorectal cancers at risk of being neglected. Ann Oncol 2018;29:1394–401. https://doi.org/10.1093/annonc/mdy090.
[99] Wu J, Subbiah V. RETooling the RET inhibitor pralsetinib for ESR1 fusion–positive breast cancer and beyond. Cancer Res 2023;83:3159–61. https://doi.org/10.1158/0008-5472.CAN-23-1021.
[100] Mulligan LM GDNF. and the RET receptor in cancer: new insights and therapeutic potential. Front Physiol 2019;9:1873. https://doi.org/10.3389/fphys.2018.01873.
[101] Plaza-Menacho I, Morandi A, Robertson D, Pancholi S, Drury S, Dowsett M, et al. Targeting the receptor tyrosine kinase RET sensitizes breast cancer cells to tamoxifen treatment and reveals a role for RET in endocrine resistance. Oncogene 2010;29:4648–57. https://doi.org/10.1038/onc.2010.209.
[102] Pecar G, Liu S, Hooda J, Atkinson JM, Oesterreich S, Lee AV. RET signaling in breast cancer therapeutic resistance and metastasis. Breast Cancer Res 2023;25: 26. https://doi.org/10.1186/s13058-023-01622-7.
[103] Paratala BS, Chung JH, Williams CB, Yilmazel B, Petrosky W, Williams K, et al. RET rearrangements are actionable alterations in breast cancer. Nat Commun 2018;9:4821. https://doi.org/10.1038/s41467-018-07341-4.
[104] Martínez-Galán J, Torres-Torres B, Núñez MI, López-Peñalver J, Del Moral R, Ruiz De Almodóvar JM, et al. ESR1 gene promoter region methylation in free circulating DNA and its correlation with estrogen receptor protein expression in tumor tissue in breast cancer patients. BMC Cancer 2014;14:59. https://doi.org/10.1186/1471-2407-14-59.
[105] Mastoraki S, Strati A, Tzanikou E, Chimonidou M, Politaki E, Voutsina A, et al. ESR1 methylation: a liquid biopsy–based epigenetic assay for the follow-up of patients with metastatic breast cancer receiving endocrine treatment. Clin Cancer Res 2018;24:1500–10. https://doi.org/10.1158/1078-0432.CCR-17-1181.
[106] Gerratana L, Basile D, Franzoni A, Allegri L, Viotto D, Corvaja C, et al. Plasma-based longitudinal evaluation of ESR1 epigenetic status in hormone receptor-positive HER2-negative metastatic breast cancer. Front Oncol 2020;10:550185. https://doi.org/10.3389/fonc.2020.550185.
[107] Will M, Liang J, Metcalfe C, Chandarlapaty S. Therapeutic resistance to anti-oestrogen therapy in breast cancer. Nat Rev Cancer 2023;23:673–85. https://doi.org/10.1038/s41568-023-00604-3.
[108] Tsimberidou AM, Fountzilas E, Nikanjam M, Kurzrock R. Review of precision cancer medicine: evolution of the treatment paradigm. Cancer Treat Rev 2020;86: 102019. https://doi.org/10.1016/j.ctrv.2020.102019.
[109] Tan AC, Tan DSW. Targeted therapies for lung cancer patients with oncogenic driver molecular alterations. J Clin Oncol 2022;40:611–25. https://doi.org/10.1200/JCO.21.01626.
[110] Uson Junior PLS, Bearss J, Babiker HM, Borad MJ. Novel precision therapies for cholangiocarcinoma: an overview of clinical trials. Expet Opin Invest Drugs 2023; 32:69–75. https://doi.org/10.1080/13543784.2023.2173064.
[111] Finn RS, Martin M, Rugo HS, Jones S, Im S-A, Gelmon K, et al. Palbociclib and letrozole in advanced breast cancer. N Engl J Med 2016;375:1925–36. https://doi.org/10.1056/NEJMoa1607303.
[112] Hortobagyi GN, Stemmer SM, Burris HA, Yap YS, Sonke GS, Paluch-Shimon S, et al. Updated results from MONALEESA-2, a phase III trial of first-line ribociclib plus letrozole versus placebo plus letrozole in hormone receptor-positive, HER2-negative advanced breast cancer. Ann Oncol 2018;29:1541–7. https://doi.org/10.1093/annonc/mdy155.
[113] Goetz MP, Toi M, Campone M, Sohn J, Paluch-Shimon S, Huober J, et al. MONARCH 3: abemaciclib as initial therapy for advanced breast cancer. J Clin Oncol 2017;35:3638–46. https://doi.org/10.1200/JCO.2017.75.6155.
[114] Hachem GE, Gombos A, Awada A. Recent advances in understanding breast cancer and emerging therapies with a focus on luminal and triple-negative breast cancer. F1000Res 2019 Apr 30;30. https://doi.org/10.12688/f1000research. F1000 Faculty Rev-591.
[115] Crimini E, Repetto M, Aftimos P, Botticelli A, Marchetti P, Curigliano G. Precision medicine in breast cancer: from clinical trials to clinical practice. Cancer Treat Rev 2021;98:102223. https://doi.org/10.1016/j.ctrv.2021.102223.
[116] Jeselsohn R, Buchwalter G, De Angelis C, Brown M, Schiff R. ESR1 mutations—a mechanism for acquired endocrine resistance in breast cancer. Nat Rev Clin Oncol 2015;12:573–83. https://doi.org/10.1038/nrclinonc.2015.117.
[117] Turner NC, Swift C, Kilburn L, Fribbens C, Beaney M, Garcia-Murillas I, et al. ESR1 mutations and overall survival on fulvestrant versus exemestane in advanced hormone receptor–positive breast cancer: a combined analysis of the phase III SoFEA and efect trials. Clin Cancer Res 2020;26:5172–7. https://doi.org/10.1158/1078-0432.CCR-20-0224.
[118] Gennari A, André F, Barrios CH, Cortés J, de Azambuja E, DeMichele A, et al. ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer. Ann Oncol 2021;32:1475–95. https://doi.org/10.1016/j.annonc.2021.09.019.
[119] Bidard F-C, Kaklamani VG, Neven P, Streich G, Montero AJ, Forget F, et al. Elacestrant (oral selective estrogen receptor degrader) 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. J Clin Oncol 2022;40:3246–56. https://doi.org/10.1200/JCO.22.00338.
[120] Tolaney SM, Chan A, Petrakova K, Delaloge S, Campone M, Iwata H, et al. AMEERA-3: randomized phase II study of amcenestrant (oral selective estrogen receptor degrader) versus standard endocrine monotherapy in estrogen receptor–positive, human epidermal growth factor receptor 2–negative advanced breast cancer. J Clin Oncol 2023;41:4014–24. https://doi.org/10.1200/JCO.22.02746.
[121] Jimenez MM, Lim E, Gregor MCM, Bardia A, Wu J, Zhang Q, et al. 211MO Giredestrant (GDC-9545) vs physician choice of endocrine monotherapy (PCET) in patients (pts) with ER+, HER2– locally advanced/metastatic breast cancer (LA/mBC): primary analysis of the phase II, randomised, open-label acelERA BC study. Ann Oncol 2022;33:S633–4. https://doi.org/10.1016/j.annonc.2022.07.250.
[122] Oliveira M, Pominchuck D, Nowecki Z, Hamilton E, Kulyaba Y, Andabekov T, et al. Abstract GS3-02: GS3-02 Camizestrant, a next generation oral SERD vs fulvestrant in post-menopausal women with advanced ER-positive HER2-negative breast cancer: results of the randomized, multi-dose Phase 2 SERENA-2 trial. Cancer Res 2023;83. https://doi.org/10.1158/1538-7445.SABCS22-GS3-02. GS3-02.
[123] Wong NZH, Yap DWT, Ong RJM, Zhao JJ, Chan YH, Tey JCS, et al. Efficacy of Oral SERDs in the treatment of ER+, HER2 - metastatic breast cancer, a stratified analysis of the ESR1 wild type and mutant subgroups. Ann Oncol 2023. https://doi.org/10.1016/j.annonc.2023.10.122.
[124] Bidard F-C, Hardy-Bessard A-C, Dalenc F, Bachelot T, Pierga J-Y, de la Motte Rouge T, 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. Lancet Oncol 2022;23:1367–77. https://doi.org/10.1016/S1470-2045(22)00555-1.
[125] Cabel L, Delaloge S, Hardy-Bessard A-C, Andre F, Bachelot T, Bieche I, et al. Dynamics and type of *ESR1* mutations under aromatase inhibitor or fulvestrant combined with palbociclib after randomization in the PADA-1 trial. J Clin Oncol 2023;41. https://doi.org/10.1200/JCO.2023.41.16_suppl.1002. 1002–1002.
[126] Mayer EL, Ren Y, Wagle N, Mahtani R, Ma C, DeMichele A, et al. Abstract GS3-06: GS3-06 palbociclib after CDK4/6i and endocrine therapy (PACE): a randomized phase II study of fulvestrant, palbociclib, and avelumab for endocrine pre-treated er+/HER2- metastatic breast cancer. Cancer Res 2023;83:GS3–6. https://doi.org/10.1158/1538-7445.SABCS22-GS3-06.
[127] Gerratana L, Davis AA, Velimirovic M, Reduzzi C, Clifton K, Bucheit L, et al. Cyclin-dependent kinase 4/6 inhibitors beyond progression in metastatic breast cancer: a retrospective real-world biomarker analysis. JCO Precis Oncol; 2023, e2200531. https://doi.org/10.1200/PO.22.00531.
[128] Bidard F-C, Kalinsky K, Cristofanilli M, Bianchini G, Chia SK, Janni W, et al. Abstract OT2-11-05: SERENA-6: a Phase III study to assess the efficacy and safety of AZD9833 (camizestrant) compared with aromatase inhibitors when given in combination with palbociclib or abemaciclib in patients with HR+/HER2-metastatic breast cancer with detectable ESR1m who have not experienced disease progression on first-line therapy. Cancer Res 2022;82. https://doi.org/10.1158/1538-7445.SABCS21-OT2-11-05. OT2-11–05.
[129] Turner N, Huang-Bartlett C, Kalinsky K, Cristofanilli M, Bianchini G, Chia S, et al. Design of SERENA-6, a phase III switching trial of camizestrant in *ESR1* -mutant breast cancer during first-line treatment. Future Oncol 2023;19:559–73. https://doi.org/10.2217/fon-2022-1196.
[130] Bose R, Kavuri SM, Searleman AC, Shen W, Shen D, Koboldt DC, et al. Activating HER2 mutations in HER2 gene amplification negative breast cancer. Cancer Discov 2013;3:224–37. https://doi.org/10.1158/2159-8290.CD-12-0349.
[131] Hyman DM, Piha-Paul SA, Won H, Rodon J, Saura C, Shapiro GI, et al. HER kinase inhibition in patients with HER2- and HER3-mutant cancers. Nature 2018;554: 189–94. https://doi.org/10.1038/NATURE25475.
[132] Ma CX, Luo J, Freedman RA, Pluard TJ, Nangia JR, Lu J, et al. The phase II MutHER study of neratinib alone and in combination with fulvestrant in HER2-mutated, non-amplified metastatic breast cancer. Clin Cancer Res 2022;28: 1258–67. https://doi.org/10.1158/1078-0432.CCR-21-3418.
[133] Smyth LM, Piha-Paul SA, Won HH, Schram AM, Saura C, Loi S, et al. Efficacy and determinants of response to HER kinase inhibition in HER2-mutant metastatic breast cancer. Cancer Discov 2020;10:198–213. https://doi.org/10.1158/2159-8290.CD-19-0966.
[134] Jhaveri K, Eli LD, Wildiers H, Hurvitz SA, Guerrero-Zotano A, Unni N, et al. Neratinib + fulvestrant + trastuzumab for HR-positive, HER2-negative, HER2-mutant metastatic breast cancer: outcomes and biomarker analysis from the SUMMIT trial. Ann Oncol 2023;34:885–98. https://doi.org/10.1016/j.annonc.2023.08.003.
[135] Ma CX, Bose R, Gao F, Freedman RA, Telli ML, Kimmick G, et al. Neratinib efficacy and circulating tumor DNA detection of HER2 mutations in HER2 nonamplified metastatic breast cancer. Clin Cancer Res 2017;23:5687–95. https://doi.org/10.1158/1078-0432.CCR-17-0900.

[136] Tolaney S, Toi M, Neven P, et al. Abstract 4458: clinical significance of PIK3CA and ESR1 mutations in ctDNA and FFPE samples from the MONARCH 2 study of abemaciclib plus fulvestrant. Cancer Res 2019;79. 4458-4458.
[137] André F, Su F, Solovieff N, Hortobagyi G, Chia S, Neven P, et al. Pooled ctDNA analysis of MONALEESA phase III advanced breast cancer trials. Ann Oncol 2023; S0923–7534(23). https://doi.org/10.1016/j.annonc.2023.08.011. 00826-8.
[138] Cristofanilli M, Turner NC, Bondarenko I, Ro J, Im SA, Masuda N, et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phas. Lancet Oncol 2016;17:425–39. https://doi.org/10.1016/S1470-2045(15)00613-0.
[139] Cristofanilli M, Rugo HS, Im SA, Slamon DJ, Harbeck N, Bondarenko I, et al. Overall survival with palbociclib and fulvestrant in women with HR+/HER2-ABC: updated exploratory analyses of PALOMA-3, a double-blind, phase III randomized study. Clin Cancer Res 2022;28:3433–42. https://doi.org/10.1158/1078-0432.CCR-22-0305.
[140] Yardley DA, Noguchi S, Pritchard KI, Burris HA, Baselga J, Gnant M, et al. Everolimus plus exemestane in postmenopausal patients with HR+ breast cancer: BOLERO-2 final progression-free survival analysis. Adv Ther 2013;30:870–84. https://doi.org/10.1007/s12325-013-0060-1.
[141] Piccart M, Hortobagyi GN, Campone M, Pritchard KI, Lebrun F, Ito Y, 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;25:2357–62. https://doi.org/10.1093/annonc/mdu456.
[142] André F, Ciruelos EM, Juric D, Loibl S, Campone M, Mayer IA, et al. Alpelisib plus fulvestrant for PIK3CA-mutated, hormone receptor-positive, human epidermal growth factor receptor-2–negative advanced breast cancer: final overall survival results from SOLAR-1. Ann Oncol 2021;32:208–17. https://doi.org/10.1016/j.annonc.2020.11.011.
[143] Juric D, Ciruelos E, Rubovszky G, et al. Abstract GS3-08: alpelisib + fulvestrant for advanced breast cancer: subgroup analyses from the phase III SOLAR-1 trial. Cancer Res 2019;79:GS3–8.
[144] Ciruelos E, Loibl S, Mayer I, et al. Abstract PD2-06: clinical outcomes of alpelisib plus fulvestrant in hormone receptor-positive, human epidermal growth factor receptor-2-negative advanced breast cancer with PIK3CA alterations detected in plasma ctDNA by next-generation sequencing: biomark. Cancer Res 2021;81. PD2-06-PD2-06.
[145] Rugo HS, Lerebours F, Ciruelos E, Drullinsky P, Ruiz-Borrego M, Neven P, 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. Lancet Oncol 2021;22:489–98. https://doi.org/10.1016/S1470-2045(21)00034-6.
[146] Jacobson A. Alpelisib plus fulvestrant or letrozole demonstrates sustained benefits across subgroups of patients with PIK3CA-mutated HR+/HER2-Advanced breast cancer. Oncologist 2022;27:S13–4. https://doi.org/10.1093/oncolo/oyac011.
[147] De Laurentiis M, Costa L, Gligorov J, Knop A, Senkus-Konefka E, García-Sáenz J, et al. EPIK-B5: a phase III, randomized study of alpelisib (ALP) plus fulvestrant (FUL) in patients with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2-), PIK3CA -mutated advanced breast cancer (ABC) progressing on/after an aromatase inhibitor (AI) with a cyclin-dependent kinase 4/6 inhibitor (CDK4/6i). J Clin Oncol 2022;40. https://doi.org/10.1200/JCO.2022.40.16_suppl.TPS1109. TPS1109–TPS1109.
[148] Juric D, Kalinsky K, Im S-A, 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). J Clin Oncol 2023;41. https://doi.org/10.1200/JCO.2023.41.16_suppl.TPS1123. TPS1123-TPS1123.
[149] Jones RH, Casbard A, Carucci M, Cox C, Butler R, Alchami F, et al. Fulvestrant plus capivasertib versus placebo after relapse or progression on an aromatase inhibitor in metastatic, oestrogen receptor-positive breast cancer (FAKTION): a multicentre, randomised, controlled, phase 2 trial. Lancet Oncol 2020;21:345–57. https://doi.org/10.1016/S1470-2045(19)30817-4.
[150] Howell SJ, Casbard A, Carucci M, Ingarfield K, Butler R, Morgan S, et al. Fulvestrant plus capivasertib versus placebo after relapse or progression on an aromatase inhibitor in metastatic, oestrogen receptor-positive, HER2-negative breast cancer (FAKTION): overall survival, updated progression-free survival, and expanded biomar. Lancet Oncol 2022;23:851–64. https://doi.org/10.1016/S1470-2045(22)00284-4.
[151] Lord CJ, Ashworth A. PARP inhibitors: synthetic lethality in the clinic. Science 2017;355:1152–8. https://doi.org/10.1126/science.aam7344.
[152] Farmer H, McCabe N, Lord CJ, Tutt ANJ, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 2005;434:917–21. https://doi.org/10.1038/nature03445.
[153] Gerratana L, Davis AA, Shah AN, Lin C, Corvaja C, Cristofanilli M. Emerging role of genomics and cell-free DNA in breast cancer. Curr Treat Options Oncol 2019;20:68. https://doi.org/10.1007/s11864-019-0667-9.
[154] Robson M, Im S-A, Senkus E, Xu B, Domchek SM, Masuda N, et al. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. N Engl J Med 2017;377:523–33. https://doi.org/10.1056/NEJMoa1706450.
[155] Robson ME, Tung N, Conte P, Im S-A, Senkus E, Xu B, et al. OlympiAD final overall survival and tolerability results: olaparib versus chemotherapy treatment of physician's choice in patients with a germline BRCA mutation and HER2-negative metastatic breast cancer. Ann Oncol 2019;30:558–66. https://doi.org/10.1093/annonc/mdz012.
[156] Litton JK, Rugo HS, Ettl J, Hurvitz SA, Gonçalves A, Lee K-H, et al. Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. N Engl J Med 2018;379:753–63. https://doi.org/10.1056/NEJMoa1802905.
[157] Litton JK, Hurvitz SA, Mina LA, Rugo HS, Lee K-H, Gonçalves A, et al. Talazoparib versus chemotherapy in patients with germline BRCA1/2-mutated HER2-negative advanced breast cancer: final overall survival results from the EMBRACA trial. Ann Oncol 2020;31:1526–35. https://doi.org/10.1016/j.annonc.2020.08.2098.
[158] Senkus E, Delaloge S, Domchek SM, Conte P, Im S-A, Xu B, et al. Olaparib efficacy in patients with germline BRCA-mutated, HER2-negative metastatic breast cancer: subgroup analyses from the phase III OlympiAD trial. Int J Cancer 2023;153:803–14. https://doi.org/10.1002/ijc.34525.
[159] Condorelli R, Mosele F, Verret B, Bachelot T, Bedard PL, Cortes J, et al. Genomic alterations in breast cancer: level of evidence for actionability according to ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). Ann Oncol 2019;30:365–73. https://doi.org/10.1093/annonc/mdz036.
[160] McCabe N, Turner NC, Lord CJ, Kluzek K, Białkowska A, Swift S, et al. Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. Cancer Res 2006;66:8109–15. https://doi.org/10.1158/0008-5472.CAN-06-0140.
[161] Galland L, Roussot N, Desmoulins I, Mayeur D, Kaderbhai C, Ilie S, et al. Clinical utility of genomic tests evaluating homologous recombination repair deficiency (HRD) for treatment decisions in early and metastatic breast cancer. Cancers 2023;15:1299. https://doi.org/10.3390/cancers15041299.
[162] Frenel J-S, Lusque A, Delaloge S, Ferrero J-M, Bachelot T, Desmoulins I, et al. Efficacy of front-line treatment for hormone receptor-positive HER2-negative metastatic breast cancer with germline BRCA1/2 mutation. Br J Cancer 2023;128:2072–80. https://doi.org/10.1038/s41416-023-02248-4.
[163] Frenel J-S, Dalenc F, Pistilli B, Rouge T de LM, Levy C, Mouret-Reynier M-A, et al. 304P ESR1 mutations and outcomes in BRCA1/2 or PALB2 germline mutation carriers receiving first line aromatase inhibitor + palbociclib (AI+P) for metastatic breast cancer (MBC) in the PADA-1 trial. Ann Oncol 2020;31:S364. https://doi.org/10.1016/j.annonc.2020.08.406.
[164] Blanch S, Balmaña J, Roso LL, Saenz JÁG, Vegas EF, Jiménez-Rodríguez B, et al. 260P luzern: phase II trial of niraparib and aromatase inhibitors (AI) for pretreated hormone receptor-positive/HER2-negative germline BRCA-mutated advanced breast cancer. ESMO Open 2024;9. https://doi.org/10.1016/j.esmoop.2024.103281.
[165] Guiu S, Balmaña J, Lemercier P, Follana P, Gonçalves A, Bigot F, et al. 179O Combination of olaparib, durvalumab and fulvestrant in patients with advanced ER-positive, HER2-negative breast cancer harboring homologous recombination repair (HRR) deficiency or microsatellite instability (MSI): results of the international phase II DOLAF trial. ESMO Open 2024;9. https://doi.org/10.1016/j.esmoop.2024.103201.
[166] Albarrán V, Chamorro J, Pozas J, San Román M, Rosero DI, Saavedra C, et al. Maintained complete response to talazoparib in a BRCA-2 mutated metastatic luminal breast cancer: case report and review of literature. Front Oncol 2023;13. https://doi.org/10.3389/fonc.2023.1158981.
[167] Piezzo M, Chiodini P, Riemma M, Cocco S, Caputo R, Cianniello D, et al. Progression-free survival and overall survival of CDK 4/6 inhibitors plus endocrine therapy in metastatic breast cancer: a systematic review and meta-analysis. Int J Mol Sci 2020;21:6400. https://doi.org/10.3390/ijms21176400.
[168] O'leary B, Cutts RJ, Huang X, Hrebien S, Liu Y, André F, et al. Circulating tumor DNA markers for early progression on fulvestrant with or without palbociclib in ER+ advanced breast cancer. J Natl Cancer Inst 2021;113:309–17. https://doi.org/10.1093/jnci/djaa087.
[169] Turner NC, Liu Y, Zhu Z, Loi S, Colleoni M, Loibl S, et al. Cyclin E1 expression and palbociclib efficacy in previously treated hormone receptor–positive metastatic breast cancer. J Clin Oncol 2019;37:1169–78. https://doi.org/10.1200/JCO.18.00925.
[170] Mai N, dos Anjos CH, Razavi P, Safonov A, Patil S, Chen Y, et al. Predictors of response to CDK4/6i retrial after prior CDK4/6i failure in ER+ metastatic breast cancer. Npj Breast Cancer 2024;10:1–9. https://doi.org/10.1038/s41523-024-00699-3.
[171] McCartney A, Bonechi M, De Luca F, Biagioni C, Curigliano G, Moretti E, et al. Plasma thymidine kinase activity as a biomarker in patients with luminal metastatic breast cancer treated with palbociclib within the TREnd trial. Clin Cancer Res 2020;26:2131–9. https://doi.org/10.1158/1078-0432.CCR-19-3271.
[172] Cabel L, Rosenblum D, Lerebours F, Brain E, Loirat D, Bergqvist M, et al. Plasma thymidine kinase 1 activity and outcome of ER+ HER2− metastatic breast cancer patients treated with palbociclib and endocrine therapy. Breast Cancer Res 2020;22:98. https://doi.org/10.1186/s13058-020-01334-2.
[173] Malorni L, Tyekucheva S, Hilbers FS, Ignatiadis M, Neven P, Colleoni M, et al. Serum thymidine kinase activity in patients with hormone receptor-positive and HER2-negative metastatic breast cancer treated with palbociclib and fulvestrant. Eur J Cancer 2022;164:39–51. https://doi.org/10.1016/j.ejca.2021.12.030.
[174] Malorni L, Bianchini G, Caputo R, Zambelli A, Puglisi F, Bianchi GV, et al. Serum thymidine kinase activity in patients with HR-positive/HER2-negative advanced breast cancer treated with ribociclib plus letrozole: results from the prospective BioItaLEE trial. Eur J Cancer 2023;186:1–11. https://doi.org/10.1016/j.ejca.2023.03.001.
[175] Bidard F-C, Jacot W, Kiavue N, Dureau S, Kadi A, Brain E, et al. Efficacy of circulating tumor cell count–driven vs clinician-driven first-line therapy choice in hormone receptor–positive, ERBB2-negative metastatic breast cancer: the STIC CTC randomized clinical trial. JAMA Oncol 2021;7:34. https://doi.org/10.1001/jamaoncol.2020.5660.

[176] Bidard F-C, Kiavue N, Jacot W, Bachelot T, Dureau S, Bourgeois H, et al. Overall survival with circulating tumor cell count–driven choice of therapy in advanced breast cancer: a randomized trial. J Clin Oncol 2024;42:383–9. https://doi.org/10.1200/JCO.23.00456.

[177] Gerratana L, Ren Y, Reduzzi C, Regan MM, Mahtani RL, Ma CX, et al. Circulating tumor cells (CTCs) dynamics after CDK4/6i for hormone-receptor positive (HR+) metastatic breast cancer (MBC): a biomarker analysis of the PACE randomized phase II study. J Clin Oncol 2023;41. https://doi.org/10.1200/JCO.2023.41.16_suppl.1059. 1059–1059.

[178] Kasimir-Bauer S, Gruber C, Hoffmann O, Kimmig R, Keup C. Abstract P5-13-33: longitudinal transcriptional profiling of CTCs in metastatic breast cancer patients receiving the CDK4/6 inhibitor Palbociclib to predict therapy response. Cancer Res 2022;82. https://doi.org/10.1158/1538-7445.SABCS21-P5-13-33. P5-13–33.

[179] Schrijver WAME, Suijkerbuijk KPM, van Gils CH, van der Wall E, Moelans CB, van Diest PJ. Receptor conversion in distant breast cancer metastases: a systematic review and meta-analysis. J Natl Cancer Inst 2018;110:568–80. https://doi.org/10.1093/jnci/djx273.

[180] Modi S, Jacot W, Yamashita T, Sohn J, Vidal M, Tokunaga E, et al. Trastuzumab deruxtecan in previously treated HER2-low advanced breast cancer. N Engl J Med 2022;387:9–20. https://doi.org/10.1056/NEJMoa2203690.

[181] Bardia A, Hu X, Dent R, Yonemori K, Barrios CH, O'Shaughnessy JA, et al. Trastuzumab Deruxtecan after Endocrine Therapy in Metastatic Breast Cancer. N Engl J Med 2024. https://doi.org/10.1056/NEJMoa2407086.

[182] Aktas B, Kasimir-Bauer S, Müller V, Janni W, Fehm T, Wallwiener D, et al. Comparison of the HER2, estrogen and progesterone receptor expression profile of primary tumor, metastases and circulating tumor cells in metastatic breast cancer patients. BMC Cancer 2016;16:522. https://doi.org/10.1186/s12885-016-2587-4.

[183] D'Amico P, Reduzzi C, Qiang W, Zhang Y, Gerratana L, Zhang Q, et al. Single-cells isolation and molecular analysis: focus on HER2-low CTCs in metastatic breast cancer. Cancers 2021;14:79. https://doi.org/10.3390/cancers14010079.

[184] Wang L, Hong R, Shi S, Wang S, Chen Y, Han C, et al. The prognostic significance of circulating tumor cell enumeration and HER2 expression by a novel automated microfluidic system in metastatic breast cancer. BMC Cancer 2024;24:1067. https://doi.org/10.1186/s12885-024-12818-1.

[185] Fehm T, Mueller V, Banys-Paluchowski M, Fasching PA, Friedl TWP, Hartkopf A, et al. Efficacy of lapatinib in patients with HER2-negative metastatic breast cancer and HER2-positive circulating tumor cells—the DETECT III clinical trial. Clin Chem 2024;70:307–18. https://doi.org/10.1093/clinchem/hvad144.

[186] Boscolo Bielo L, Trapani D, Repetto M, Crimini E, Valenza C, Belli C, et al. Variant of allele frequency: a decision-making tool in precision oncology? Trends Cancer 2023;S2405–8033(23). https://doi.org/10.1016/j.trecan.2023.08.011. 00171-1.

[187] Strom SP. Current practices and guidelines for clinical next-generation sequencing oncology testing. Cancer Biol Med 2016;13:3–11. https://doi.org/10.28092/j.issn.2095-3941.2016.0004.

[188] Lebofsky R, Decraene C, Bernard V, Kamal M, Blin A, Leroy Q, et al. Circulating tumor DNA as a non-invasive substitute to metastasis biopsy for tumor genotyping and personalized medicine in a prospective trial across all tumor types. Mol Oncol 2015;9:783–90. https://doi.org/10.1016/j.molonc.2014.12.003.

[189] O'Leary B, Hrebien S, Morden JP, Beaney M, Fribbens C, Huang X, et al. Early circulating tumor DNA dynamics and clonal selection with palbociclib and fulvestrant for breast cancer. Nat Commun 2018;9:1–10. https://doi.org/10.1038/s41467-018-03215-x.

[190] Darrigues L, Pierga JY, Bernard-Tessier A, Bièche I, Silveira AB, Michel M, et al. Circulating tumor DNA as a dynamic biomarker of response to palbociclib and fulvestrant in metastatic breast cancer patients. Breast Cancer Res 2021;23:1–10. https://doi.org/10.1186/s13058-021-01411-0.

[191] Jacob S, Davis AA, Gerratana L, Velimirovic M, Shah AN, Wehbe F, et al. The use of serial circulating tumor DNA to detect resistance alterations in progressive metastatic breast cancer. Clin Cancer Res 2021;27:1361–70. https://doi.org/10.1158/1078-0432.CCR-20-1566.

[192] Chiu J, Su F, Joshi M, Masuda N, Ishikawa T, Aruga T, et al. Potential value of ctDNA monitoring in metastatic HR +/HER2 − breast cancer: longitudinal ctDNA analysis in the phase Ib MONALEESASIA trial. BMC Med 2023;21:306. https://doi.org/10.1186/s12916-023-03017-z.

[193] Razavi P, Li BT, Brown DN, Jung B, Hubbell E, Shen R, et al. High-intensity sequencing reveals the sources of plasma circulating cell-free DNA variants. Nat Med 2019;25:1928–37. https://doi.org/10.1038/s41591-019-0652-7.

[194] Slavin TP, Banks KC, Chudova D, Oxnard GR, Odegaard JI, Nagy RJ, et al. Identification of incidental germline mutations in patients with advanced solid tumors who underwent cell-free circulating tumor DNA sequencing. J Clin Oncol 2018;36:3459–65. https://doi.org/10.1200/JCO.18.00328.