The evolving role of receptors as predictive biomarkers for metastatic breast cancer

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Abstract

Introduction: In breast cancer, oestrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2) are essential biomarkers to predict response to endocrine and anti-HER2 therapies, respectively. In metastatic breast cancer, the use of these receptors and targeted therapies present additional challenges: temporal heterogeneity, together with limited sampling methodologies, hinders receptor status assessment and the constant evolution of the disease invariably leads to resistance to treatment.

Areas covered: We summarise genomic abnormalities in ER and HER2, such as mutations, amplifications, translocations and alternative splicing, emerging as novel biomarkers that provide an insight into underlying mechanisms of resistance and hold potential predictive value to inform treatment selection. We also describe how liquid biopsies for sampling of circulating markers and ultrasensitive detection technologies have emerged which complement ongoing efforts for biomarker discovery and analysis.

Expert commentary: While evidence suggests that genomic aberrations in ER and HER2 could contribute to meeting the pressing need for better predictive biomarkers, efforts need to be made to standardise assessment methods and better understand the resistance mechanisms these markers denote. Taking advantage of emerging technologies, research in upcoming years should include prospective trials incorporating these predictors into the study design to validate their potential clinical value.

1. Introduction

Breast cancer is the most common cancer type and the leading cause of cancer-related death in women worldwide. In 2012, there were almost 1.7 million diagnoses and over half a million deaths worldwide, equivalent to more than 1 woman dying from breast cancer every minute[1]. A recent study has estimated that more than 330,000 new breast cancer cases and over 40,000 breast cancer-related deaths are expected in 2018 in the US alone[2].

Breast cancer is a heterogeneous disease comprising several well-characterised molecular subtypes. Patients vary widely in their prognosis and response to different treatments, so biomarkers, defined as characteristics which can be objectively measured and evaluated as indicators of normal biological processes, pathogenesis or responses to therapy[3], have been used for decades to assist in diagnosis, prognosis and treatment decision-making for breast cancer. Biomarkers can be classified as: prognostic, those that estimate the likelihood of an event, such as disease recurrence or progression; or predictive, those that identify patients likely to be responsive or resistant to a specific type of therapy[4].

Prognostic biomarkers include clinicopathological factors, such as axillary node status, tumour grade and size, patient ethnicity and age at diagnosis[5–7]. Other prognosticators are molecular biomarkers, which have been incorporated into a number of multifactor prognostic tests like Oncotype DX and Mammaprint [5,8,9]. These tests enable patient stratification according to risk and are now used in the clinic to varying extents following endorsement by American and European expert panels[5,10].

Both clinicopathological and molecular prognostic biomarkers are useful in the decision-making process for breast cancer management. They help identify patients with worse prognosis or higher risk of disease recurrence who might be more likely to benefit from treatments such as radio or chemotherapy[5]. However, this review will focus on the discussion of predictive biomarkers in breast cancer.

2. Predictive biomarkers in breast cancer: the central role of receptors

Biomarkers with predictive value are often direct indicators of the role of a certain pathway or molecular mechanism in governing cancer growth or progression. Thus, these variables can be useful in predicting the benefit from treatments that specifically tackle the pathway or

mechanism in question. Such targeted therapies are normally advantageous for their efficacy and low toxicity in comparison to other treatment modalities such as radio- or chemotherapy.

Predictive biomarkers can play an essential role in treatment decision-making, to move towards a more personalised and targeted clinical management of the disease. A recent meta-analysis of 570 phase II clinical trials in a diverse range of cancer types has supported the selection of treatment according to the molecular characteristics of each patient's tumour. This study found that the use of personalised targeted therapies is an independent predictor of better outcomes (with higher response rates and longer progression-free and overall survival) and fewer toxic deaths[11].

2.1. Hormone receptors

Oestrogen receptor α (ERα or ER) is the oldest and most important predictive biomarker in breast cancer. Despite the vast heterogeneity in the disease, the majority of breast tumours are hormone-dependent, relying on oestrogen for both carcinogenesis and tumour progression. Oestrogen can exerts its regulatory effect by directly binding the transcription factor ERα, causing its translocation to the nucleus, where it interacts with co-activators and binds specific DNA regions called oestrogen response elements (EREs) which regulate the transcription of oestrogen-responsive genes linked to proliferation, apoptosis, angiogenesis and invasion. This mechanism of action of oestrogen and ER as a transcription factor is referred to as nuclear-initiated steroid signalling (NISS)[12].

Oestrogen and ER can also modulate gene expression and promote cancer progression indirectly through non-genomic signalling, also referred to as membrane-initiated steroid signalling (MISS). Following ligand binding, the oestrogen-ER complex can interact with and activate other transcription factors, such as activating protein 1 (AP1), nuclear factor-κB (NF-κB) and p53, which in turn promote the transcription of their target genes in the nucleus[13–15]. Membrane-bound ER complexes can also trigger rapid activation of signal transduction pathways such as ERK/MAPK and PI3K/Akt, thus modulating protein function in the cell and also indirectly regulating gene expression[13–15].

The genomic and non-genomic actions of ER have been shown to converge at certain regulatory sites by directly and indirectly modulating the same target genes. Ultimately, this dual mechanism expands and diversifies the effect of ER on gene expression. This regulatory

role of ER can also be enhanced by cross-talk with other oncogenic pathways, particularly with growth factor-dependent signalling involving EGFR and HER tyrosine kinases[14,16,17]. Such interactions can lead to activation of ER signalling (including ER itself, even in the absence of ligand), thus enhancing ER-modulated changes in gene expression and have been linked to the development of resistance to endocrine therapy.

Endocrine therapy is used to treat the majority of patients with ER+ breast cancers and is normally the most effective therapy for these tumours[18]. The different endocrine therapy strategies have been well characterised and continue to be investigated[18,19] and the most appropriate endocrine therapy for each patient is normally selected by the multidisciplinary clinical team based on pre- or post-menopausal status, clinicopathological factors and other patient factors such as possible side effects.

Endocrine therapy can target hormone regulation in two distinct ways. Firstly, it can be administered to block oestrogen synthesis. In pre-menopausal women, this can be achieved through surgical ovarian ablation (by oophorectomy) or chemical ovarian suppression (using luteinizing hormone releasing hormone agonists, such as goserilin). In post-menopausal women, the ovaries cease to be the main source of oestrogen production, with oestrogen being instead synthesised from androgens in tissues of the bone, fat and breast through the activity of the enzyme aromatase[20,21]. This can be blocked through the administration of aromatase inhibitors (AI) such as anastrazole, letrozole and exemestane. Secondly, endocrine therapy can directly influence the effect of oestrogen in cancer cells. ER function can be chemically blocked in pre- and post-menopausal women using antioestrogens, including selective oestrogen receptor modulators (SERMs) that act as partial oestrogen agonists, like tamoxifen, or selective oestrogen receptor down-regulators or degraders (SERDs) that act as full oestrogen antagonists, like fulvestrant.

Resistance to endocrine therapy eventually occurs in a large proportion of patients, leading to recurrence or progression[19]. As agents act via different mechanisms, resistance to a specific drug does not necessarily result in resistance to related compounds[22]. Thus, different classes of endocrine treatment are often administered sequentially in order to address the significant hurdle of acquired resistance.

Progesterone receptor (PR) is another nuclear hormone receptor which can be assessed simultaneously with ER. PR is known to regulate epithelial proliferation[23] and can alter

which genes are stimulated by ER binding[24,25]. Its expression is strongly dependent on the presence of ER, so the majority of ER-positive (ER+) cancers are also PR-positive (PR+)[26]. While the independent predictive value of PR has been questioned[27–32], several studies have reported its role as an independent prognosticator of risk of recurrence[33–38].

The rare clinical subgroup of tumours that express ER but not PR (ER+/PR-) have been reported to derive less benefit from certain types of endocrine therapy, specifically SERMs, Response rates are about half of those in the ER+/PR+ group, in both primary and metastatic settings[31,39,40]. Research has suggested that PR loss results from crosstalk between ER and growth factor-related signalling pathways. This means that ER+/PR- cancers are dependent on the higher activity of different pathways than ER+/PR+ tumours, such as the PI3K/Akt/mTOR and EGFR signalling pathways, and would explain why modulation of ER alone using SERMs can be insufficient to treat ER+/PR- cancers.

While hormone-based treatment is widely recommended for all ER+ breast cancers, it has been suggested that PR status should be considered for the selection of the optimal form of endocrine or combination therapy. The ER+/PR- group may be better tackled by complete blockade of ER signalling, either by AI-induced oestrogen withdrawal or SERD-induced ER degradation, or by the combination of a SERM with an agent targeting growth factor-related signalling pathways, such as EGFR or HER2 inhibitors[41,42].

In short, the expression of hormone receptors is indicative of dependence on oestrogen for tumour progression and, consequently, susceptibility to treatment by targeting this hormonal regulation. ER and PR have been used as diagnostic and predictive markers for response to this type of treatment since the 1970s[43,44] and their assessment by immunohistochemistry (IHC) is now mandatory in all breast cancer diagnoses to assist in treatment selection[10]. A recent UK-based large population study reported average positivity rates for ER and PR of 85% and 67%, respectively[45].

2.2. Human epidermal growth factor receptor 2

The human epidermal growth factor receptor 2 (ERBB2 or HER2) is another important predictive biomarker for breast cancer. This receptor, which belongs to a family of transmembrane receptor tyrosine kinases (RTKs), is amplified or overexpressed in 20-25% of

breast cancers[46,47], where it drives tumour growth by activation of the MAPK and PI3K/Akt signalling pathways that lead to proliferation, invasion and metastasis[48].

HER2+ breast cancers are typically more aggressive and have worse clinical prognosis than other subtypes[47,49], so HER2 level has historically been used as a prognostic marker. The development of HER2-targeted therapy led to its gaining predictive value. HER2 overexpression/amplification detection is now used as a biomarker and its measurement is mandatory for all new breast cancer diagnoses. HER2 status can be assessed at protein, RNA or DNA level [50–52] using numerous methodologies, with IHC or fluorescence *in situ* hybridisation (FISH) being the most commonly used. Several studies have reported some discordance across different sites in clinical trials in the assessment of HER2 overexpression using either of these methods[53–57]. This is partly due to variations in reagents, protocols, scoring and the semi-quantitative nature of both methods[58]. Other studies have reported a better degree of internal concordance (>90%) between IHC and FISH when performed within a single site[56,59]. Discordance between IHC and FISH assessment is most commonly found in cases which are borderline for HER2+ status[52]. Accordingly, guidelines recommend both methodologies should be used concomitantly to more accurately evaluate the HER2 status of such cases (e.g., samples with intermediate IHC score should be further assessed using FISH)[58,60–62].

The advent of HER2-targeted therapy has vastly improved the outcome of patients with HER2-overexpressing breast cancers[63,64]. Four different anti-HER2 agents have been approved for clinical use in the US and Europe: trastuzumab and pertuzumab are anti-HER2 monoclonal antibodies, lapatinib is a tyrosine kinase inhibitor and trastuzumab-emtansine (TDM-1) is a conjugate of trastuzumab with a cytotoxic agent [65,66]. Trastuzumab (trade name Herceptin) is the best characterised and most commonly used agent, since studies have shown that patients with HER2-enriched breast cancers gain benefit from co-treatment with trastuzumab and chemotherapy in the neoadjuvant, adjuvant and advanced settings[64,65,67–70]. Other trials have shown that dual therapy with trastuzumab and a second anti-HER2 agent can lead to superior HER2 signalling blockage and better patient outcome in the neoadjuvant and advanced settings[70–73]. Research continues to study other anti-HER2 agents such as the pan-HER inhibitor neratinib or HER2-targeted drug delivery and immunotherapy[74].

About half of HER2-overexpressing (HER2+) breast tumours are also ER+[75–78]. Patients in this ER+/HER2+ subgroup derive less benefit from endocrine therapy alone than ER+/HER2- patients, possibly due to cross-talk between ER and HER2 signalling[76]. However, studies have demonstrated benefit from the combination of anti-HER2 and endocrine therapy for this group[75,76,78].

2.3. Intratumoural heterogeneity in receptor expression

The inherent heterogeneity of cancer means that the disease can be vastly different between patients, but also between multiple lesions in a single patient or within the cell population of a single tumour[79]. The existence of such intratumoural (or spatial) heterogeneity in the expression of receptors with predictive value has been a source of concern, casting doubts on the validity of single rather than multiple biopsies, particularly for large lesions[9,80]. Needle biopsy has long been an essential tool in the diagnosis and management of cancer, with reports of its first documented use over 1,000 years ago[81] and is the current standard for preoperative diagnosis and sampling for histological and receptor status assessment in breast cancer. However, our increasing understanding of the impact of disease heterogeneity has raised concerns that a single needle biopsy might underestimate the molecular complexity and varying genomic landscape of the disease[80], as this methodology provides only a snapshot of a small subset of tumour cells at a given moment in time[82].

Recent guidelines from the European Group on Tumour Markers (EGTM) have suggested that negative results in the assessment of ER, PR or HER2 in primary tumours using core needle biopsies should be corroborated by re-assay of the corresponding surgical sample[10]. This recommendation is not evidence-based, but precautionary based on concern that a core biopsy might not be representative of the receptor status for the whole tumour.

Nevertheless, two different recent studies have reported high accuracy of core needle biopsies in the assessment of the true ER and PR status when compared with the results from whole surgical samples[83,84]. On the other hand, these studies reported poorer specificity in the measurement of HER2 overexpression by core biopsy, particularly in high grade tumours[83,84], most likely due to the higher intratumoural heterogeneity of expression recognised for this receptor[85,86]. These observations suggest that corroboration of negative core biopsy results in surgical specimens might be justified for HER2 assessment, as the ASCO guidelines also suggest[62], but not necessary for ER evaluation.

Also in relation to receptor status heterogeneity, recent UK guidelines have recommended individual assessment of HER2 in all co-existing bilateral, distinct ipsilateral or widely separated primary cancers[60]. Reviewers have suggested that this guideline should be followed for the multi-location testing of all tissue-based biomarkers in synchronous primary cancers[9].

3. Challenges in the management of metastatic breast cancer

While outcomes for patients with primary breast cancer have improved significantly in the last few decades, metastatic breast cancer (MBC) is still a common occurrence. In fact, the incidence of cancers that are metastatic at first presentation has remained stable since the 1970s[87]. The frequent development of acquired resistance to treatments, including in patients with ER+ cancers receiving endocrine therapy[19,88] and those with HER2+ cancers who are treated with anti-HER2 therapy[89,90], often leads to recurrence as either local or advanced disease[19,90]. Additionally, more aggressive breast cancer subtypes (which are typically ER- and HER2- and thus inherently not susceptible to targeted therapies), along with the well-established challenge of intratumoural heterogeneity, also contribute to the incidence of metastases.

Despite the advances in prevention, diagnosis and treatment, the clinical management of MBC still presents many challenges and prognosis continues to be poor: MBC is the most frequent cause of cancer death for women worldwide[91,92], with current median survival time of only 18-24 months[93]. This is partly due to the fact that resistance to therapy occurs invariably in MBC, rendering it virtually incurable[94,95]. Current efforts are centred in improving survival and quality of life[96].

The next sections will summarise the role that receptors and targeted therapy continue to play in the management of breast cancer in the metastatic setting, as well as considerations and the main challenges faced for their application in advanced disease.

3.1. Biological challenges: temporal heterogeneity and resistance to treatment in MBC

The well-established receptors ER, PR and HER2 are currently the only universally used predictive biomarkers for MBC[91], as they are essential in the treatment decision-making process in all clinical settings (neoadjuvant, adjuvant and advanced or metastatic). Clinical

trials have shown that the genetic landscape of metastatic breast lesions can determine their susceptibility to different therapeutic agents and treatment selection based on molecular markers or abnormalities can lead to improved outcome[97,98].

Despite the almost inevitable development of resistance to treatment, targeted therapy is also a useful tool in the management of advanced disease. For instance, endocrine therapy has been shown to be at least as effective as chemotherapy[37,99] in ER+ MBC. Evidence suggests that treatment with endocrine therapy in the adjuvant setting does not significantly influence the rate of response of subsequent recurrent cancers[100]. Endocrine therapy is now the recommended first option for treatment of ER+ MBC, except in cases with visceral involvement, which warrant the administration of chemotherapy instead[94,101,102]. Patients with HER2+ MBC can also benefit from targeted treatment, with median survival having more than doubled since the advent of anti-HER2 therapy[74]. The current treatment consensus includes the use of multiple lines of HER2-targeted therapy beyond progression, often using dual blockade with trastuzumab and pertuzumab combined with chemotherapy as first line therapy, followed by treatment with different anti-HER2 agents. Patients have also been shown to derive benefit from combination of anti-HER2 drugs with endocrine therapy in ER+/HER2+ MBC cases[75,76,78]. Research continues to assess the potential of treatment strategies that combine anti-HER therapies with agents targeting other pathways and signalling components in the HER network, such as CDK4/6, PI3K and mTOR inhibitors [74,75,103–105].

Currently, many treatment recommendations for the management of MBC are still often based on the histological and molecular characteristics of the primary tumour[92]. This represents a significant hurdle for treatment optimisation, since studies have shown that cancer evolution leads to vast variation in the genetic landscape between primary and metastatic lesions[106–108].

In addition to the inherent intratumoural heterogeneity in primary breast cancers, tumour cells can accumulate driver mutations that are either present at diagnosis or emerge during treatment of the primary cancer[109–111]. A recent meta-analysis of 560 breast cancers has recently provided an insightful overview of this process, showing how somatic mutations contribute to the genetic evolution of breast cancer and the progression towards metastatic disease[112]. As part of the invasion-metastasis cascade, selected subclones can then

disseminate to distant sites and lead to the development of secondary lesions, which can be molecularly distinct from the originating primary breast cancers. This phenomenon is referred to as temporal heterogeneity[92,109].

Temporal heterogeneity can include changes in the hormone receptor status and sensitivity to different treatments in metastatic lesions compared to primary tumours, partly due to evolution through exposure to standard treatments. This leads to metastases that are more aggressive and resilient than primary cancers. As the disease continues to evolve and tumour clones with different sets of aberrations often co-exist within a single lesion, in metastatic cancer resistance to treatment occurs invariably and repeatedly under exposure to different agents[79]. This complex tumour evolution and clonal selection and expansion contribute to the poorer patient outcomes in advanced disease, particularly if primary tumour characteristics only are considered for treatment selection.

The well-established occurrence of temporal heterogeneity[113,114] suggests that receptor expression status ought to be re-assessed prior to treatment selection in the metastatic setting[79]. A recent meta-analysis of 33 studies has reported rates of discordance between primary and metastatic sites of 20% for ER, 33% for PR and 8% for HER2. For ER, 24% of tumours converted from ER+ to ER-, while 14% gained ER positivity[114].

Accordingly, guidelines from both European and American expert panels recommend that receptor status should be retested for both primary and distant lesions upon diagnosis of any *de novo* metastatic disease[10,115]. Realistically, this is dependent on the feasibility of tissue sampling, since biopsy of the metastasis may not be possible due to its location and the risk to the patient[9] (see following section).

Interestingly, guidelines differ in the recommendations for cases in which primary and metastatic lesions present discordant receptor status. The American Society of Clinical Oncology (ASCO) recommends following the receptor status of the metastatic lesion for treatment selection if supported by the clinical scenario and patient's goals[115], while both the National Comprehensive Cancer Network (NCCN) and the European School of Oncology and European Society of Molecular Oncology (ESO-ESMO) consensus recommend that endocrine or anti-HER2 therapy should be administered if the receptor status of any of the biopsies (primary or metastatic) supports it[10,96].

3.2. Technical challenges: limitations of current assessment methodologies in MBC

The clinical management of advanced breast cancer is also hindered by practical limitations in the sampling and diagnosis of metastatic lesions. As previously discussed, needle biopsy is the current standard for tissue sampling and receptor assessment in new breast cancer lesions (see section 2.3.). Although a quick and relatively simple procedure, needle biopsies present other limitations and risks particularly relevant to the advanced setting.

While primary breast cancers are normally easily accessible for core needle biopsy, the location of metastatic lesions may render tissue sampling by this method unfeasible. Together with the fact that core needle biopsy can cause discomfort to the patient, this limits the practicality of repeated biopsies, which would allow for the monitoring of disease evolution in MBC.

It has been suggested that needle biopsy might not be advisable for some patients receiving antiangiogenic treatment, which alters blood vessel growth and healing and thus leads to increased risk of bleeding[116], although there would obviously also be contraindications associated with surgical biopsy for these and other patients. Additionally, the procedure itself could increase the risk of cancer spreading due to seeding of malignant cells along the needle track[117]. A systematic review of 15 breast cancer studies using core needle biopsy reported needle track seeding of cancer cells in 22% of cases[118,119], although it ultimately concluded that tumour seeding from needle is very unlikely and it remains unclear whether this phenomenon would actually lead to the development of metastases.

Research has also looked at the possible effect of sampling method on the tissue collected itself, which can be relevant for its subsequent analysis. A recent publication showed that, although tissue sampling did not lead to significant immune or wound responses, biopsy could induce significant changes in the transcriptomic profile of a sample in the form a hypoxia-like response[120]. This effect was greater following surgical excision rather than needle biopsy, as it derives from the technical issue of warm ischemia during the surgical extirpation and subsequent handling of the tissue prior to its processing and storage. While the changes induced by this surgical stress are most likely small compared to those due to response to treatment and disease heterogeneity and evolution, the potential confounding effect that biopsy method may introduce for subsequent sample analysis should be considered.

Finally, sampled breast tissue needs to undergo processing prior to histological and receptor status assessment. Fixation in formalin has been used for decades as a simple and inexpensive way of preserving tissue. Although this processing could delay sample assessment, this is likely negligible in the larger timescale of the treatment schedule. Possibly more importantly, fixation can affect the molecular integrity of DNA and mRNA and create artefacts, limiting the use of nucleic acids as tissue biomarkers[121,122].

4. Alterations in receptors as novel biomarkers to improve management of MBC

As previously discussed, the unavoidable development of resistance to treatment is a hallmark of and the ultimate hurdle in the clinical management of MBC (see section 3). Accordingly, extensive research has gone into studying the different mechanisms of resistance to both endocrine[123–125] and anti-HER2 therapies[90,126,127], leading to an increasing understanding of how alterations in the proteins targeted by treatment, their co-activators or related signalling pathways contribute to the development of the resistant phenotype. These studies may help identify novel treatment targets and inform new combinatorial therapeutic strategies. Importantly, markers for these underlying mechanisms can be identified and validated as candidates to better predict the development of resistance and aid in the selection of the best possible treatment for MBC.

While numerous prognostic and predictive biomarkers have been studied, here we will focus on genomic alterations identified in the established receptors which may hold additional predictive value beyond the well-known, traditional roles of wild-type ER expression and HER2 overexpression. Genomic alterations in PR are less relevant given that the predictive value of wild-type PR expression is not as well established, PR mutations and polymorphisms are infrequent and there is a lack of evidence of their significance in breast cancer[128]. It has been suggested that changes in PR expression might be a consequence of genetic alterations in ER, the expression of which PR is well known to depend on[128].

4.1. Genetic and genomic alterations in ER as emerging biomarkers

In addition to the central role of the expression of wild-type (WT) ER as a predictor for the benefit from endocrine therapy, numerous genomic aberrations in ER have been described in relation to differential response to treatment and possible mechanisms for the development of resistance. This grants these altered forms of ER potential value as prognostic or predictive

biomarkers in their own right. This section will summarise the accumulated evidence on ESR1 point mutations, amplifications, alternative splicing and translocations, their clinical impact and potential role in improving the management of ER+ MBC.

4.1.1. *ESR1* mutations

The ER genomic alterations best characterised to date are single nucleotide mutations in the *ESR1* gene. These alterations were first identified in cell line models[129] and clinical metastatic samples[130] in the 1990s. In recent years, the advancement of sequencing techniques has enabled further study into mutant receptors, with accumulating evidence on their frequency and role in the development of acquired resistance to endocrine therapy[131–137].

Numerous missense, gain-of-function mutations that lead to constitutive activation of ER have been described in the ligand-binding domain (LBD) of the receptor, such as E380Q, Y537C/N/S and D538G (see Figure 1 for diagram). These LBD-ER mutants are stabilised in the agonistic conformation, enabling hormone-independent induction of transcription and proliferation. Studies have shown that these alterations lead to resistance to AIs, whose effect is based in the deprivation of oestrogen, as well as reduced sensitivity to SERMs and SERDs[131–134,138].

These mutations are absent or extremely rare in primary or treatment-naïve ER+ breast cancers and in ER- breast cancers[131–133,139–141], but can be found in about 10-50% (depending on the specific mutation) of metastatic ER+ patients who have been previously treated with certain types of endocrine therapy. Numerous studies have reported not only on the presence but also the increase in the relative frequency of these alterations in advanced disease[132,141,142]. For instance, a recent study screening breast cancer patients for LBD-ER mutations found mutant allele frequencies (i.e., the proportion of copies of ER that are aberrant) were very low (0.07-0.2%) in primary lesions but much higher in subsequent metastases (1.4% for bone and 34.3-44.9% in brain metastases)[142].

These findings support the hypothesis that very rare pre-existent mutant clones may expand under the selective pressure of treatment for which the aberrant ER variant confers an advantage[140,143]. This notion is also supported by the correlation between mutation frequency and the number of endocrine treatments received[131]. Mutations rarely arise in

patients treated only with the SERM tamoxifen[131,143,144], but appear to be selected by treatment with AIs[131–137]. Interestingly, a recent study has shown that frequency of LBD-ER mutations is much greater among patients who received AIs in the metastatic rather than only the adjuvant setting[144].

The exact clinical implications of *ESR1* mutations have been the focus of numerous recent studies. The frequency of LBD-ER mutations has been shown to be directly correlated with tumour progression in different patient cohorts[131]. Several studies have reported that the presence of *ESR1* mutations in metastatic disease is a prognosticator for poor prognosis and shorter progression-free survival (PFS) under subsequent AI treatment[138,144–146].

While the predictive value of *ESR1* mutations still requires further study, several studies have shown mutant status is linked to differential response to combination treatments[143]. Results from the SoFEA trial reported that patients with LBD-ER mutants, but not WT ER, benefit from fulvestrant-containing treatment compared to treatment with the AI exemestane alone[143]. Despite reduced sensitivity to SERMs or SERDs, studies have suggested that high-dose tamoxifen or fulvestrant may effectively inhibit tumour progression in cancers expressing these mutant receptors[131,132,134–136,147].

Importantly, different mutations in LBD, even in adjacent residues, can lead to different degrees of resistance[133]. For instance, Y537S mutants exhibited significantly greater resistance to ER antagonists than that observed in other mutants: Y537S required a 70-fold higher dose of fulvestrant (relative to WT ER), compared to other mutants which only required a 2-fold increase[133]. Y537S mutants were also less effectively inhibited by fulvestrant than by the novel SERD AZD9496, whereas WT ER and other mutants exhibited similar response to both SERDs[133]. Other interesting results from the BOLERO-2 trial showed that, while both Y537S and D538G mutations were associated with worse prognosis in MBC, only patients with the latter type of mutation derived benefit from the addition of everolimus, an inhibitor for the mammalian target of rapamycin (mTOR), to exemestane[145].

Research of *ESR1* mutations has led to the proposal of potential treatment strategies that tackle resistance to endocrine therapy due to constitutive ER activation, which could be of use to target aberrant ER variants derived from point somatic mutations or other abnormalities. Firstly, evidence to date has suggested that new agents with greater potency could be promising therapeutic alternatives, so current efforts are focused in the

development and assessment of novel anti-oestrogens. The third-generation SERM with SERD activity bazedoxifene has been shown to inhibit Y537S mutant-driven tumour growth and also exhibits greater potency than tamoxifen against other mutants[138,148–150]. Two novel orally bioavailable SERDs, elacestrant (RAD1901) and bilanestrant (GDC-0810 or ARN-810), have exhibited promising preclinical growth-inhibiting effects in ER-mutant tumours[151–154]. Both these agents and GDC-0927 (SRN-927), a third new SERD with improved potency, will be assessed in currently ongoing phase I clinical trials that will include screening for *ESR1* mutant status[155–157]. A second treatment strategy consists of inhibiting ER co-activator proteins. For instance, the inhibitors bufalin or verrucarin are being studied for their activity blocking the recruitment of the steroid receptor co-activator 3 (SRC-3)[158,159]. Thirdly, another approach is inhibiting the effect of constitutive ER activation downstream. For example, cyclin D1 regulates the cell cycle in complex with cyclin-dependent kinases 4 and 6 (CDK4/6) and, as a well-known ER transcriptional target, its expression is strongly correlated with that of the receptor[160]. Studies have reported the efficacy of CDK4/6 inhibitors palbociclib in combination with SERMs tamoxifen and bazedoxifene[148,161]. Importantly, it has been shown that LBD-ER mutants do not exhibit reduced sensitivity to CDK4/6 inhibitors. Trials to assess the effect of palbociclib in combination with endocrine therapy (PALOMA trials) showed that mutant-carrying patients benefited from the addition of the CDK4/6 inhibitor to the AI letrozole[143,162] or the SERD fulvestrant[143,163].

4.1.2. *ESR1* amplification

Gene amplification, by which the copy number of a chromosomal region is multiplied, leads to overexpression of the affected genes and is a prevalent mechanism for acquired resistance to treatment in cancer[164]. Amplification of the gene *ESR1* was first described as a possible mechanism for increased expression of ER in 1990[165]. Given the importance of ER in breast cancer, this initiated considerable research and discussion of the clinical implications of this finding. *ESR1* amplification has since been an object of debate due to the conflicting evidence reported by different researchers[166,167].

In 2007, Holst *et al* reported that *ESR1* amplification was a frequent event in breast cancer: using FISH, they detected amplification in about 21% of a large cohort of more than 2000 breast cancer patients, and reported this was significantly associated with ER overexpression[168]. Amplification was found in benign and precancerous breast lesions,

suggesting this could be a very early genetic alteration. Importantly, Holst also reported that women presenting this alteration benefit from significantly longer survival under treatment with tamoxifen, suggesting the potential role of amplification as a predictor for better response to endocrine therapy[168].

However, other studies swiftly reported conflicting results: 5 different studies reported varying, but consistently much lower prevalence or complete absence of *ESR1* amplification[166,169–173]. These authors also criticised the methodology and analysis of the earlier work and, importantly, reported significant discrepancies between quantification by FISH and alternative biochemical methods[169,170].

Other studies added to the controversy by supporting results from the original study, reporting similar *ESR1* amplification rates as measured by FISH (21-23%) and supporting the association of amplification with higher ER expression levels[174,175]. The study by Tomita *et al* showed amplification was associated with longer survival[174], while Tsiambas *et al* reported that this was only true for a subset of the cases with amplified *ESR1*[175]. The former study also found that it was negatively correlated with factors associated with poor prognosis, such as tumour size and lymph node involvement[174].

In contrast, a Dutch study reported that true amplification was rare (2%), further supported the issue of methodological discrepancies (with only 60% concordance between techniques) and, importantly, showed that amplification was correlated with high grade and proliferation[176]. However, a subsequent molecular profiling study by the same group reported significantly higher *ESR1* amplification rates (16%)[177,178].

In short, evidence has accumulated on both sides of the debate, with contradictory findings in terms of both the frequency and prognostic or predictive value of *ESR1* amplification[167,179–181].

Finally, a study by Ooi *et al* showed that some FISH signals may actually correspond to hybridisation to *ESR1* mRNA from highly transcribed chromatin areas[182], an issue that had been previously suggested as a plausible source of artefacts[166,183]. This study reported relatively infrequent (6%) low level amplification[182] and has been considered as an explanation for the wide discrepancies between studies and methodologies[181].

One obvious conclusion of these studies has been the considerable technical challenges for the assessment of ESR1 amplification[169,170,183], particularly by FISH, in which artefacts are common and slight differences in scoring thresholds can lead to significantly different conclusions[180,181,183]. Ooi's findings provided an answer to the decade-long debate on incidence and an important lesson on the need for standardisation and robust validation of methodologies for translational cancer applications[181].

Whether increases in ER expression could be an early alteration in cancer or how this might be indicative of dependence on the receptor for tumour progression in ER+ cancer still remains unclear[184–186]. It has been suggested that amplification could be linked to better or worse response to treatment and prognosis depending on the underlying mechanism leading to the chromosomal aberration in the first place[167]. However, the only current point of consensus seems to be that further studies with appropriate, robust methodology are needed before we can determine the actual clinical relevance of *ESR1* amplification and its potential prognostic or predictive value[140,180,181,184,187].

4.1.3. *ESR1* alternative splicing

Since the 1990s, numerous studies have described multiple *ESR1* splice variants[188,189], which are expressed heterogeneously and often in co-expression in both normal and cancerous breast tissue[189,190]. These variants are truncated versions of the normal transcript that arise through different exon deletions, with the resulting frame-shift alterations, from the full-length WT ER[191].

As a result of these deletions, splice variants have been shown to exhibit differential transcriptional activity: some variants are constitutively activated, some compete with and inhibit the effect of WT ER, and others are altogether inactive due to lacking both ligand and DNA-binding domains[188,191–195]. The best characterised variant, labelled ΔE5, presents a deletion of exon 5 of *ESR1* that affects the LBD [192,194]. This results in constitutive, hormone-independent activation and has been linked to the development of resistance to endocrine treatment and tumour progression[194–198].

However, the prevalence and role of these abnormal ER isoforms in the development of acquired resistance has not been well characterised in metastatic disease. A recent study by Beije *et al*[141] analysing circulating tumour cells in liquid biopsies from patients with MBC to

screen for a range of *ESR1* variants only detected the ΔE5 isoform. Results reported expression of this variant was higher in cancer patients than in healthy blood donors, but showed no differences between a baseline cohort and another one comprising patients progressing on palliative endocrine therapy[141]. Consequently, this work did not show a significant association of ΔE5 ER with differential response to treatment or progression in patients with MBC. However, given the previous evidence in preclinical and primary breast cancer studies, the potential role of *ESR1* splice variants as prognostic or predictive biomarkers merits further investigation. This might be aided by the emergence of better sampling and diagnosis tools (see section 5).

4.1.4. *ESR1* translocations

Genomic rearrangements are gross alterations in the chromosomes or large chromosomal regions in the form of deletions, duplications, insertions, inversions or translocations. This phenomena can lead to dysregulation of transcription or the generation of fusion gene products[199]. Such alterations have long been shown to take place in the development and advancement of many diseases, including breast cancers[200], where the rearrangements involving the BRCA gene family have been well characterised[201].

Recent years have seen the discovery of several in-frame fusion genes involving *ESR1*. Genomic characterisation of xenografts derived from a patient with endocrine-resistant MBC described a translocation leading to the fusion of the first 6 exons of *ESR1* with the *YAP1* (Yes-associated protein 1) gene, whose WT product is involved in the regulation of organ size and tumorigenesis[134]. The resulting *ESR1-YAP1* product has been shown to modulate growth and transcription of classic oestradiol-regulated genes in a hormone-independent manner. Indeed, the aberrant receptor lacks an LBD, which represents a very plausible mechanism for the development of acquired resistance to SERMs and SERDs.

A study by Veeraraghavan *et al* in 2014[202] reported a genomic rearrangement involving *ESR1* and its adjacent gene *CCDC170* (Coiled-Coil Domain-Containing Protein 170), which has been shown to be co-expressed with ER but whose function remains unknown[203]. This gain-of-function aberration involves an *ESR1-CCDC170* gene fusion that leads to overexpression of a truncated CCDC170, which in turn has been shown to induce growth factor signalling, motility, tumourigenicity and resistance to endocrine therapy through the GRB2-associated binding protein 1 (GAB1) signalling pathway. This is consistent with the fact that, with a

prevalence of 4% of cases in the study cohort, this fusion product was found to be significantly enriched in the ER+ luminal B subtype, characterised by a typically more aggressive and treatment-resistant phenotype[202]. This suggests the potential prognostic value of the aberrant CCDC170 form.

Following the discovery of the *ESR1-YAP1* and *ESR1-CCDC170* fusion gene products, further study is now needed to better characterise how they exert their activities. This could help assess these aberrant proteins or related pathways as both markers to determine the mechanism enabling resistance and potential therapeutic targets to tackle these.

4.2. Somatic mutations in HER2 as biomarkers

Despite the improvement in prognosis of patients with HER2+ breast cancer since the introduction of anti-HER2 therapy (see sections 2.2. and 3.1), challenges remain in the management of these tumours, particularly in the advanced setting where overall response rates are often relatively limited and the high rates of *de novo* and acquired resistance frequently lead to tumour progression. While researchers continue to investigate alternative anti-HER2 and combination therapies for first and second line treatment[74,76,104,204], there is also a need to identify additional predictors to help improve treatment selection[205]. Most of the research to date has described alterations in associated pathways and downstream effectors of HER2[206]. For instance, *PIK3CA* (phosphatidylinositide 3-kinase) mutations that lead to resistance through constitutive activation of the PI3K signalling pathway targeted by anti-HER2 therapies have been described[207–209], although they have not yet been shown to have predictive value to help guide treatment selection[210,211].

More importantly for the focus of this review, research has also identified genomic alterations in HER2 itself. Among the mechanisms leading to acquired resistance to anti-HER2 therapies are obstacles preventing binding of different drugs to HER2[212–215]. Trastuzumab resistance can arise from overexpression of glycoproteins such as mucin that mask the receptor from binding, but also from genetic alterations that lead to the loss of the targeted epitope. Research has described how mutations in the extracellular domain, alternative RNA processing or alternative translation-initiation sites lead to the expression of a truncated form of the receptor named p95HER2[216], which has been shown to correlate with outcome in patients treated with trastuzumab and thus holds prognostic and predictive value[217,218]. Additionally, several mutations affecting the RTK domain have been identified that are linked

to resistance to tyrosine kinase inhibitors such as lapatinib, used as anti-HER2 therapy in combination with other agents[213].

In summary, preclinical and translational studies have described genetic aberrations in HER2+ MBC that lead to the loss or alteration of the epitopes or domains targeted by different anti-HER2 treatments. While further work is needed to validate these findings, evidence to date suggests specific mutations could act as predictive markers, helping identify patients unlikely to respond to these therapies who may instead derive greater benefit from alternative treatment strategies.

Interestingly, somatic mutations in HER2 could be useful as biomarkers in cancers that would normally be considered HER2 negative (HER2-). Meta-analysis of 8 sequencing studies identified that 1.6% of newly-diagnosed breast cancers may harbour HER2 somatic mutations[219]. Importantly, most of these patients did not exhibit HER2 amplification or overexpression. These activating mutations lead to an overactive form of HER2 and represent an alternative mechanism by which signalling through this receptor leads to disease progression in patients that would normally be considered HER2-.

Studies have shown that tumours carrying these activating HER2 mutations respond to treatment with the irreversible pan-HER kinase inhibitor neratinib[219,220]. Preliminary results from the ongoing SUMMIT trial, exploring the efficacy of neratinib in patients carrying activating HER2 and HER3 mutations, have been encouraging[221,222], while other work continues to investigate how specific mutations may impact responsiveness to this and other inhibitors[223].

In short, HER2 genetic alterations could also be useful markers in HER2- cancers. Screening for these activating HER2 mutations could help identify a subpopulation of MBC patients who are likely to benefit from anti-HER2 therapy despite presenting no overexpression of the receptor. Under current standard practices, these patients would miss out on treatment likely to improve their outcomes. Ongoing studies will need to validate the predictive value of these mutations and assess the feasibility of their potential clinical application.

5. The need for new methodologies for better biomarker assessment in MBC

There is a need for better sampling and measurement methods before promising novel biomarkers can be considered for regular implementation in the clinic[224] (see section 3.2.).

These technologies are needed, firstly, to help identify and validate novel circulating biomarkers and, secondly, to establish standardised protocols that enable a more accurate, sensitive, quick and ideally frequent screening for predictors in often inaccessible and continuously evolving metastatic lesions. As the better characterised receptor genomic alterations to date (see section 4.1.1.), *ESR1* mutations represent a good example of how both better biopsies and analysis methods have aided and will continue to drive research on this kind of novel predictive factors. The following paragraphs will summarise the impact and evolving role of these technologies.

5.1.1.1. Liquid biopsies for better sampling

The term "liquid biopsy" refers to the use of blood samples to study existing tumours[225–227]. Blood often contains cell-free DNA (cfDNA), circulating tumour DNA (ctDNA), circulating tumour cells (CTCs), exosomes and tumour-educated platelets (TEPs), the analysis of which can provide an insight into cancers.

While needle biopsy continues to be routinely used in the clinic, the use of liquid biopsies (LBs) to sample and analyse cancer tissues has proven instrumental to the discovery and study of emerging biomarkers. LBs can enable better biomarker assessment and monitoring of genomic changes in tumours. This holds particular potential in the metastatic setting, where early detection of emerging resistance mechanisms could help guide treatment correction and thus improve patient outcomes[139].

LBs have the advantage of circumventing many of technical limitations of traditional biopsies (see section 3.2.): they are minimally invasive, enable repeated sampling for constant monitoring of tumour evolution and provide an insight into cancers that would normally be inaccessible for needle biopsy[225]. Additionally, LBs might also allow for the detection of circulating biomarkers which would be absent or harder to accurately detect in fixed tumour tissue, such as mutant DNA or transcripts[228].

In recent years, the use of LBs has become more prevalent in cancer studies. While CTCs have the potential to provide an insight into multiple molecular dimensions (DNA, RNA and protein), several authors have remarked on the technical complexity of their analysis[141,229]. For instance, CTC analysis can be complicated by issues such as the presence of leukocytes in the blood: despite the use of procedures for CTC enrichment,

patient blood can still contain contaminating blood cells, which interfere with the gene expression profiling of CTCs and significantly reduce the sensitivity for detection of biomarkers such as *ESR1* mutations found only in these cancerous cells[141,230].

Instead, much of the research to date using LBs in breast cancer has focused on the analysis of ctDNA, which is any tumour-related circulating free DNA that is released into the blood by cancer cells undergoing necrosis[227,231–233], and is often more easily detectable. Indeed, a recent study has shown that sensitivity for detection of *ESR1* mutations is greater in ctDNA than in CTCs[141] and, in line with this, analysis of ctDNA from LBs has been a key tool in most of the studies looking at *ESR1* mutations to date[141,143–145,233–237]. The availability of a method for repeated, non-invasive sampling is of particular relevance to these markers, since mutation status would ideally be determined at diagnosis, after each recurrence or progression and possibly at regular intervals even without clinical signs of disease advancement[139].

Some evidence to date has shown good concordance in the assessment of mutational status (i.e., positive or negative for the presence of mutants) between traditional tissue biopsies and LBs. For instance, studies looking at detection of *PI3KCA* and *ESR1* mutations found 95% and 97% concordance, respectively, in mutational status as assessed by allele-specific PCR in tumour DNA from tissue biopsies or ctDNA analysis[144,238]. However, a different study found that in some cases mutations can be detected in cfDNA from LBs that were not found in analysis of tissue from metastatic biopsies[142]. This study also reported discrepancies in the quantification of the frequency (rather than positive or negative status) of LBD-ER mutations in both sample types, with differences of several fold changes between the mutant allele frequencies in those cases in which mutations were detected in both paired metastatic biopsies and cfDNA[142].

These results evidence that, despite the promising results to date from the implementation of LBs, further work is still needed before we can bring their potential technical advantages to the clinic. Research will need to better determine how accurately LBs can quantify biomarkers and, by extension, monitor disease evolution in MBC. For instance, the concept of “mutational status” relies on the definition of thresholds that define how frequent an alteration (such as a specific LBD-ER mutation) must be to be classified as positive. Although detection technologies already allow for ultrasensitive measurements (see next section),

there is a current lack of consensus in the definition of cut-offs for mutation positivity[132,141,142,239,240] and studies are needed to establish rigorous and standardised thresholds. Larger studies with paired blood and metastatic tumour biopsy samples are also required to investigate the correlation in biomarker levels between both sample types and ensure that detection in LBs does not misrepresent the actual frequency of genetic alterations in the metastases. Work to assess the potential discrepancies and influencing factors will likely require multicentre research efforts, given the difficulty of obtaining a large cohort of metastatic breast biopsies with paired LBs.

5.1.1.2. Improved detection technologies

As previously mentioned, the revolution in sequencing technologies in the last decade was instrumental in recent advances in the research of mutant ER variants in particular. In the past, adequate mutation detection has faced the challenge of achieving high enough sensitivity[140,241]. Even if a considerable proportion of metastatic cancers eventually carry an *ESR1* mutation, the aberrant *ESR1* copy might be present in a very small fraction of the tumour cell population in primary cancers or at the early stages of clonal evolution, where mutants would ideally be detected for optimum prognosis and treatment selection, so ultrasensitive detection methods are needed. Inherent error rates of up to 1% limit the suitability of massively parallel next-generation sequencing and targeted sequencing techniques for detection of rare mutations[242,243].

Improved tools include the development of sequencing methods with reduced background error and greater sensitivity for screening of liquid biopsies[241,244–247]. PCR-based methods for allele-specific mutant detection have also been developed[245,246,248,249] and digital droplet PCR (ddPCR) in particular has been used for *ESR1* mutation assessment in many of the studies to date[142,145,253,235–237,239,240,250–252].

6. Conclusion

ER expression and HER2 overexpression are well-established predictive markers for response to endocrine and anti-HER2 therapies, respectively, in both primary and advanced breast cancers. However, the management of metastatic disease continues to face significant challenges. The constant evolution of advanced disease leads to temporal heterogeneity, complicating receptor status assessment, and contributes to the invariable development of

resistance to treatment and thus poor prognosis in advanced disease. Additionally, traditional sampling and assessment techniques are often insufficient for their use in metastatic disease, which is often inaccessible and would ideally require frequent testing for continued monitoring of the evolving disease. These biological and technical limitations are the main reasons why tumour profiling for the management of MBC is still in the early stages.

As a result of extensive research in recent years, genomic alterations in ER and HER2 have emerged which evidence suggests hold the potential to greatly add to the well-established predictive value of the expression or overexpression of the wild type variants of these receptors. Here we have summarised the evidence to date on the predictive value of *ESR1* point mutations, amplification, alternative splicing and translocations, as well as *HER2* somatic mutations (see Figure 2 for summary diagram).

We have also described the new methodologies that have emerged in recent years to address the practical limitations for assessment of metastatic cancers. Liquid biopsies and specifically isolation of ctDNA have shown great potential for study of circulating biomarkers when complemented by ultrasensitive methods for their detection and monitoring, although further work is still needed.

In short, the accumulating evidence suggests that, in their evolving role as predictive markers, genomic alterations in ER and HER2 hold potential to help meet the need for better biomarkers. By providing an insight into the underlying mechanisms of resistance, these predictors might help monitor the loss of response to treatment in the metastatic setting, helping select alternative agents, adjust dosage or identify patients likely to benefit from extended or combination treatments, as well as allowing for the identification of novel potential treatment targets and new combinatorial strategies for multiple pathway targeted therapy.

A good example of these advances are LBD-mutant variants of ER: as the best characterised novel predictors in this field, prospective retrospective studies have already yielded evidence on their promise for patient stratification and treatment selection, while the better understanding of the underlying mechanisms of resistance has led to the proposal of alternative combination therapeutic strategies.

7. Expert commentary

As summarised in this review, the optimal application of targeted therapies and the overall clinical management of metastatic breast cancer are significantly impacted by both the biological complexity of the disease and limitations in the methods available for its assessment. Ultimately, the main challenge remains in incorporating the underlying heterogeneity and genetic complexity of the disease into its clinical management.

In our opinion, a three-point strategy is required to guide and improve the use of predictive biomarkers in the metastatic setting: firstly, assessment of the status of established biomarkers (ER, PR and HER2) in advanced disease should be improved (see section 3.1.); secondly, novel biomarkers with improved predictive value need to be identified and validated (see section 4); thirdly, new tools for biomarker discovery and sensitive and accurate detection are required to aid the advancement of the two previous points (see sections 5). As presented throughout this review, work in recent years has largely followed this approach, but greater efforts are needed to bring advances closer to the clinic and to address specific limitations in the work to date.

Regarding the first point, increasing awareness on the issue of temporal heterogeneity has led to recent updates in European and American guidelines, supporting testing of receptor status in *de novo* metastases. Nevertheless, practical experience makes it clear that implementation of these guidelines is often unfeasible, particularly using traditional biopsy techniques. If we are to improve our approach to the selection of first line endocrine and anti-HER2 therapies in metastatic breast cancer, work needs to focus in establishing liquid biopsies as a suitable alternative technique. In line with this, recent studies have used CTCs from liquid biopsies to assess receptor status with promising results[254,255], exemplifying how such approaches could improve receptor status assessment in metastatic cancers. The DETECT study, a large prospective trial comprising clinical phase III and one phase II studies, is currently undergoing to investigate treatment personalisation for patients with MBC based on the HER2 status of CTCs[256–258].

On the second point, despite significant advances made in the characterisation of genomic traits of ER and HER2 with novel predictive value, several aspects need to be improved upon. Importantly, the methodologies used in these studies must be standardised to enable robust validation of the findings and a better description of these aberrant receptors, so a consensus can be reached on their clinical implications and potential implementation. For instance,

despite the initial description of *ESR1* amplifications in the 1990s, their study has been set back by inconsistencies in their assessment and debate in their incidence and clinical relevance, including disagreement on whether amplifications are indicative of better or poorer response to endocrine therapy. The study of *ESR1* point mutations exemplifies a better case, as emerging sequencing technologies have provided greater insight into their significance in recent years. We expect that similar approaches will help advance our understanding of the other genomic alterations described here.

Finally, the next stage to move these novel biomarkers closer to their clinical application must be larger dedicated studies. Most findings to date have largely been the result of preclinical studies or *post hoc* retrospective analyses, such as in the case of *ESR1* mutation assessment in the SoFEA and PALOMA trials. More comprehensive and prospective trials including large patient cohorts and incorporating biomarker assessment for patient stratification into their study design are now needed.

Just as the DETECT studies are implementing liquid biopsies to better assess temporal heterogeneity in receptor status, similar prospective approaches are also needed that study not only established hormone and HER2 status, but also emerging biomarkers. Some currently ongoing studies mentioned in this review suggest that researchers are beginning to move towards these important prospective trials: phase I trials testing novel SERDs include screening for *ESR1* mutations, while the SUMMIT trial is investigating the effect of pan-HER inhibitor neratinib in patients carrying *HER2* mutations.

This type of molecularly-driven studies are required to confirm the potential predictive and clinical value of these and other biomarkers, define standardised methodologies and establish significant cut-off values and guidelines that will be essential before any biomarkers can be translated into new clinical tools. If such clinical trials are successful in upcoming years, we might be able to implement the recent technological advances to turn somatic mutations and other genetic alterations in ER and HER2 into clinically useful biomarkers that provide insight into the complexity of MBC and help guide the selection of the optimal treatment (or sequential treatments) for each patient.

8. 5-year view

The recent advances in both preclinical research and prospective studies suggest that the next few years could see significant developments in establishing some of the novel biomarkers described here in the clinical and research settings. *ESR1* mutations hold particular promise for their inclusion in more prospective trials to assess how mutant status can assist treatment selection in patients with MBC. If successful, in the longer term these trials could eventually lead to the approval of some of these new predictors for their clinical application. As previously discussed, the accuracy of new sampling and measurement techniques must be confirmed first before methodological standards for detection of these biomarkers can be established. This should then be followed by expert description of guidelines for their implementation, as well as studies to assess their cost-effectiveness.

Further research is also likely to shed light on how specific genomic aberrations in ER or HER2 arise. Clonal evolution plays a well-established role in the development of resistance and evidence has shown how treatment with aromatase inhibitors correlates with the subsequent increased abundance of LBD-ER mutants in ER+ MBC. Further research might provide a better understanding of how exposure to certain treatments in the adjuvant or first line metastatic setting might be linked to the emergence of specific mechanisms of resistance.

This may also help to improve our understanding of how genomic aberrations circumvent the mechanism of action of current standard-of-care therapeutic agents. In turn, this may highlight potential novel targets to tackle resistance and help develop novel drug candidates or propose dose escalation strategies or alternative therapeutic approaches using combination treatments, such as have been proposed to target resistance led by the specific LBD-ER mutants.

For less well characterised genomic aberrations, further basic research and retrospective studies could help clarify their clinical value. For instance, it remains unclear whether *ESR1* amplifications are predictive of a better or worse response to endocrine therapy and this remains a subject of debate. Work in the coming years including larger studies with standardised methodologies could resolve such debates and help come to a consensus.

To conclude, much work is needed to incorporate novel biomarkers with improved predictive value into the treatment decision-making process for patients with MBC. However, preclinical and early clinical studies to date have provided encouraging evidence suggesting that ER and HER2 aberrations could one day fulfil this role. In the future, we could see several of these

predictors translated into the clinic or even incorporated into multifactor tests that, from a liquid biopsy, could provide important information to select and adapt a patient's treatment as their disease evolves, in a move towards truly personalised medicine, thus helping delay progression and extend survival in patients with MBC.

9. Key issues

- The unavoidable development of resistance to treatment renders MBC virtually incurable, making it the leading cause of cancer-related death.
- While ER and HER2 can still inform treatment selection in MBC, the receptor status of *de novo* metastases is often different from that of primary tumours (temporal heterogeneity).
- Biomarker assessment in MBC is difficult due to the frequent inaccessibility to lesions for sampling.
- We need better biomarkers to predict the development of resistance to treatment, so therapeutic strategies can be adapted to the evolving biology of the disease.
- Genomic aberrations in ER and HER2 have emerged as potential predictive biomarkers, although they require further study so we can gain a better understanding of the underlying mechanisms of resistance and reach a consensus on their clinical implications.
- Validation of the findings to date and standardisation of sampling and detection methodologies are also required to continue advancing the study of these novel predictors.
- Prospective trials that incorporate biomarker assessment for treatment selection into their study design are essential to validate these novel predictors and advance towards their potential clinical implementation.

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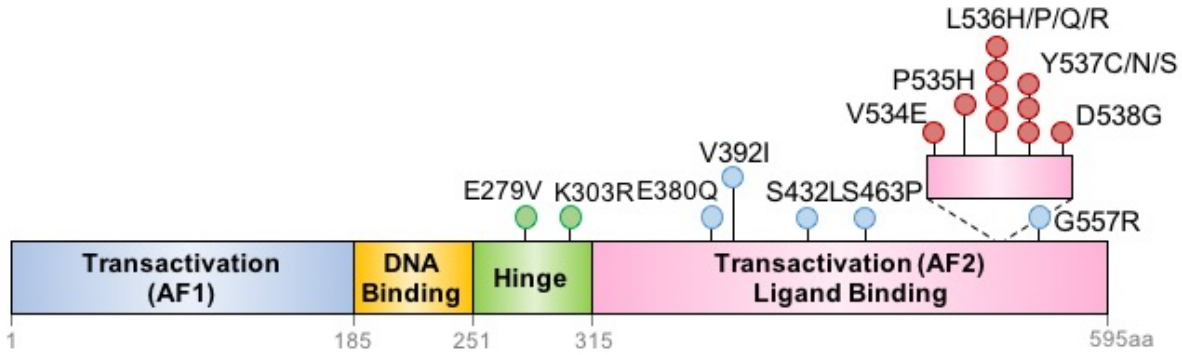

Figure 1. Diagram summarising known point mutations in the sequence of oestrogen receptor. *First discovered in the 1990s, somatic mutations are the best characterised genomic aberrations in ESR1. In recent years, more dedicated studies and technological advances have led to the description on numerous alterations in primary and metastatic breast cancer. Many of the most common mutations are located in a hotspot in the ligand binding domain and lead to constitutively active forms of the receptor that have been linked to reduced sensitivity to endocrine therapies.*

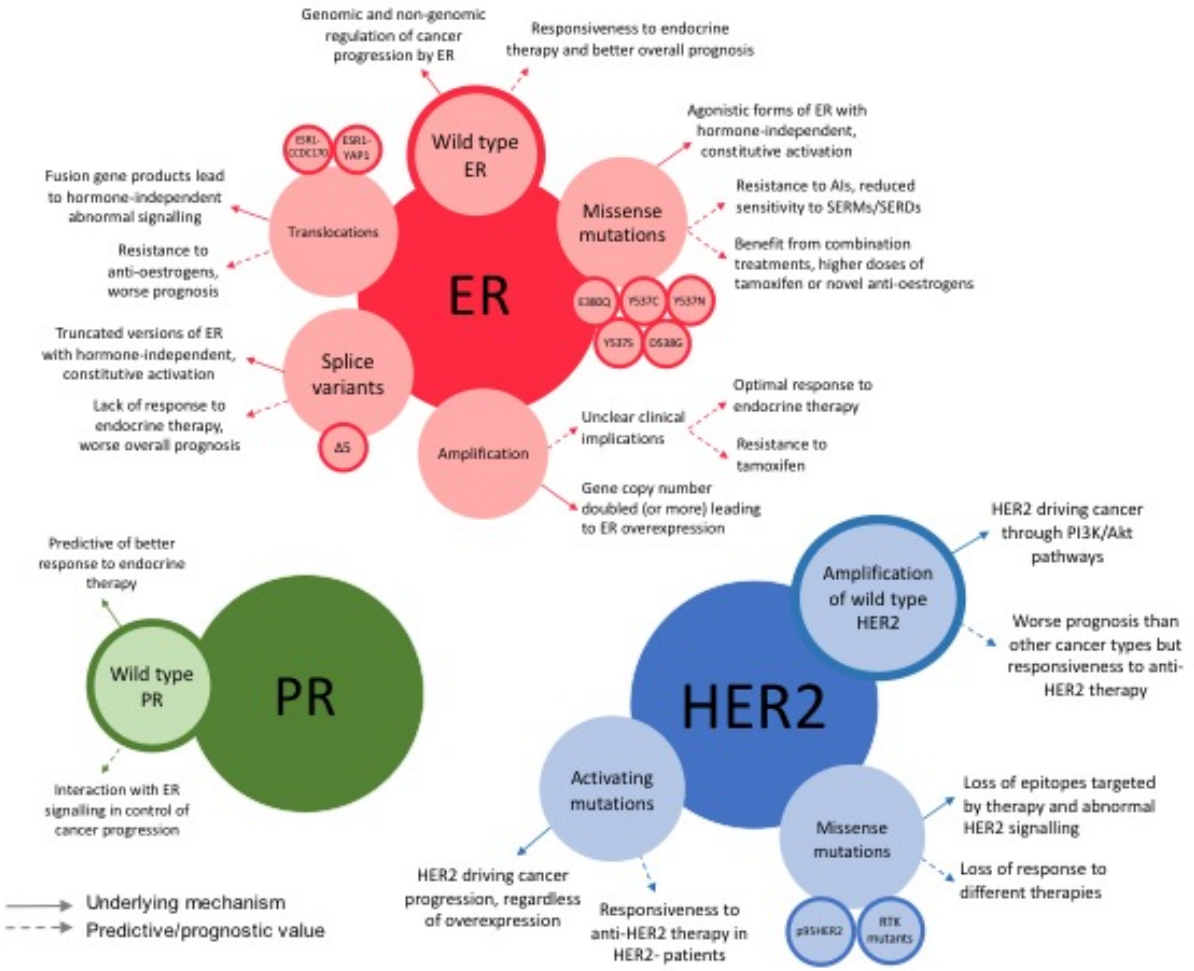

Figure 2. Diagram summarising the evolving role of receptors as biomarkers. The expression (or overexpression) of oestrogen receptor (ER), progesterone receptor (PR) and the human epidermal growth factor receptor 2 (HER2) has long been used to guide treatment decision-making. Recent years have seen the description of genomic aberrations in ER and HER2 which are linked to cancer progression and the development of resistance to treatment and thus hold additional potential to aid the clinical management of the disease. This diagram summarises these traditional and novel predictive traits, the underlying mechanism they denote (solid arrows), associated predictive and prognostic value (dashed arrows) and some examples of the best characterised genomic alterations.