



## Tumour Review

## Triple positive breast cancer: A distinct subtype?



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## ABSTRACT

Breast cancer is a heterogeneous disease, and within the HER-2 positive subtype this is highly exemplified by the presence of substantial phenotypical and clinical heterogeneity, mostly related to hormonal receptor (HR) expression. It is well known how HER-2 positivity is commonly associated with a more aggressive tumor phenotype and decreased overall survival and, moreover, with a reduced benefit from endocrine treatment. Preclinical studies corroborate the role played by functional crosstalks between HER-2 and estrogen receptor (ER) signaling in endocrine resistance and, more recently, the activation of ER signaling is emerging as a possible mechanism of resistance to HER-2 blocking agents. Indeed, HER-2 positive breast cancer heterogeneity has been suggested to underlie the variability of response not only to endocrine treatments, but also to HER-2 blocking agents. Among HER-2 positive tumors, HR status probably defines two distinct subtypes, with dissimilar clinical behavior and different sensitivity to anticancer agents. The triple positive subtype, namely, ER/PgR/Her-2 positive tumors, could be considered the subset which most closely resembles the HER-2 negative/HR positive tumors, with substantial differences in biology and clinical outcome. We argue on whether in this subgroup the "standard" treatment may be considered, in selected cases, i.e., small tumors, low tumor burden, high expression of both hormonal receptors, an overtreatment. This article review the existing literature on biologic and clinical data concerning the HER-2/ER/PgR positive tumors, in an attempt to better define the HER-2 subtypes and to optimize the use of HER-2 targeted agents, chemotherapy and endocrine treatments in the various subsets.

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## Introduction

Breast cancer is a heterogeneous disease, with substantial genotypic and phenotypic diversity [1]. HER-2 protein overexpression or gene amplification is reported in ~15–20% of primary breast carcinomas and is associated with decreased disease free survival (DFS) and overall survival (OS) [2]. Approximately 70% of invasive breast cancers express estrogen receptor (ER), and the majority of ER positive cancers also express progesterone receptor (PgR). Although the presence of normal PgR levels suggests an intact ER signal transduction pathway in breast cancer cells, discrepant ER and PgR expression patterns (ER positive/PgR negative and ER negative/PgR positive) are sometimes observed. Overall, the ER positive breast cancers are classified as luminal cancers. These cancers are further subclassified based on their HER-2 status and proliferation rate into the ER positive/PgR positive/HER-2 positive (“triple positive”) and ER positive/PgR positive/HER-2 negative subtypes [3–5].

Initially, an inverse association was described between HER-2 positivity and the presence of hormonal receptors (HR), but subsequently it was reported that ~50% of the patients with HER-2 positive tumors are also HR positive [6], even if HER-2 positive tumors often, though not always, express HR at lower levels compared with HR positive/HER-2 negative tumors [7], and approximately one tenth of HR positive tumors are also HER-2 positive [8].

With regard to treatment, it is commonly believed that HER-2 blocking agents are effective in patients with HER-2-positive disease, irrespective of HR status [9]. A retrospective evaluation on the pivotal and other trastuzumab trials showed efficacy independently on HR status, thus confirming the paradigm of chemotherapy and trastuzumab in all the subsets of HER-2 positive disease. Nevertheless, there are some limitations in the analysis, such as the simplistic definition of HR status (positive vs negative), which does not take into account the degree of HR expression, and the lack of centralized evaluation [10,11]. Recent data seem to confirm the hypothesis that some heterogeneity exists among HER-2 positive subsets, mostly related to HR expression [12].

It is well known how women with HER-2/HR co-positive disease derive less benefit from endocrine therapy than women with HER-2 negative/HR positive disease [8,13,14]. Preclinical evidences seem to confirm that cross-talks between HER-2 and ER signaling pathways may contribute to resistance to endocrine therapy [15,16]. Trastuzumab concurrent with tamoxifen or fulvestrant may inhibit tumor growth and restore tumor sensitivity to these hormonal agents [17,18]. Therefore, simultaneously inhibition of both HER-2 and ER pathways is believed more effective than ER inhibition alone.

Even if treatment with HER-2 targeted agents in early-stage as well as in advanced HER2 positive BC have shown benefit across HR status, it is increasingly clear that in HER-2 positive disease the magnitude of benefit of HER-2 targeted therapy may differ by HR status. This leads to the question about whether HR status defines two or more distinct subtypes in HER-2 positive disease [19]. Moreover, among ER/HER-2 copositive group there is a subset of “triple positive” (ER/PgR/HER-2 positive) tumors, or tumors with particularly high degree of HR expression, which might represent a further and distinct subset with a particularly favorable prognosis, and for which the combination of standard chemotherapy, Her-2 blockade and endocrine treatment might be considered an overtreatment.

In this article, we summarize and critically discuss the available literature data on differences in tumor biology and clinical outcomes by ER and PgR status in HER-2 positive early and advanced breast cancer.

## Epidermal growth factor receptor family and ER pathways

Epidermal Growth Factor Receptor (EGFR) family overexpression/amplification, resulting in increased phosphorylation of ER, even in absence of its natural ligand, has been associated with tamoxifen resistance in vitro [20,21], with EGFR/HER-2 blockade being shown to both prevent the development of resistance [22], as well as restoring sensitivity to endocrine treatment in HER-2 overexpressing xenograft models [16,23]. Before the advent of HER-2 blocking agents, HER-2 positive/HR positive tumors had unfavorable prognosis compared with HR positive/HER-2 negative tumors, even if treated with endocrine therapy. In a French regional retrospective cohort study including 714 small, node-negative breast cancers treated in “pre-trastuzumab era”, the 10 years prognosis of HER-2 positive tumors was worse than that of HER-2 negative tumors, and the cohort with co-expression of HER-2 and HR showed the worst prognosis at 10 years. This seems to confirm the decreased benefit from hormone therapy in the HER-2 positive/ER positive subset, due to possible cross-talks between the two pathways, resulting in both intrinsic and acquired resistance to endocrine agents [13]. Recently, Nahta and O'Regan suggested that a subset of HER-2 positive/HR positive breast cancers could be driven primarily by high level of ER expression, and may show a behavior more similar to HER-2 negative/HR positive breast cancers [24]. In recent years, several clinical trials have focused on the association of both EGFR/HER-2 and HR pathways blockade in breast cancer patients.

### Gefitinib

A phase II randomized, placebo controlled study of 290 patients with ER positive advanced breast cancer regardless of HER-2 status compared tamoxifen in combination with either gefitinib or placebo. In patients with newly diagnosed metastatic disease or recurrence after adjuvant tamoxifen there was a numerical advantage in progression free survival (PFS) with the combination, which exceeded the predefined efficacy primary endpoint. Moreover, even in the small HER-2 positive subset a numerical advantage in PFS in the gefitinib arm was observed. However, patients recurring during/after adjuvant aromatase inhibitors (AI) or after having failed first-line AI did not benefit from combination therapy [25]. The combination of anastrozole and gefitinib vs anastrozole and placebo has been tested in 93 endocrine-naive patients, with significant improvement in PFS favoring the combination arm [26]. The effects of gefitinib have also been explored in a randomized perioperative study involving 56 patients with ER-positive and EGFR positive breast cancers, comparing 4–6 weeks of treatment with a combination of anastrozole and gefitinib vs gefitinib and placebo. A statistically significant decrease of Ki67 favoring the combination arm (92.4% vs 98% reduction,  $p = 0.005$ ) was observed [27].

### Lapatinib

The efficacy of lapatinib in combination with hormonal therapy as first line treatment for advanced breast cancer in HR positive and any HER-2 status has been explored in EGF3008, a randomized placebo controlled study of letrozole and lapatinib vs letrozole and placebo [28]. One thousand two hundred eighty-six patients were randomized, with stratification according to time since adjuvant tamoxifen therapy (<6 vs >6 months). PFS was significantly greater in the combination arm for the HR positive/HER-2 positive population. No additional benefit was seen overall with the addition of lapatinib in the HER-2 negative cohort. A subsequent blinded retrospective biomarker evaluation employing immunohistochem-

istry to semiquantify ER and PgR showed that in the HER-2 negative subgroup there was a significant improvement in PFS with lapatinib and letrozole combination in patients with low ER expression, while no benefit was observed with stronger ER expression [29]. The benefit may be related to inhibition of functional cross-talks between the two pathways, or to an increased HER-2 sensitivity related to ER inhibition [30]. This data contribute to support the dynamic interaction between HR and HER-2 signaling.

#### *Trastuzumab*

The TANDEM study was a randomized study of 207 patients which compared anastrozole plus trastuzumab vs anastrozole in HR and HER-2 positive MBC. Prior tamoxifen but not chemotherapy in the adjuvant or metastatic setting was permitted. Both PFS and response rate were significantly improved in the combination arm [31]. The eLEc-TRA study randomized 57 HR and HER2 positive patients receiving as first line treatment for advanced disease letrozole plus trastuzumab or letrozole alone. There was a numerical but non-statistically significant improvement in PFS favoring the combination arm. Interestingly, the PFS for those receiving letrozole with trastuzumab was similar to a cohort of women who were HR positive/HER2 negative tumours which received letrozole alone, 14.1 vs 15 months respectively [32].

Recent guidelines on systemic therapy for HER-2 positive/HR positive advanced breast cancer patients consider endocrine treatment with either trastuzumab or lapatinib or endocrine therapy alone as an acceptable first-line treatment. Endocrine therapy alone is included as an option because the trials of endocrine therapy with or without HER2-targeted therapy did not demonstrate a survival advantage [28,31,33].

#### **Differential efficacy of HER-2 blocking agents and chemotherapy according to HR status**

Even if in patients with HER-2 overexpressing breast cancer trastuzumab dramatically changed the disease natural history and improved outcome in all the settings, the pCR rates of neoadjuvant trastuzumab-containing regimes are still in the range of 30–60%, with 3-year relapse free survival (RFS) of 71–78% [34], clearly showing that a substantial number of HER-2 positive breast cancer patients undergoing surgery still present residual disease despite prior trastuzumab-based neoadjuvant therapy. Growing evidence indicates that the response to anti-HER-2 agents and the prognostic impact of pCR after anti-HER-2 based therapy depend on HR status. Moreover, despite major improvements in outcome, approximately 15% of patients treated with adjuvant trastuzumab-based therapy develop disease recurrence, and all the patients treated with HER-2 blocking agents for advanced disease progress, due to intrinsic or acquired resistance [35,36].

Gene expression profiling studies suggested that HER-2 positive/HR positive and HER-2 positive/HR negative tumors are two different subtypes, with different prognosis in absence of HER-2 blockade [1,37], confirming their distinct features and behavior. Moreover, emerging data suggest the involvement of the bidirectional cross-talk between the HER-2 and HR pathways not only in endocrine resistance but also in resistance to HER-2 blocking agents [23]. Cross-talks between HER-2 and HR pathways may intervene in trastuzumab and lapatinib resistance [38]. An increase in HR signaling was observed in patients with HER-2-positive/HR-positive tumor treated with lapatinib monotherapy [39–41]. These data suggest that positive HR status might be also a marker of lower sensitivity to anti-HER-2 therapies. Recently, ER pathways have been postulated as means of escape to HER-2 directed therapy. Wang et al. showed that, following lapatinib and trastuzumab

treatment, ER and its downstream effectors increased in all but one ER positive/HER-2 positive cell lines, and the acquisition of HER-2 directed agents resistance is mediated by activation of ER pathway, via Bcl2 family members [42]. Another recent study investigated the cross-talks between HER-2 and ER pathways and the effect of HER-2 blocking agents on the tyrosine kinase effector transcription factor Myc, showing that elevated Myc protein was inversely associated with pCR. In HER-2 positive cells trastuzumab can repress Myc transcriptional activity, inhibiting its target gene survivin, and this correlates with favorable response. Conversely, the co-expression of ER leads to upregulation of survivin expression and increased ER transcriptional activity, with subsequent lower response [43].

#### *Neoadjuvant setting*

In the neoadjuvant setting, data from the retrospective study by Guarneri et al. showed that the addition of trastuzumab to chemotherapy produced different outcomes according to HR status, with pCR rates 1.5 and 2-fold lower in ER positive than in ER negative cases [44]. More recently, data from the Neo-Sphere, NeoALTO, GeparQuinto, ACOSOG Z1041, CALGB 40601, NOAH and TBCRC006 trials further confirmed these findings, showing that pCR rates were significantly lower in ER-positive than ER-negative tumors, regardless the type of HER-2 targeted treatment [45–51]. Moreover, the pooled analysis of the German neoadjuvant studies suggested that pCR may be a suitable surrogate endpoint for HER-2 positive/HR negative but not for HER-2 positive/HR positive breast cancer patients [52].

Thus, patients with HER-2 positive/HR positive tumors not achieving a pCR are not necessarily at poor prognosis, as are triple negative subgroup, since adjuvant hormonal treatment may further improve clinical outcome. However, in this subgroup the prognostic value of pCR is lower than in patients with HER-2 positive/HR negative tumors, the achievement of pCR is infrequent and, when reported, it does not translate into the favorable outcome as in the HER-2 positive/HR negative subset [53]. A recent retrospective study, investigating the prognostic significance of pCR according to HR status in 366 patients, showed that among 204 patients treated with neoadjuvant trastuzumab, the achievement of pCR was confirmed to be not significantly prognostic in HR positive subgroup, suggesting that patients with “triple positive” tumors had a good prognosis despite the lower rate of pCR [54]. Due to the low rate of pCR in “triple positive” tumors, and its relatively small prognostic impact, we might speculate to reconsider the use of hormonal therapy combined with HER-2 blocking agents, without adding chemotherapy-related toxicity in this subset of patients.

The HER-2 dual blockade without concurrent chemotherapy was tested in two phase II neoadjuvant trials [45,51]. TBCRC006 results are particularly intriguing, since 49% of pCR/downstaging was reported with trastuzumab–lapatinib without chemotherapy, 54% in patients with HR positive tumors receiving also letrozole, while this percentage was 40% in HR negative group. According to pCR definition standard criteria, it was 27% overall, 21% in HR positive tumors, versus 36% in HR negative tumors. Moreover, the efficacy of trastuzumab and pertuzumab combination, without chemotherapy, was evaluated in one arm of the NeoSphere trial, reporting 17% of pCR, but in the subset of patients with HR positive tumors, not receiving any endocrine treatment, this percentage was only 6%. Even if pCR rates observed in these two trials are lower than those reported in studies with chemotherapy, they raise the hope that HER-2 dual blockade, with endocrine therapy whenever appropriated (HR positive tumors) might be a reasonable and well tolerable choice even in neoadjuvant setting. Recently, alterations of the PI3K and ER pathway genes have been

correlated with poor outcome in terms of RFS and lower pathological response rate in patients with HR positive tumors (while not in HR negative tumors) receiving neoadjuvant chemotherapy and trastuzumab, suggesting that cross-talks between the two pathways are bidirectional and may influence chemotherapy and trastuzumab efficacy [55].

A recent retrospective analysis of histopathologic features of 450 HER-2 positive breast cancer patients treated with neoadjuvant chemotherapy and trastuzumab confirmed that HR positive and HR negative tumors show distinct histopathologic features, that may be relevant to their distinct clinical behavior [56].

#### Adjuvant setting

Similar data are reported also in the adjuvant setting, where a retrospective evaluation from the HERA trial indicate a clear advantage in DFS for the trastuzumab arm, but with wider confidence intervals of hazard ratios among patients with HER-2 positive/HR positive tumors [9].

As a confirm of the differential behavior of the two subtypes, HR positive/HER-2 positive tumors have usually a different timing and pattern of relapse, since recurrences occur at a relatively constant rate over time and continue occurring after more than 10–15 years of follow up. Conversely, HER-2 positive/HR negative tumors relapse more commonly within the first 5 years. Moreover, sites of relapse are different, since bone and soft tissue are more common in ER positive disease, conversely, visceral sites are more frequently observed in ER negative subset, and sensitivity to some chemotherapy drugs, i.e. paclitaxel, may be different between the two subsets [57–59]. A recent evaluation of 1187 early breast cancers compared disease characteristics among different groups according to HR and HER-2 status. Results showed that both HR and HER-2 status had a profound impact on breast cancer characteristics, and triple positive tumors were associated with lower grade and higher bone involvement, reflecting a retained impact of HR. Conversely, HER-2 impact on HR positive disease was reflected by higher grade, younger age and increased frequency of developing visceral metastases. Moreover, triple positive breast cancers occurred more frequently in younger patients compared to ER positive/PgR negative/HER-2 positive subset [30].

Among 123,780 early breast cancers from the California Cancer Registry, the surrogate classification using ER/PgR/HER-2 and tumor grade showed a variability in survival among HER-2 subtypes within each stage of disease, and while survival was superior across all the stages for ER/PgR positive/HER-2 negative subtype, the difference was less than 1–2.2% between ER/PgR positive/HER-2 negative and ER/PgR positive/HER-2 positive subtypes in early stages, with no significant difference between the two subtypes in stage 3 [60].

A retrospective evaluation of records from 897 patients with HER-2 positive/HR positive breast cancers treated with adjuvant chemotherapy followed or not by hormonal therapy and trastuzumab reported higher DFS and OS in patients who had received chemotherapy, trastuzumab and endocrine therapy than those observed in patients treated with chemotherapy and trastuzumab without subsequent endocrine therapy. At multivariate analysis, administration of endocrine therapy in addition to chemotherapy and trastuzumab resulted the only independent prognostic factor for DFS, with a trend in OS, confirming that endocrine treatment confers benefit when added to chemotherapy and trastuzumab in patients with HR positive/HER-2 positive early breast cancer [61].

#### Advanced setting

Some retrospective analyses showed differential sensitivity to combined HER-2 blocking agents and chemotherapy according to

HR status in the advanced setting. A relationship between quantitative immunohistochemical HR expression and response to first-line chemotherapy and trastuzumab was reported in a retrospective evaluation on 111 out of 227 HER-2 positive advanced breast cancer patients, suggesting that an expression of ER in  $\geq 30\%$  of tumor cells was predictive of reduced response to chemotherapy and trastuzumab; moreover, a maintenance endocrine treatment, added to trastuzumab after chemotherapy in one third of the patients, translate into significant PFS benefit, indicating a relevant role of endocrine therapy combined to anti HER-2 agents in this subset of patients. Conversely, when considering a HR cut off  $\geq 1\%$ , in the absence of maintenance endocrine therapy, no difference in PFS was observed between patients with HR positive and HR negative tumors. Moreover, a non significant trend toward a better PFS was observed in patients with tumor expressing high levels of ER and/or PgR, even in the absence of the maintenance endocrine therapy [62].

Since the magnitude of benefit of trastuzumab in advanced HER-2 positive breast cancer varies widely, the clinicopathological features associated with prolonged first-line trastuzumab-based treatment duration have been recently investigated in a retrospective study including 164 patients. Results have shown that long-term benefit of trastuzumab-based therapy was associated with HR positivity and the absence of previous adjuvant trastuzumab. It is noteworthy that a subgroup of patients with HER-2 positive/HR positive tumors received maintenance trastuzumab and/or endocrine therapy, which may have favorably influenced the outcome [63].

A prospective observational study (registHER) on a cohort of more than 1000 patients with HER-2 positive advanced breast cancer, including 530 patients with HER-2 positive/HR positive tumors, showed that, with or without chemotherapy, outcomes were more favorable for the HR positive subset, since dual targeting of HR and HER-2 pathways was associated with more prolonged PFS and OS compared with HER-2 based therapy alone [64].

#### PgR expression role

The role of PgR expression is not completely clarified in terms of prognostic and predictive significance in breast cancer. So far, the available literature data suggest its value in predicting endocrine response in the advanced setting, and retrospective evaluation from large endocrine adjuvant trials confirm a more favorable prognosis of patients with tumors expressing both HR [12,65,66], even when considering numerous bias related to quality testing. ER positive/PgR negative tumors, as defined by RNA profiling, represent a distinct subset of breast cancer with more aggressive features and poor outcome despite being clinically ER positive [67]. The prognostic value of PgR expression has been already reported in several studies but, to our knowledge, no prospective study has focused on HER-2 positive subgroup. Clinical and biological features of 31,415 patients with ER/PgR positive tumors were retrospectively compared with 13,404 patients with ER positive/PgR negative tumors. Results showed that the PgR negative subgroup expressed higher levels of EGFR and HER-2 and displayed more aggressive features than the ER/PgR copositive subgroup, suggesting that the loss of PgR expression in ER positive tumors may be a marker of activated EGFR/HER-2 pathway signaling [12]. A significant correlation between absence of PgR expression and poorer outcome in luminal breast cancer was shown in a retrospective series of 4837 patients with luminal B tumors by immunohistochemical classification. The subset of patients with ER positive/PgR negative/HER-2 positive tumors had a reduced breast cancer related survival when compared with the HR positive/HER-2 negative subgroup; conversely, no statistically significant differences

were found among patients with “triple positive” tumors and patients with HR positive/HER-2 negative tumors, even if it must be taken into account that the HER-2 positive subset received more chemotherapy than Her-2 negative subgroup. On this basis, PgR loss may be considered an unfavorable event even in the HER-2 positive subset [68].

Several studies evaluated the impact of PgR status on recurrence and mortality [69–71]. In particular, one trial reported that women with PgR-negative tumors had a higher risk of mortality independent on the different characteristics, compared with women with both HR positive tumors [69]. A recent population-based study on 1074 patients with early breast cancer confirmed that the absence of PgR expression was a powerful independent prognostic variable even in ER positive breast cancer receiving endocrine therapy, and cancers PgR negative were significantly more likely to be HER2 positive than PgR positive tumors ( $p < 0.001$ ) [72]. The results of the previous reported trial from the California Cancer Registry showed that the survival of ER/PgR positive/HER-2 positive subtype was superior to that of ER positive/PgR negative/HER-2 positive subtype across all stages, confirming the relevant role of PgR on survival even in HER-2 positive tumors [60].

### HER2 blocking plus chemotherapy always?

To date, the paradigm of chemotherapy plus anti-HER-2 agents is still the mainstay of treatment of all the HER-2 positive breast cancer subsets, regardless of HR status, even if the molecular heterogeneity of HER-2 positive breast cancer may have some therapeutic implications. It is largely known how HR positive status, and the degree of ER expression, reduce chemosensitivity in breast cancer [73–75].

It is reasonable that the co-expression of both HRs, along with a high extent of hormonal expression, even if in HER-2 positive breast cancer, may identify a subset of tumors with a particularly favorable prognosis, and perhaps less sensitive to chemotherapy and, probably, to HER-2 blocking agents. We have recently performed a retrospective evaluation on 441 “triple positive” tumors, all treated with adjuvant chemotherapy and subsequent hormonal therapy. The series included 158 patients treated with chemotherapy and endocrine treatment without HER-2 blocking agent in the “pre-trastuzumab era”, and 283 patients treated with adjuvant trastuzumab. The relapse rate at 3 years was 15% in the chemotherapy and sequential hormonal adjuvant therapy without trastuzumab, and 6.4% in the trastuzumab treated patients ( $p 0.005$ ). Kaplan Meyer curves indicated a 5-year RFS and a 5-year OS of 71% and 92%, respectively, in the “pre-trastuzumab” group, while these estimates raised to 91% and 96.6% in patients having received chemotherapy, endocrine therapy and trastuzumab. This clearly confirmed the advantage conferred by trastuzumab administration even in real practice outside of clinical trials. Conversely, in a small subset analysis of patients with tumors with ER staining in 50% or more of cancer cells, the relapse rate at 3 years was 6.2% in the cohort having received chemotherapy and sequential endocrine adjuvant therapy without trastuzumab and 5.4% in the cohort having received also trastuzumab ( $p 0.84$ ); Kaplan Meier curves showed a 5 years RFS of 89.7% and a 5-year OS of 95.7% in the cohort without trastuzumab, and a 5-year RFS of 92.3% and a 5-year OS of 94.9% in the cohort having received trastuzumab. Notwithstanding the limitations related to the small sample size and the low number of recurrences, this evidence seems to suggest that in “triple positive” patients with tumors expressing very high degree of HR the addition of trastuzumab to conventional adjuvant therapy does not provide benefit [76]. We are currently conducting a larger observational retrospective analysis on “triple positive”

tumors to better evaluate the correlation among HR status, adjuvant treatments, endocrine therapy, trastuzumab administration and outcome, and to better define the role of trastuzumab in this specific patient subset.

Indeed, HER-2 positive breast cancer heterogeneity has been suggested to underlie the variability of response to HER-2 blocking agents, although much remains unknown regarding the precise genomic and biological features of HER-2 positive cancers, and underlying mechanisms of *de novo* or acquired resistance.

Recently, the Cancer Genome Atlas (TCGA) Network has evidenced the existence of two genetically distinct HER-2 positive subtypes, with different mRNA expression, HER-2 enriched or luminal [77]. Since at least two different HER-2 positive subtypes exist, HR positive and HR negative, and HR positive/HER-2 positive tumors are usually enriched with luminal gene cluster, falling into the luminal B subtype, it is reasonable hypothesizing also distinct clinical behavior and different sensitivity to anticancer agents.

On the basis of what above reported, the precise identification and characterization of a HER-2 positive/HR positive subset may be essential to avoid overtreatment, mainly in patients with small tumors who could benefit from adjuvant hormonal treatment combined to anti HER-2 agents, possibly without prolonged chemotherapy, or without chemotherapy at all. Whether the term “overtreatment” is more strictly related to trastuzumab or to chemotherapy is still to be clarified. This raises the question if, in selected cases or specific subgroups of patients, omission of chemotherapy may be the most appropriate choice and, whether or not, endocrine treatment combined with HER-2 blocking agents or HER-2 blockade alone have to be administered, even in the early setting [78]. Results from neoadjuvant trials testing the HER-2 dual blockade without chemotherapy seem to confirm this hypothesis, suggesting that a subgroup exists for which anti-HER-2 treatment alone in absence of chemotherapy may be effective [45,46], even if in the HR positive subset the pCR rate in the chemotherapy-free arms was lower than in HR negative tumors, possibly because these regimens did not contain endocrine therapy. Moreover, the results from the small phase II study of Rimawi et al. clearly confirmed in fact the benefit of adding endocrine manipulation [51].

In the advanced setting, the dual blockade with trastuzumab and pertuzumab without chemotherapy produced considerable response rate and clinical benefit even in patients with HR positive tumors [79,80]. The combination of trastuzumab and lapatinib showed significantly longer PFS and OS compared to lapatinib alone in trastuzumab-refractory patients, half of them with HR positive tumors [81,82]. However, in the Cleopatra trial patients with HER-2 positive and HR positive tumors had reduced benefit in PFS and OS from dual blockade with pertuzumab and trastuzumab compared to patients with HR negative tumors, as a confirmation of a possible negative interference between HER-2 blocking agents and endocrine pathway [83,84].

Recent ASCO guidelines still recommend as first line treatment for advanced breast cancer the combination of HER-2 blocking agents and chemotherapy as the optimal choice, but also consider the use of endocrine treatment combined with HER-2 target therapy [33]. To date, no trials have directly compared endocrine therapy plus HER2-targeted therapy with chemotherapy plus HER-2-targeted therapy. Although there seems to be no OS benefit from adding HER-2-targeted therapy to endocrine therapy, two out of three studies did show a PFS benefit for the combination therapy groups [28,31,32], suggesting this combination as a reasonable option in advanced disease.

The identification of potential biomarkers predictive of HER-2 blocking agents benefit in absence of chemotherapy could significantly improve the management of HER-2 positive breast cancer patients, and a number of studies are ongoing. Potential biomark-

ers of response to trastuzumab or lapatinib have been recently investigated by Montemurro et al. in 19 HER-2 positive advanced breast cancer patients by expression analysis of more than 100 genes from primary tumor samples; PAM-50 intrinsic subtypes were also identified, along with quantitative HER-2 (H2T) and p95 protein expression. Median PFS and OS were 7.3 and 43 months, respectively. Biological evaluations showed that high expression of 17q12-21 amplicon genes HER-2 and belonging to the PAM50 HER-2 enriched intrinsic profile were associated with better outcome, suggesting that a subset of patients could be treated without chemotherapy [85].

## Conclusions

Molecular classification is playing an increasingly essential role in the personalized care of breast cancer, and the three key molecular determinants, HER-2, ER and PgR, are commonly evaluated in routine clinical practice. However, the biological significance and complex interactions of their related pathways should be investigated further to determine intrinsic heterogeneity of breast cancer and inform treatment decisions in the complexities of the clinical setting, with the identification of further tumor subtypes amenable to targeted and innovative treatments representing a research priority.

Overall, the above reported data suggest the possibility that a subset of small HER-2 positive, ER/PgR positive (“triple positive”), breast cancer might be driven primarily by HR status, and biologically behave more likely HER-2 negative, HR positive breast cancers: should these patients receive endocrine treatment in combination with HER-2 blocking agents, without chemotherapy? Is the administration of anti-HER-2 agent always necessary? The identification and characterization of this subset of HER-2 positive cancers may be essential to avoid possible overtreatment, and define the role of both HR and HER-2 pathways in the development of treatment resistance is one of the hallmarks for further investigation, to optimize the use of HER-2 targeting agents, endocrine therapy and chemotherapy.

## Conflict of interest

The authors declare that they have no conflict of interest.

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None.

## Author contribution

The outline was conceived by PV. All authors participated in the preparation of the manuscript and contributed to initial drafts, edited version, and final version. All the authors read and approved the final version before submission.

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