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REVIEW ARTICLE

New Insights to Reshape the Management of Patients with Metastatic Breast Cancer - Focus on Overcoming Challenges in HER2 Status Interpretation

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Abstract:

Approximately 20% of invasive Breast Cancers (BCs) are characterized by Human Epidermal growth factor Receptor 2 (HER2) protein overexpression or *HER2* gene amplification. HER2 represents a standard diagnostic test and a predictive biomarker for the use of HER2-directed treatments in patients with BC. At present, the HER2 Immunohistochemistry (IHC) assay is applied for screening purposes, and the *In Situ* Hybridization (ISH) test serves as a confirmation, when HER2 IHC results are equivocal.

However, an accurate assessment and interpretation of the HER2 status can be complicated in many women with BC. These difficulties can be attributed to various factors such as HER2 Intratumoral Heterogeneity (ITH) and changes of HER2 in the process of BC metastatic progression or post neoadjuvant Chemotherapy (CHT). In particular, the status of biomarkers (*e.g.*, HER2 and co-expressed Hormone Receptor (HR)) can be altered in patients with metastatic BC and such receptor changes influence the therapeutic responses and clinical outcomes.

The goal of this article is to present challenges in the assessment of HER2 expression and to underscore a need for the biomarker status reevaluation in patients with metastatic BC. This mini-review also provides some insights into the interpretation of equivocal HER2 status in women with metastatic BC and discusses the impact of HER2 and HR biomarker conversions on therapeutic decision-making and the patient prognosis in metastatic BC.

It is crucial to correctly interpret the HER2 biomarker status and to assess conversions of HER2 and HR in the BC metastatic lesions since timely detection of such alterations is critical to management modifications of individual patients with metastatic BC.

Keywords: Breast Cancer (BC), Metastasis, Human Epidermal growth factor Receptor 2 (HER2), Hormone Receptor (HR), Biomarker conversion, Intratumoral Heterogeneity (ITH).

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1. INTRODUCTION

Breast Cancer (BC), which begins as a local tumor, can potentially metastasize, and thus, during an initial diagnostic workup, various biomarkers are checked to help evaluate the probability of the metastatic progression [1]. In approximately 20% of patients with BC, Human Epidermal growth factor Receptor 2 (HER2) (a proto-oncogene located on chromosome 17) is amplified or HER2 protein is overexpressed [1]. On the one hand, a positive HER2 status is related to the increased metastatic potential of BC and poor patients outcomes [2]. However, on the other hand, a positive HER2 biomarker predicts a favorable response to standard HER2-targeted therapies (*e.g.*, anti-HER2 monoclonal antibodies, such as trastuzumab or pertuzumab, and Tyrosine Kinase Inhibitors (TKIs), such as lapatinib or neratinib) in women with BC [2].

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Since HER2-directed therapy has beneficial effects on patients with BC, characterized by HER2-overexpression or *HER2*-gene amplification, an accurate and updated HER2 status evaluation is of utmost importance for correct diagnostic and therapeutic management, especially in the metastatic BC setting [3].

Traditionally, in women with metastatic BC, the assessment of HER2 status would be done predominantly on the primary tumor (according to the opinion that the HER2 status remains stable during the metastatic progression) [4]. However, in more recent studies, discordant HER2 status findings between the primary and metastatic BC sites have been noted [5 - 7]. Such a discrepancy in biomarkers can be relevant to the genetic instability that accompanies BC progression and may affect the changes of the *HER2* gene and chromosome 17 (Chr17). For instance, chromosomal rearrangements that take place during the BC metastatic transformation and progression can interfere with the diagnosis and therapy of patients with metastatic BC [8].

Similarly, Hormone Receptors (HR), such as Estrogen Receptors (ER) and Progesterone Receptors (PR), which can be co-expressed together with HER2, are important prognostic and predictive factors in patients with BC, and thus, their assessment in both early and metastatic BC stages allows to more precisely guide the Endocrine Therapy (ET) [9].

Consequently, women with HR-positive breast tumors experience beneficial effects from adjuvant ET, in the form of tamoxifen (a Selective Estrogen Receptor Modulator (SERM)) or Aromatase Inhibitors (AI) (*e.g*., anastrozole, letrozole, and exemestane) [10]. In contrast, patients with HR-negative breast tumors have no advantage from ET and their prognoses are usually worse [10]. However, it should be highlighted that the response to systemic therapies, among breast tumors co-expressing HER2 and HR is very complicated, because of the bilateral cross-communication between the HER2 and HR signaling pathways, which can lead to a decreased sensitivity or resistance to the HER2-targeted therapy and ET [10]. According to a recent meta-analysis, the receptor features of the primary BC tumors are frequently preserved in metastatic lesions [11]. However, the HER2 and HR conversion can take place during the BC progression, and thus, a reevaluation of their status in BC metastases is critically important, since it affects the patient management [11]. A need for the repeated assessment of BC biomarkers (*e.g*., in samples from primary and metastatic BC lesions), in order to precisely inform treatment decision-making for patients with metastatic BC, was highlighted in the American Society of Clinical Oncology (ASCO) practice guidelines [12].

The goal of this article is to present challenges in the assessment of HER2 expression and to underscore a need for the reevaluation of biomarker status in patients with metastatic BC. This mini-review also provides some insights into the interpretation of equivocal HER2 status in women with metastatic BC and discusses the impact of HER2 and HR biomarker conversions on therapeutic decision-making and the patient prognosis in metastatic BC.

2. COMMON CHALLENGES TO HER2 STATUS ASSESSMENT AND INTERPRETATION

At present, HER2 Immunohistochemistry (IHC) is applied for screening purposes, and *in situ* Hybridization (ISH) serves as a confirmation, if the HER2 IHC test results are equivocal (this has usually been determined *via* HER2 Fluorescence *In Situ* Hybridization (FISH) or Silver *In Situ* Hybridization (SISH) probes) [12]. Furthermore, when there is a discrepancy between the results of HER2 status between primary and metastatic BC lesions, FISH or SISH tests should be conducted [12].

In response to these challenging problems, over the past years, the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines have incorporated some modified recommendations for a precise interpretation of the HER2 status [12, 13]. For instance, according to the ASCO/CAP guidelines (2018), it has been recommended that tumors with double-equivocal HER2 status (based on IHC and in ISH assays) should be considered as HER2-negative (since there is no evidence that the HER2-targeted treatment is beneficial in such cases) [12]. Some important suggestions relevant to the assessment of HER2 status in metastatic BC are summarized in Table **1** [12, 13].

3. HER2 AND HR CONVERSIONS AND THEIR CLINICAL CONSEQUENCES FOR PATIENTS WITH BC

HER2 and HR (ER and PR) represent important biomarkers for the management of patients with metastatic BC, and thus, any changes in HER2 and HR expression need to be addressed [11]. “Receptor conversion”, which means an alteration of the receptor status during BC metastatic progression, can take place in both HER2 and HR (with different incidence rates) [11]. For instance, the frequency of HER2 conversion from positive to negative was 21.3%, but in the opposite direction (negative to positive), it was only 9.5% [11].

According to a recent meta-analysis, different studies have reported various discordance rates in HER2 and HR expression between BC primary tumors and metastases [11]. For instance, changes in HER2 and HR (ER and PR) biomarker status have been estimated as approximately 21.9% and 34.2%, respectively. Such conversions merit careful diagnostic evaluations and should be followed by individualized therapeutic approaches in patients with metastatic BC [10]. It should be pointed out that a negative conversion basically implies that the present therapy is not effective anymore, and thus, another management strategy needs to be introduced. In contrast, a positive conversion implies that there is a possibility of some specific treatments that could be tried [11]. As an illustration of the HER2 and HR expression changes, another meta-analysis has indicated that the prevalence rate of negative conversion outnumbered that of positive conversion (13 *vs*. 5%) [14]. Similarly, it has been reported that the HER2 discordance rate was about 3%, and all the observed cases switched from a positive HER2 status in the primary tumors to a negative one in the metastatic lesions [15].

Concurrently, based on another review of discordant BC cases, it has been shown that changes in HER2 and HR status more often included negative conversions (*e.g*., from HER2-positive and/or HR-positive to HER2-negative and/or HR-negative status), which are frequently associated with adverse BC outcomes [16]. The biomarker conversion that leads to a difference in the HER2 and HR expression has an impact on the selection of treatment options. It should be emphasized that in cases of the discordant HER2 expression, a conversion from HER2-negative primary BC tumors to HER2-positive metastases can also occur, and such a positive conversion

Table 1. Updated interpretation of HER2 status by using immunohistochemistry and dual-probe *in situ* hybridization test (based on the ASCO/CAP 2018 guidelines) [12, 13].

HER2 by Immunohistochemistry (IHC)[12]			
Negative [-]	**Negative [1+]**	**Equivocal [2+]**	**Positive [3+]**
No staining observed, incomplete membrane staining (faint) & within ≤10% of the invasive tumor cells	Incomplete membrane staining (faint) & within >10% of the invasive tumor cells	Weak to moderate complete membrane staining in >10% of tumor cells	Circumferential membrane staining (complete, intense) & in >10% of tumor cells
HER2 by in situ Hybridization (ISH) [12]			
Negative [-]		**No equivocal status**	**Positive [+]**
HER2/CEP17 ratio < 2.0 & average HER2 copy number < 4.0 (group 5) HER2/CEP17 ratio ≥ 2.0 & average HER2 copy number < 4.0 (group 2) with concurrent IHC 2+ HER2/CEP17 ratio < 2.0 with average HER2 copy number ≥ 4.0 and < 6.0 (group 4) with concurrent IHC 2+ Groups 2, 3, and 4 with concurrent IHC 0 or 1+		-	HER2/CEP17 ratio ≥ 2.0 & average HER2 copy number ≥ 4.0 (group 1) HER2/CEP17 ratio ≥ 2.0 & average HER2 copy number < 4.0 (group 2) with concurrent IHC 3+ HER2/CEP17 ratio < 2.0 & average HER2 copy number ≥ 6.0 (group 3) with concurrent IHC 2+ HER2/CEP17 ratio < 2.0 & average HER2 copy number ≥ 6.0 (group 3) with concurrent IHC 3+ HER2/CEP17 ratio < 2.0 with average HER2 copy number ≥ 4.0 and < 6.0 (group 4) with concurrent IHC 3+
Helpful Hints for Evaluation of HER2 Heterogeneity in BC [13]		**Recommended Actions [13]**	
Review the entire HER2 IHC slide to find areas with potential HER2 amplification; Scan the entire HER2 ISH slide prior to counting;		Use SISH or CISH that is beneficial for assessment of HER2 heterogeneity (since such tests can be conveniently matched with HER2 IHC slide under the optical microscope)	
In the ISH report, provide a proportion of amplified cells within a tumor.		In case of finding a subpopulation of tumor cells with HER2 amplification (including > 10% of tumor cells on the slide), perform a separate counting in this subpopulation; Provide separate calculations for the HER2/CEP17 ratios and *HER2* gene copy number for the amplified & non-amplified areas	

Abbreviations: ASCO, American Society of Clinical Oncology; BC, Breast Cancer; CAP, College of American Pathologists; HER2, Human Epidermal Growth Factor Receptor 2; IHC, Immunohistochemistry; ISH, *in situ* Hybridization; CEP17, Chromosome Enumeration probe 17; CISH, Chromogenic *in situ* Hybridization; SISH, Silver *in situ* Hybridization.

opens a new therapeutic possibility that should not be overlooked. At this point, a prompt reevaluation of the receptor status in the metastatic BC lesions is critical to precisely adjust the therapeutic regimen and possibly improve the patient outcome [16]. In addition, it should be kept in mind that the discrepant findings between the HER2-negative primary BC tumors and the HER2-positive metastases may be due to some inaccurate procedural issues, which need be clarified [16]. With regard to the HR (ER and PR) status, it has been reported that its changes are associated with the prognosis of women with metastatic BC, and in particular, a negative conversion of HR status has been linked to adverse patient outcomes [17].

4. POSSIBLE MECHANISMS OF HER2 STATUS CHANGES DURING BC METASTATIC PROGRESSION

Although the exact reasons for the receptor conversion during BC metastatic progression are still unknown, there are some possible explanations of this phenomenon. For instance, according to a concept of Intratumoral Heterogeneity (ITH), BC cells in a given tumor mass may display heterogeneity (in terms of genotype and phenotype), and only a few of such cells can create a metastasis [1, 11]. Consequently, when in the primary BC tumor, the biomarker's status is heterogeneous, there is a likelihood of their alterations during the metastatic progression [1, 11]. A similar phenomenon can occur under the circumstances of selection pressure from therapy when a patient with BC is given HER2-targeted therapy (*e.g.*, trastuzumab) or ET (depending on the BC receptor status). In consequence, only those BC cells, which were able to survive the treatment's selective pressure could subsequently create a metastatic lesion, which might reveal a changed biomarker status (compared to a primary BC tumor) [1, 11, 12].

Furthermore, heterogeneity of *HER2* gene amplification that has been shown in some women with BC, contributed to inaccurate HER2 status evaluation, and in consequence, could have some influence on the effects of HER2-directed therapy [18]. In addition, it has been revealed that HER2 ITH is more common in women with HER2-positive metastatic BC, who initially had demonstrated HER2-positive BC, and then, were determined to have equivocal HER2 protein expression and

low-level *HER2* gene amplification [19]. Such patients have responded poorly to trastuzumab therapy and had worse outcomes [19]. In addition, it has been reported that BC cases with HER2 ITH displayed a reduced rate of the Disease-Free Survival (DFS), in comparison to the ones without HER2 ITH [20]. It was particularly evident in the HR-positive subset of women with BC, who received trastuzumab therapy in the adjuvant setting [20]. Similarly, it has been reported that HER2 ITH was a predictive factor for an incomplete therapeutic response to anti-HER2 agents (*e.g*., in the neoadjuvant setting) [21].

Even though the exact mechanism of HER2 status conversion during BC metastatic progression still remains unclear, it is conceivable that HER2 ITH and therapeutic selection pressure play a contributing role [22]. In the instance of positive to negative HER2 conversion, a lower degree of HER2 protein expression and heterogeneous *HER2* gene amplification has been revealed, compared to the cases, which continuously displayed HER2-positive test results [22]. These findings suggest that the breast tumors, which are characterized by HER2 ITH, are able to present different HER2 status in metastatic lesions, since the HER2-directed therapy had eliminated susceptible clones [22]. Moreover, it should be highlighted that the timely detection of HER2 conversion is of utmost importance, since it may have a profound impact on future treatment choices. For instance, some studies have revealed a beneficial response to trastuzumab therapy, among women who switched to HER2-positive status in the metastatic sites [23, 24] Furthermore, in patients with metastatic BC, in whom the HER2 status was changed from negative to positive, a longer Time To Progression (TTP) was reported (*e.g*., 10 months *vs*. 4 months) with trastuzumab treatment, compared to those who did not receive trastuzumab [24]. Therefore, it is crucial to reassess the HER2 status in BC metastatic lesions (particularly in patients, in whom the primary BC tumors display HR-positive expression) to be able to apply the most optimal targeted therapy and improve patient outcomes [12].

5. *HER2* GENE CHANGES IN THE BC PRIMARY TUMOR AND METASTASES – THE INTERFERING ROLE OF CHEMOTHERAPY

The status of the *HER2* gene in BC (that is usually determined at the time of BC diagnosis) can be changed in both the BC primary tumor and metastases (*e.g*., local lymph nodes and distant sites) [25]. Such alterations can be a result of the tumor evolution itself or the therapeutic intervention [25]. At present, no specific recommendations exist with regard to the modification of treatment, depending on changed biomarker status after applying neoadjuvant Chemotherapy (CHT) (standard therapy for locally advanced BC) [22]. However, a change of the HER2 status can affect the therapeutic approach in individual patients, and thus, a reassessment of the HER2 status post neoadjuvant CHT is advisable [22].

The mechanism of HER2 conversion after neoadjuvant CHT is not clear, but it may be related to the selection of HER2-positive or HER2-negative clones after neoadjuvant CHT, tumor heterogeneity, and some analytical or procedural errors [22].

For instance, it has been reported that women with breast tumors, who underwent HER2 negative conversion, after neoadjuvant CHT, had decreased Disease-Free Survival (DFS) compared to those who presented continuous HER2 positivity [26].

Furthermore, it should be underlined that an interpretation of HER2 ISH, after neoadjuvant CHT is really needed for correct distinguishing between a true *HER2* amplification and an increase of *HER2* copy number by chromosome 17 polysomy [25]. In fact, under these circumstances, the increase in the *HER2* copy number might not be attributed to the true *HER2* amplification, but it could rather reflect a polyploidization event due to CHT (that can happen in all of the chromosomes) [25]. At this point, an accurate HER2 status assessment, using dual-probe ISH with concurrent IHC assay has been recommended [25]. In addition, it should be underscored that the increase of centromere17 copy number is predominantly related to the amplification of the centromeric region, and thus, correcting the *HER2* gene copy number with Centromere17 Enumeration Probe (CEP17) may lead to an inaccurate assessment of *HER2* amplification [27].

6. THE ROLE OF BIOMARKERS IN INFORMING THE MANAGEMENT DECISIONS FOR PATIENTS WITH METASTATIC BC

The aim of the ASCO guidelines has been to present the concise evidence-based recommendations for the oncology community (*e.g*., medical, surgical, or radiation oncology specialists, pathologists, as well as primary care physicians, nursing staff and patients with BC) [12, 28]. To keep abreast of the newest trends in this dynamically developing area, the focused ASCO recommendations highlight selected BC biomarkers and indications for their practical applications (*e.g*., using the updated HER2 and HR status to help decide whether to switch or to continue a given targeted therapy) (Table **2**) [12, 28]. Consequently, in women with BC metastases, a biopsy for confirmation of the BC process and reevaluation of the HER2 and HR (ER and PR) status has been recommended. However, the available evidence is still not sufficient to decide about potential changes in BC treatment for such patients.

Nevertheless, in the face of the discordant results between BC primary tumor and metastatic lesions, it has been recommended to use preferentially the HER2 and HR status of the metastases to guide treatment. Moreover, such a decision needs to be convergent with the patient's clinical context and management goals (*e.g*., medical condition and personal preferences) (Table **2**) [12, 28]. In addition, it should be kept in mind by medical practitioners (and clearly conveyed to their patients with BC) that evidence is still limited to determine if switching the oncology treatment, based on BC biomarker status alterations would influence patient outcomes. Furthermore, it needs to be underscored that the use of circulating tumor biomarkers depends on clinical experience and judgment, and thus, physicians should not apply such biomarkers only as additional therapeutic indications (Table **2**) [12, 28]. In practical terms, (Table **3**) [29 - 45] briefly summarizes current and emerging treatment strategies for patients with HER2-positive metastatic BC, including anti-

HER2 monoclonal antibodies, Antibody-Drug Conjugates (ADCs), and Tyrosine Kinase Inhibitors (TKIs). In addition, possible combinations of anti-HER2 agents and endocrine therapies (depending on the HR status) with different therapeutic modalities, such as Cyclin-Dependent Kinase (CDK) 4/6 inhibitors, phosphatidylinositol-3 kinase/ mammalian target of rapamycin signaling pathway (PI3K/mTOR) inhibitors, and immunotherapy, are also being considered. In this light, the right "points of attack", meaning molecular targets, related to HER2 expression can facilitate an adequate selection of candidates for these new and emerging treatment options. Since safe and effective therapeutic strategies for HER2-positive and/or HR-positive metastatic BC are urgently needed, the future clinical trials should investigate the use of innovative therapies, guided by biomarkers (*e.g.*, related to gene expression or molecular targets) for metastatic BC, which is so difficult to manage.

Table 2. Practical recommendations for biomarker applications to guide management decisions in patients with metastatic BC.

The main Clinical Questions [28]	Key Recommendations [12,28]	Type and Quality of Evidence [28]	Strength of Advice [28]
1. Should metastases be biopsied to assess changes from the primary tumor HER2 or HR (ER or PR) status?	1 Women with newly diagnosed metastases from primary BC should have a biopsy for confirmation of BC and testing of HER2, ER, and PR status; if results between BC primary and metastatic lesions are discordant, the HER2, ER, and PR status from the metastasis should be preferentially used to guide therapy (in accordance with the clinical context/patient's management goals)	1.T: evidence based on biomarker change from primary to metastasis; lack of evidence to determine if outcomes are better with treatment options based on receptor status in the metastases *vs*. in the primary tumor Q: insufficient	1.moderate
2. For patients with metastatic BC & known HER2, ER, and PR status, which additional tumor biomarkers are clinically useful to start systemic therapy or select a new systemic treatment ?	2. Decisions on starting systemic therapy for metastatic BC should be predominantly based on clinical exam, medical judgment, and patient preferences	2.T: evidence based Q: low.	2.moderate
3. For patients with metastatic BC & known HER2, ER, and PR status, which additional tumor biomarkers are clinically useful to guide decisions on changing to a different therapy or terminating it?	3. Recommendations for tissue biomarkers: In patients already receiving systemic therapy for metastatic BC, decisions on switching to a new therapy or terminating it should be based on clinical exam, assessment of BC progression/response to treatment, and the patient's management goals	3.T: evidence based Q: low.	3.moderate
4. For biomarkers that have clinical utility to guide decisions on systemic therapy for metastatic BC, in questions 2 & 3, what are the indicated tests and frequency of monitoring?	4. Recommendations for circulating tumor biomarkers: In patients already receiving systemic therapy for metastatic BC, decisions on switching to a new therapy or terminating it should be based on clinical exam, assessment of BC progression/response to treatment, and the patient's management goals	4.T: evidence based Q: intermediate	4.moderate
	5. Biomarkers: CEA, CA 15-3, and CA 27-29 can be used as adjuncts to help with therapeutic decisions for metastatic BC; However, evidence is insufficient to recommend their application alone for monitoring therapeutic response	5.T: informal consensus Q: insufficient.	5.moderate

Abbreviations: BC, Breast Cancer; CEA, Carcinoembryonic Antigen, CA 15-3, Cancer Antigen 15-3; CA 27-29, Cancer Antigen 27-29; ER, Estrogen Receptor; HER2, Human Epidermal Growth Factor Receptor 2; HR, Hormone Receptor; PR, Progesterone Receptor; Q, Quality; T, Type; *vs*., *versus*.

Table 3. Selected biomarkers as therapeutic targets in the management of patients with HER2-positive metastatic breast cancer – helpful implications from recent clinical trials.

Therapeutic Target	Targeted Medication, Class	Trial Name, Phase Identifier	Clinical Implications of the Trial Relevant to the Patient Management [Author, Year; Reference Number]
HER2	Trastuzumab HER2 inhibitor	HERA (HERceptin Adjuvant), P 3 NCT00045032	1 year of adjuvant trastuzumab after CHT for pts with HER2-positive BC significantly improved long-term Disease-Free [Cameron *et al*. 2017; 29]
HER2	Pertuzumab HER2 inhibitor	CLEOPATRA, P 3 NCT00567190	Combination of pertuzumab with docetaxel and trastuzumab, in pts with HER2-positive mBC increased PFS [Baselga *et al*. 2012; 30]

(Table 3) contd.....

Therapeutic Target	Targeted Medication, Class	Trial Name, Phase Identifier	Clinical Implications of the Trial Relevant to the Patient Management [Author, Year; Reference Number]
HER2	Trastuzumab emtansine (T-DM1), Anti-HER2 ADC: HER2 inhibitor, microtubule inhibitor	EMILIA P 3 NCT00829166	T-DM1 prolonged PFS and OS, with less toxicity (compared to lapatinib plus capecitabine), in patients with HER2-positive mBC, previously treated with trastuzumab and a taxane [Verma *et al*. 2012; 31]
HER2	Trastuzumab deruxtecan (DS-8201) Anti-HER2 ADC, Topoisomerase I inhibitor	DESTINY-Breast01 P 2 NCT03248492	Trastuzumab deruxtecan revealed durable antitumor activity in pretreated (with T-DM1) pts with HER2-positive mBC; in the trastuzumab deruxtecan arm: ORR = 60.3%; median PFS = 16.4 ms; interstitial lung disease is a serious AE that requires vigilant monitoring/intense therapy [Modi *et al*. 2020; 32]
HER2	Trastuzumab duocarmazine (SYD985); Anti-HER2 ADC Duocarmycin derivative – an alkylating agent	P 1 (ongoing) NCT02277717 (SYD985 dose-escalation/dose-expansion)	Trastuzumab duocarmazine has shown clinical activity in pretreated pts with HER2-positive mBC (HER2-positive, T-DM1-resistant, HER2-low expression BC), with manageable safety; ORR in HER2-positive BC = 33%; ORR in HER2-low, HR-positive BC = 27%; ORR in HER2-low, HR-negative BC = 40% [Banerji *et al*. 2019; 33]
HER2	Margetuximab novel Anti-HER2 mAB; (Fc-engineered)	SOPHIA P 3 NCT02492711	In pts with HER2-positive mBC (after anti-HER2 therapy, *e.g*. pertuzumab), margetuximab + CHT improved PFS compared to trastuzumab + CHT; PFS benefits were stronger in low-affinity CD16A-158F allele carriers [Rugo *et al*. 2019; 34]
HER2	Tucatinib Novel selective HER2 TKI	HER2CLIMB P 3 NCT02614794	In pretreated pts with HER2-positive mBC (with CNS metastases) adding tucatinib to a combination of trastuzumab/capecitabine resulted in longer median PFS (7,6 *vs*. 5,4 ms) and OS (21,9 *vs*. 17,4 ms) compared to the placebo arm; tucatinib exerts a stronger activity (than other TKIs) for CNS metastases and has a lower rate of AEs (*e.g*., skin reactions, diarrhea) [Murthy *et al*. 2020; 35]
HER2	Neratinib An irreversible pan HER2 TKI	SUMMIT basket trial P 2 NCT01953926	Neratinib (+ fulvestrant) is clinically active in pretreated pts with HER2-mutant, HR-positive mBC; median PFS = 5.4 months; ORR = 30%; CBR = 47%; Synergistic effects with trastuzumab exist in pts with HER2-positive mBC (including those with brain metastases); Neratinib/capecitabine improved median PFS (with a trend for improved OS) compared to Lapatinib/capecitabine; Neratinib/capecitabine delayed time to intervention for brain metastases [Smyth *et al*. 2019; 36]
HER2	Neratinib An irreversible pan HER2 TKI	NALA P 3 NCT01808573	In pretreated pts with HER2-positive mBC, in the Neratinib/capecitabine arm *vs*. Lapatinib/capecitabine arm: PFS rates = 28.8% *vs*. 14.8%; OS rates = 72.5% *vs*. 66.7%; ORR = 32.8% vs 26.7%; CBR = 44.5% *vs*. 35.6% activity for CNS metastases [Saura *et al*. 2019; 37]
HER2	Poziotinib An irreversible pan HER2 TKI	NOV120101-203 P 2 NCT02418689	In pretreated pts with HER2-positive mBC, Poziotinib (as monotherapy, in a single-arm trial) revealed median PFS = 4 ms; DCR = 75% [Park *et al*. 2018; 38]
HER2	Pyrotinib An irreversible pan HER2 TKI	P 2 NCT003080805	Pyrotinib or lapatinib (with capecitabine) in pts with HER2-positive mBC (post treatment with anthracyclines/taxanes/trastuzumab); in the arm: Pyrotinib/capecitabine: ORR = 78.5%; median PFS = 18.1 ms, *vs*. the arm Lapatinib/capecitabine: ORR = 57.1%; median PFS = 7.0 ms [Ma *et al*. 2019; 39]
CDK4/6	Palbociclib CDK4/6 inhibitor	PATINA P 3 (ongoing) NCT02947685	Palbociclib added to trastuzumab, pertuzumab, and an AI *vs*. anti-HER2 therapy + ET, after induction treatment for HR-positive/HER2-positive mBC; evaluation of PFS with using the combination of palbociclib with anti-HER2 therapy + ET *vs*. anti-HER2 therapy + ET alone; (pending OS, tumor control measurements, safety, and QoL) [Loibl *et al*. 2018; 40]
CDK4/6	Abemaciclib CDK4/6 inhibitor	MonarcHER P 2 (ongoing) NCT02675231	Combination of abemaciclib + trastuzumab and fulvestrant in pts with pretreated HR-positive, HER2-positive mBC has shown benefits: median PFS = 8.3 ms, compared to PFS =5.7 ms for trastuzumab + CHT; response rate with the combination of abemaciclib + trastuzumab and fulvestrant = 33% [Tolaney *et al*. 2019; 41]
mTOR	Everolimus mTOR inhibitor	BOLERO-1 P 3 NCT00876395	Everolimus in combination with trastuzumab + paclitaxel, as first-line treatment for pts with HER2-positive mBC, resulted in median PFS that was 7,2 ms longer upon adding everolimus in HR-negative, HER2-positive mBC) [Hurvitz *et al*. 2015; 42]

(Table 3) contd.....

Therapeutic Target	Targeted Medication, Class	Trial Name, Phase Identifier	Clinical Implications of the Trial Relevant to the Patient Management [Author, Year; Reference Number]
mTOR	Everolimus mTOR inhibitor	BOLERO-3 P 3 NCT01007942	Everolimus in combination with trastuzumab + vinorelbine in pts with HER2-positive mBC (pretreated with a taxane) has shown benefits: median PFS = 7.0 ms in the combination arm *vs*. PFS = 5.78 ms the placebo arm (trastuzumab + vinorelbine) [Andre *et al*. 2014; 43]
PD-L1	Pembrolizumab PD-L1 inhibitor	PANACEA P 1b-2 NCT02129556	Pembrolizumab + trastuzumab (single-arm trial) in trastuzumab-resistant, HER2-positive mBC; in PD-L1-positive subgroup of pts, the combination therapy revealed durable clinical benefits/acceptable safety; ORR = 15% [Loi *et al*. 2019; 44]
PD-L1	Atezolizumab PD-L1 inhibitor	KATE2 P 2 NCT02924883	Atezolizumab added to T-DM1 in pts with HER2-positive mBC did not significantly increase median PFS compared to T-DM1 (placebo arm) in the ITT group; however, the median PFS was longer in PD-L1-positive subgroup [Emens *et al*. 2019; 45]

Abbreviations: ADC, Antibody Drug Conjugate; AEs, Adverse Events; AI, Aromatase Inhibitor; BC, Breast Cancer; CDK, Cyclin Dependent Kinase; CHT, Chemotherapy; CNS, Central Nervous System; DFS, Disease-Free Survival; ER, Estrogen Receptor; ET, Endocrine Therapy; HER2, Human Epidermal Growth Factor Receptor 2; HR, Hormone Receptor; ITT, Intent-To-Treat; m, Metastatic; mAb, Monoclonal Antibody; ms, Months; mTOR, Mechanistic Target of Rapamycin; ORR, Overall Response Rate; OS, Overall Survival; pts, Patients; PD-L1, Programmed Death Ligand 1; PFS, Progression-Free Survival; P, Phase; QoL, Quality of Life; ref., Reference; T-DM1, Trastuzumab Emtansine; TKI, Tyrosine Kinase Inhibitor; *vs*., *versus*.

7. EFFECTIVE COMMUNICATION WITH PATIENTS ABOUT BC BIOMARKERS STATUS - A VALUABLE HELP IN THEIR CARE

To successfully distribute and practically implement the updated ASCO guidelines, it is necessary to raise awareness of these recommendations among both oncology treatment teams and BC patients/survivors (as well as their families and caregivers). In the face of a large number of patients with multiple chronic diseases, designing evidence-based recommendations to direct therapy for such patients is very difficult [12, 28]. For instance, it should be underlined that in a population of patients with comorbidities (*e.g*., hepatic and renal insufficiency), the test results of some serum biomarkers of malignant tumors can be altered, and thus, their diagnostic value may be controversial [46]. In such cases, the oncology teams need to carefully consider the specific comorbidities (with their poly-pharmacotherapy) in preparing the therapeutic plans. For practical purposes, this could mean that many of the recommended treatment options should be modified or used only after a comprehensive analysis of individual patient cases. Moreover, to facilitate the BC management, the afflicted patients need to be educated about the results of pathology tests (including the main BC biomarkers), and their impact on the targeted treatment plans.

This is especially important since women diagnosed with BC (*e.g*., advanced or metastatic stages) often experience severe emotional distress and are overwhelmed by confusing medical terminology. To help overcome these obstacles, effective communication and a trustworthy relationship with the treatment team members are essential (*e.g*., using easy to understand language, patient-friendly brochures, clear written notes, or reminders, and asking patients to repeat the main information about therapy plan) [12, 28]. For instance, patients should understand that HER2 and HR status determines if certain medications (*e.g*., trastuzumab, pertuzumab, lapatinib, or tamoxifen) are recommended for them. In addition, the physician should review the test results with them, interpret the main data, clearly explain their impact on the patient's treatment course, inform about available clinical trials in this field, and answer any relevant questions, which the patient may have [12, 28, 47]. Such patient-centered care should augment adherence to medical advice. Some basic, most practical recommendations focused on biomarker application, to guide management decisions in patients with metastatic BC are summarized in Table **2** [12, 28].

As patients move along the trajectory of their metastatic BC course, no definite cure is available for them. Therefore, searching for therapies, which will not only control their malignancy for prolonged intervals of time but also extend their quality lifespan, is of utmost importance. Simultaneously, it is imperative to find biomarkers beyond HER2, which will accurately predict both the response and resistance to various treatment options and help identify which patients can particularly benefit from the specific therapies. Many challenges still remain with the treatment of patients with HER2-positive BC. For instance, availability, accuracy, and cost of genetic and molecular testing present serious limitations. Since this is not "a one size fits all" approach, it is critical to establish criteria, according to which the patients would be selected for the use of particular therapeutic agents. It is also crucial to spare a sufficient amount of time for a discussion with each patient about the pros and cons of the different proposed therapies, considering an individual patient's clinical context, characteristics of a given therapy, as well as personal preferences, goals, and needs.

CONCLUSION

HER2 overexpression/amplification in patients with BC is crucial for establishing a precise diagnosis and individualized HER2-targeted treatment plan. It is of utmost importance to accurately interpret the HER2 biomarker status, including assessment of conversions of HER2 and HR biomarkers in the BC primary tumor and metastatic lesions (because detection of these changes is necessary for the specific management modifications of individual patients with metastatic BC). If such alterations in the biomarker status are timely detected, there is a chance for selecting the most optimal, targeted therapeutic agents for the afflicted women with metastatic BC. To shed some more light on this area, large prospective studies, assessing the impact of HER2 and HR biomarker conversions on the treatment selection and efficacy of HER2-targeted therapy/ET and patient outcomes are warranted in the future.

Although HER2 is usually evaluated in BC primary tumor, knowledge of the HER2 status in metastases and the extent of HER2 changes between primary and metastatic BC is extremely valuable for further therapeutic decision making.

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REFERENCES

[1] Weigelt B, Peterse JL, van 't Veer LJ. Breast cancer metastasis: Markers and models. Nat Rev Cancer 2005; 5(8): 591-602. [http://dx.doi.org/10.1038/nrc1670] [PMID: 16056258]

[2] Nielsen DL, Kümler I, Palshof JA, Andersson M. Efficacy of HER2-targeted therapy in metastatic breast cancer. Monoclonal antibodies and tyrosine kinase inhibitors. Breast 2013; 22(1): 1-12. [http://dx.doi.org/10.1016/j.breast.2012.09.008] [PMID: 23084121]

[3] Maennling AE, Tur MK, Niebert M, *et al.* Molecular targeting therapy against egfr family in breast cancer: Progress and future potentials. Cancers (Basel) 2019; 11(12): E1826. [http://dx.doi.org/10.3390/cancers11121826] [PMID: 31756933]

[4] Vincent-Salomon A, Jouve M, Genin P, *et al.* HER2 status in patients with breast carcinoma is not modified selectively by preoperative chemotherapy and is stable during the metastatic process. Cancer 2002; 94(8): 2169-73. [http://dx.doi.org/10.1002/cncr.10456] [PMID: 12001113]

[5] Santinelli A, Pisa E, Stramazzotti D, Fabris G. HER-2 status discrepancy between primary breast cancer and metastatic sites. Impact on target therapy. Int J Cancer 2008; 122(5): 999-1004. [http://dx.doi.org/10.1002/ijc.23051] [PMID: 17973263]

[6] Guarneri V, Giovannelli S, Ficarra G, *et al.* Comparison of HER-2 and hormone receptor expression in primary breast cancers and asynchronous paired metastases: Impact on patient management. Oncologist 2008; 13(8): 838-44. [http://dx.doi.org/10.1634/theoncologist.2008-0048] [PMID: 18650259]

[7] Lower EE, Glass E, Blau R, Harman S. HER-2/neu expression in primary and metastatic breast cancer. Breast Cancer Res Treat 2009; 113(2): 301-6. [http://dx.doi.org/10.1007/s10549-008-9931-6] [PMID: 18273700]

[8] Marchiò C, Lambros MB, Gugliotta P, *et al.* Does chromosome 17 centromere copy number predict polysomy in breast cancer? A fluorescence *in situ* hybridization and microarray-based CGH analysis J Pathol 2009; 219(1): 16-24. [http://dx.doi.org/10.1002/path.2574] [PMID: 19670217]

[9] VanderLaan PA. Molecular markers: Implications for cytopathology and specimen collection. Cancer Cytopathol 2015; 123(8): 454-60. [http://dx.doi.org/10.1002/cncy.21560] [PMID: 25964216]

[10] Francis IM, Altemaimi RA, Al-Ayadhy B, *et al.* Hormone receptors and human epidermal growth factor (her2) expression in fine-needle aspirates from metastatic breast carcinoma - role in patient management. J Cytol 2019; 36(2): 94-100. [http://dx.doi.org/10.4103/JOC.JOC_117_18] [PMID: 30992644]

[11] Schrijver WAME, Suijkerbuijk KPM, van Gils CH, van der Wall E, Moelans CB, van Diest PJ. Receptor conversion in distant breast cancer metastases: A systematic review and meta-analysis. J Natl Cancer Inst 2018; 110(6): 568-80. [http://dx.doi.org/10.1093/jnci/djx273] [PMID: 29315431]

[12] Wolff AC, Hammond MEH, Allison KH, *et al.* Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update. J Clin Oncol 2018; 36(20): 2105-22. [http://dx.doi.org/10.1200/JCO.2018.77.8738] [PMID: 29846122]

[13] Starczynski J, Atkey N, Connelly Y, *et al.* HER2 gene amplification in breast cancer: A rogues' gallery of challenging diagnostic cases: UKNEQAS interpretation guidelines and research recommendations. Am J Clin Pathol 2012; 137(4): 595-605. [http://dx.doi.org/10.1309/AJCPATBZ2JFN1QQC] [PMID: 22431536]

[14] Aurilio G, Disalvatore D, Pruneri G, *et al.* A meta-analysis of oestrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 discordance between primary breast cancer and metastases. Eur J Cancer 2014; 50(2): 277-89. [http://dx.doi.org/10.1016/j.ejca.2013.10.004] [PMID: 24269135]

[15] Hou Y, Shen R, Chaudhary S, Gao F, Li Z. Correlation of expression of breast biomarkers in primary and metastatic breast carcinomas: A single-institution experience. Acta Cytol 2016; 60(5): 481-9. [http://dx.doi.org/10.1159/000449400] [PMID: 27681712]

[16] Rossi S, Basso M, Strippoli A, *et al.* Hormone receptor status and HER2 expression in primary breast cancer compared with synchronous axillary metastases or recurrent metastatic disease. Clin Breast Cancer 2015; 15(5): 307-12. [http://dx.doi.org/10.1016/j.clbc.2015.03.010] [PMID: 25922284]

[17] Meng X, Song S, Jiang ZF, *et al.* Receptor conversion in metastatic breast cancer: A prognosticator of survival. Oncotarget 2016; 7(44): 71887-903. [http://dx.doi.org/10.18632/oncotarget.12114] [PMID: 27655689]

[18] Moeder CB, Giltnane JM, Harigopal M, *et al.* Quantitative justification of the change from 10% to 30% for human epidermal growth factor receptor 2 scoring in the American Society of Clinical Oncology/College of American Pathologists guidelines: tumor heterogeneity in breast cancer and its implications for tissue microarray based assessment of outcome. J Clin Oncol 2007; 25(34): 5418-25. [http://dx.doi.org/10.1200/JCO.2007.12.8033] [PMID: 18048824]

[19] Lee HJ, Seo AN, Kim EJ, *et al.* HER2 heterogeneity affects trastuzumab responses and survival in patients with HER2-positive metastatic breast cancer. Am J Clin Pathol 2014; 142(6): 755-66. [http://dx.doi.org/10.1309/AJCPIRL4GUVGK3YX] [PMID: 25389328]

[20] Lee HJ, Kim JY, Park SY, *et al.* Clinicopathologic significance of the intratumoral heterogeneity of HER2 gene amplification in HER2-positive breast cancer patients treated with adjuvant trastuzumab. Am J Clin Pathol 2015; 144(4): 570-8. [http://dx.doi.org/10.1309/AJCP51HCGPOPWSCY] [PMID: 26386078]

[21] Hou Y, Nitta H, Wei L, *et al.* HER2 intratumoral heterogeneity is independently associated with incomplete response to anti-HER2 neoadjuvant chemotherapy in HER2-positive breast carcinoma. Breast Cancer Res Treat 2017; 166(2): 447-57. [http://dx.doi.org/10.1007/s10549-017-4453-8] [PMID: 28799059]

[22] Woo JW, Chung YR, Ahn S, *et al.* Changes in biomarker status in metastatic breast cancer and their prognostic value. J Breast Cancer 2019; 22(3): 439-52. [http://dx.doi.org/10.4048/jbc.2019.22.e38] [PMID: 31598343]

[23] Chang HJ, Han SW, Oh DY, *et al.* Discordant human epidermal growth factor receptor 2 and hormone receptor status in primary and metastatic breast cancer and response to trastuzumab. Jpn J Clin Oncol 2011; 41(5): 593-9. [http://dx.doi.org/10.1093/jjco/hyr020] [PMID: 21406492]

[24] Fabi A, Di Benedetto A, Metro G, *et al.* HER2 protein and gene variation between primary and metastatic breast cancer: Significance and impact on patient care. Clin Cancer Res 2011; 17(7): 2055-64. [http://dx.doi.org/10.1158/1078-0432.CCR-10-1920] [PMID: 21307144]

[25] Valent A, Penault-Llorca F, Cayre A, Kroemer G. Change in HER2 (ERBB2) gene status after taxane-based chemotherapy for breast cancer: Polyploidization can lead to diagnostic pitfalls with potential impact for clinical management. Cancer Genet 2013; 206(1-2): 37-41. [http://dx.doi.org/10.1016/j.cancergen.2012.12.001] [PMID: 23313108]

[26] Guarneri V, Dieci MV, Barbieri E, *et al.* Loss of HER2 positivity and prognosis after neoadjuvant therapy in HER2-positive breast cancer patients. Ann Oncol 2013; 24(12): 2990-4. [http://dx.doi.org/10.1093/annonc/mdt364] [PMID: 24013581]

[27] Viale G. Be precise! The need to consider the mechanisms for CEP17 copy number changes in breast cancer. J Pathol 2009; 219(1): 1-2.

[http://dx.doi.org/10.1002/path.2593] [PMID: 19670216]

[28] Van Poznak C, Somerfield MR, Bast RC, *et al.* Use of biomarkers to guide decisions on systemic therapy for women with metastatic breast cancer: American society of clinical oncology clinical practice guideline. J Clin Oncol 2015; 33(24): 2695-704. [http://dx.doi.org/10.1200/JCO.2015.61.1459] [PMID: 26195705]

[29] Cameron D, Piccart-Gebhart MJ, Gelber RD, Procter M, Goldhirsch A, de Azambuja E. Herceptin Adjuvant (HERA) Trial Study Team. 11 years' follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive early breast cancer: Final analysis of the HERceptin Adjuvant (HERA) trial. Lancet 2017; 389(10075): 1195-205. [http://dx.doi.org/10.1016/S0140-6736(16)32616-2] [PMID: 28215665]

[30] Baselga J, Cortés J, Kim SB, *et al.* Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. N Engl J Med 2012; 366(2): 109-19. [http://dx.doi.org/10.1056/NEJMoa1113216] [PMID: 22149875]

[31] Verma S, Miles D, Gianni L, *et al.* Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med 2012; 367(19): 1783-91. [http://dx.doi.org/10.1056/NEJMoa1209124] [PMID: 23020162]

[32] Modi S, Saura C, Yamashita T, *et al.* DESTINY-Breast01 investigators. Trastuzumab deruxtecan in previously treated her2-positive breast cancer. N Engl J Med 2020; 382(7): 610-21. [http://dx.doi.org/10.1056/NEJMoa1914510] [PMID: 31825192]

[33] Banerji U, van Herpen CML, Saura C, *et al.* Trastuzumab duocarmazine in locally advanced and metastatic solid tumours and HER2-expressing breast cancer: A phase 1 dose-escalation and dose-expansion study. Lancet Oncol 2019; 20(8): 1124-35. [http://dx.doi.org/10.1016/S1470-2045(19)30328-6] [PMID: 31257177]

[34] Rugo HS, Im SA, Wright GL, *et al.* SOPHIA primary analysis: A phase 3 (P3) study of margetuximab (M) + chemotherapy (C) versus trastuzumab (T) + C in patients (pts) with HER2+metastatic (met) breast cancer (MBC) after prior anti-HER2 therapies (Tx). Journal of Clinical Oncology 2019; 37(15_suppl): 1000.

[35] Murthy RK, Loi S, Okines A, *et al.* Tucatinib, trastuzumab, and capecitabine for HER2-positive metastatic breast cancer. N Engl J Med 2020; 382(7): 597-609. [http://dx.doi.org/10.1056/NEJMoa1914609] [PMID: 31825569]

[36] Smyth LM, Saura C, Piha-Paul SA, Lu J, Mayer IA, Brufksy AM. Update on the phase II SUMMIT trial: Neratinib + fulvestrant for HER2-mutant, HR-positive, metastatic breast cancer. Annals Oncol 2019; 30(suppl_3): iii1-iii26.

[37] Saura C, Oliveira M, Feng Y-H, Dai M-S, Hurvitz SA, Kim S-B. Neratinib + capecitabine *versus* lapatinib + capecitabine in patients with HER2+ metastatic breast cancer previously treated with ≥2 HER2-directed regimens: Findings from the multinational, randomized, phase III NALA trial J Clin Oncol 2019; 37(15_suppl): 1002-2. [http://dx.doi.org/10.1200/JCO.2019.37.15_suppl.1002]

[38] Park YH, Lee KH, Sohn JH, *et al.* A phase II trial of the pan-HER inhibitor poziotinib, in patients with HER2-positive metastatic breast cancer who had received at least two prior HER2-directed regimens: Results of the NOV120101-203 trial. Int J Cancer 2018; 143(12): 3240-7. [http://dx.doi.org/10.1002/ijc.31651] [PMID: 29978467]

[39] Ma F, Ouyang Q, Li W, *et al.* Pyrotinib or lapatinib combined with capecitabine in her2-positive metastatic breast cancer with prior taxanes, anthracyclines, and/or trastuzumab: A randomized, phase ii study. J Clin Oncol 2019; 37(29): 2610-9. [http://dx.doi.org/10.1200/JCO.19.00108] [PMID: 31430226]

[40] Loibl S, Metzger O, Mandrekar SJ, Mundhenke C, Seiler S, Valagussa P. PATINA: A Randomized, Open Label, Phase III Trial to Evaluate the Efficacy and Safety of Palbociclib + Anti-HER2 Therapy + Endocrine Therapy (ET) *vs*. Anti-HER2 Therapy + ET after Induction Treatment for Hormone Receptor Positive (HR+)/HER2-Positive Metastatic Breast Cancer (MBC) Annals of Oncology 2018; 29(suppl_8): viii90-viii121.

[41] Tolaney SM, Wardley AM, Zambelli S, *et al.* MonarcHER: A randomized phase II study of abemaciclib plus trastuzumab with or without fulvestrant versus trastuzumab plus standard- of care chemotherapy in women with HR+, HER2+ advanced breast cancer. ESMO Congress 2019 Abstract LBA23.

[42] Hurvitz SA, Andre F, Jiang Z, *et al.* Combination of everolimus with trastuzumab plus paclitaxel as first-line treatment for patients with HER2-positive advanced breast cancer (BOLERO-1): A phase 3, randomised, double-blind, multicentre trial. Lancet Oncol 2015; 16(7): 816-29. [http://dx.doi.org/10.1016/S1470-2045(15)00051-0] [PMID: 26092818]

[43] André F, O'Regan R, Ozguroglu M, *et al.* Everolimus for women with trastuzumab-resistant, HER2-positive, advanced breast cancer (BOLERO-3): A randomised, double-blind, placebo-controlled phase 3 trial. Lancet Oncol 2014; 15(6): 580-91. [http://dx.doi.org/10.1016/S1470-2045(14)70138-X] [PMID: 24742739]

[44] Loi S, Giobbie-Hurder A, Gombos A, *et al.* Pembrolizumab plus trastuzumab in trastuzumab-resistant, advanced, HER2-positive breast cancer (PANACEA): A single-arm, multicentre, phase 1b-2 trial. Lancet Oncol 2019; 20(3): 371-82. [http://dx.doi.org/10.1016/S1470-2045(18)30812-X] [PMID: 30765258]

[45] Emens LA, Esteva FJ, Beresford M, Saura C, De Laurentiis M, Kim S-B. Overall survival (OS) in KATE2, a phase II study of programmed death ligand 1 (PD-L1) inhibitor atezolizumab (atezo)+trastuzumab emtansine (T-DM1) *vs* placebo (pbo)+TDM1 in previously treated HER2+ advanced breast cancer (BC) Ann Oncol 2019; 30(Suppl. 5): v104. [http://dx.doi.org/10.1093/annonc/mdz242]

[46] Estakhri R, Ghahramanzade A, Vahedi A, Nourazarian A. Serum levels of CA15-3, AFP, CA19-9 and CEA tumor markers in cancer care and treatment of patients with impaired renal function on hemodialysis. Asian Pac J Cancer Prev 2013; 14(3): 1597-9. [http://dx.doi.org/10.7314/APJCP.2013.14.3.1597] [PMID: 23679242]

[47] Giordano SH, Temin S, Kirshner JJ, *et al.* Systemic therapy for patients with advanced human epidermal growth factor receptor 2-positive breast cancer: American Society of Clinical Oncology clinical practice guideline. J Clin Oncol 2014; 32(19): 2078-99. [http://dx.doi.org/10.1200/JCO.2013.54.0948] [PMID: 24799465]