

REVIEW

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Circulating HER2 Extracellular Domain: A Specific and Quantitative Biomarker of Prognostic Value in all Breast Cancer Patients?

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Abstract: The HER2 oncoprotein has emerged as an essential biomarker in the treatment of breast cancer patients. Once the primary breast cancer is removed, there is an increasing need to detect breast cancer recurrence as early as possible with the hope that earlier intervention with new anti-HER2 therapies will improve quality of life and increase overall survival. Numerous publications have shown that increasing blood levels of circulating HER2 is an early indicator of progression, particularly in HER2-positive patients and that the rise and fall parallels the clinical course of disease and independent of therapy. Many studies show that the HER2 status of the primary tumor may not fully and accurately reflect the HER2 status of recurrent cancer. Thus, elevated serum HER2 levels may be an early signal of the emergence of a HER2-positive metastatic tumor and therefore alert the physician to re-assess HER2 status using a tissue test.

Keywords: Serum HER-2, Circulating HER-2, HER-2 Biomarker, HER-2 extracellular domain, HER-2 positive, HER-2 negative

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Introduction

Currently, tumors from breast cancer patients are tested semi-qualitatively for HER2 positivity using a combination of immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) techniques and patients are separated into HER2-positive or HER2-negative groups.¹ These are collectively referred to as “tissue tests” and currently are considered to be essential for establishing the HER2 status of a breast tumor sample. Determination of the correct HER2 tumor status is critically important for guiding the therapy of patients with HER2 positive breast cancer since HER2 targeted therapies are now used in the neoadjuvant,² adjuvant,³ and metastatic breast cancer settings.⁴

The current American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP) guidelines consider a tumor to be HER2-positive if greater than 30% of the cells (defined as uniform 3 + intense membrane staining) are HER2-positive by IHC or if on FISH amplification the ratio of HER2 to CEP17 is >2.2 or the average gene copy number is $>$ six signals/nucleus for test systems without an internal control probe. Therefore, patients who do not meet these criteria are considered to have a HER2-negative tumor, although they may have a significant number of HER2-positive cancer cells within the primary tumor. Since tumors are heterogeneous in nature, tumor cells can show high or low expression of HER2 and contain significant numbers of HER2-positive cells, but not enough to be considered HER2-positive by ASCO/CAP guidelines. Therefore, this minor population of HER2-positive cancer cells may break free from the primary tumor, spread throughout the body, and become seeds that establish HER2-positive metastatic tumors. Several studies have suggested that under such circumstances, the sensitivity of tissue testing may be enhanced by combining the IHC/FISH methods with a test that quantifies the external fragment of the HER2 protein, referred to as the serum HER2 (sHER2) test.⁵⁻⁹ The test quantitatively measures the extracellular domain (ECD) of the HER2 protein which is released from cells into the blood stream and can be measured in the serum component of blood in both normal individuals and breast cancer. Similarly to other members of the HER family of transmembrane proteins, the ECD is proteolytically cleaved by metalloproteases and

quantitated using a standard enzyme-linked immunosorbent assay (ELISA). The ELISA test uses one mouse monoclonal antibody to capture the ECD and a different biotinylated mouse monoclonal antibody to detect and quantify the ECD. Both capture and detector reagents specifically bind to the extracellular domain of HER2 protein. The sHER2 test has been cleared by Food & Drug Administration (FDA) and numerous clinical studies have demonstrated that an elevated sHER2 level is ≥ 15 ng/mL and that a change of 20% or more between 2 successive blood draws is a significant difference.¹⁰⁻¹³

The sHER2 test is both quantitative and standardized and can be used in real-time to monitor changes in the blood levels of circulating HER2 ECD. It has been shown in several studies that the rise and fall of sHER2 parallels the clinical course of disease when compared with standard clinical tools such as imaging.¹⁰⁻²⁰ Since the sHER2 test monitors the concentration of ECD from HER2-positive cancer cells, it is independent of the therapy type and therefore not restricted to patients receiving only HER2-targeted therapies.¹⁰⁻²⁰ As a simple blood test, it allows the monitoring of changes in sHER2 levels once the primary tumor is removed in both early²⁰⁻²⁶ and late stage^{7-9,11-19} breast cancer patients.

Several studies have demonstrated that the HER2 status of a primary tumor may not entirely and accurately reflect the HER2 status of the metastatic tumor when both are evaluated by IHC and FISH tests. Many reports have documented that there is a significant number of breast cancer patients with a primary breast tumor that was classified as HER2-negative but who develop a recurrent HER2 tissue-positive metastatic tumor. Since selecting for HER2 targeted therapies is based on IHC/FISH results of the primary tumor, there may be a significant population of women missing an opportunity to be treated with approved HER2-targeted therapies or participate in clinical trials with new HER2-targeted therapies. Since sHER2 testing is complementary to tissue testing and is a simple blood test that can be done anytime, it can be used to help identify patients with latent HER2-positive status as pointed out by Ardavanis and colleagues²⁷ as well as other groups.⁷⁻⁹

The purpose of our review is to provide a summary of the published findings on thousands of patients studied with the sHER2 test since the 2007



publication of the ASCO guidelines on tumor markers. It is our hope that this literature review will help convince oncology leaders to re-consider the clinical utility of monitoring sHER2 levels in breast cancer patients. In this review, we focus primarily on peer-reviewed publications since 2007 that only employed the FDA cleared sHER2 test with a clearly defined cutoff of ≥ 15 ng/mL and a definition of what constitutes a significant change in sHER2 levels. This was to resolve some of the confusion with serum HER2 testing that has evolved over the past several years with the use of non-standardized and non-validated assays, some of which are no longer commercially available.

Serum HER2 Testing is Complementary to IHC/FISH Tests and Aids in Identifying HER2-Positive Patients Initially Misclassified as HER2-Negative by Tissue Testing

In general, 70–90% of all breast cancer patients are considered to be HER2-negative by standard tissue tests. This group, including triple-negative breast cancer (TNBC) patients, do not have access to approved HER2-targeted therapies, such as Trastuzumab and Lapatinib, nor are they considered for clinical trials of new HER2 targeted therapies such as Neratinib and Afatinib.

An in-depth analysis of the publications related to HER2 testing demonstrated that on average, 20% (range 10–40%) of these HER2-negative patients may be misclassified regarding HER2 status and may develop a HER2-positive recurrent breast cancer. The evidence to support this observation has been demonstrated in 3 ways. A comparison of the primary tumor with the metastatic tumor from the same patient using the standard IHC and FISH tests revealed a significant number of breast cancer patients who can have a HER2-negative primary tumor but a corresponding HER2-positive metastatic tumor.^{28–31} The conversion from HER2-negative status in the primary tumor to HER2-positive status in the metastatic tumor is also true in women with TNBC.^{32,33} It has also been shown that women with a HER2-negative primary tumor can have HER2-positive circulating tumor cells in the metastatic setting.^{6,34} Third, it has been shown in several

studies that women with an HER2-negative primary tumor can have elevated sHER2 levels ≥ 15 ng/mL with the development of metastatic breast cancer (MBC).^{5–9,17,18,27,35–40} In 2002, Yeh reported that approximately 17–20% of patients with breast cancer whose tumors initially tested negative for HER2 may experience recurrence with increasing sHER2 levels. Yeh proposed a triage system using IHC analysis for screening for HER2 positivity, FISH, as a complimentary test and ELISA for disease monitoring.⁵ In a 2009 publication, Sorensen et al evaluated 437 tissue-negative patients, 69 (15.7%) had elevated sHER2 level, and for 219 patients whose tissue status was unknown, 45 (20.5%) had an elevated sHER2 level. Sørensen et al recommended a simple algorithm in which sHER2 complements tissue testing to improve the sensitivity of determining the correct HER2 status. They recommended periodic testing of sHER2 levels in breast cancer patients who are either HER2-negative or have an unknown HER2 status. If the sHER2 level is ≥ 15 ng/mL, then the primary tumor or a metastatic tumor should be tested by IHC and FISH to determine HER2 status.⁸ If the primary breast tumor or a metastatic tumor is found to be HER2-positive, then the patient can become a candidate for HER2-targeted therapies.

Interestingly, Muller et al reported that in a population of 254 MBC patients where only 35% had a HER2-positive primary breast tumor, 47% had elevated sHER2 levels and 49% had HER2-positive circulating tumor cells at the time of metastasis.³⁹ In a report by Ardavanis et al it was shown that 73% of patients that were HER2-negative by tissue testing but who received Trastuzumab therapy based on an elevated sHER2 level derived clinical benefit.²⁷ In a similar report, 174 patients out of 1787 of patients with breast cancers that were found to be HER2-negative (9.7%) appeared to benefit from Trastuzumab, but no sHER2 levels were reported in the publication.⁴¹

According to the information described above, a significant proportion of the approximately 2.5 million breast cancer sufferers may have an incorrect HER2 status and therefore deemed not eligible for HER2-targeted therapies. Thus, there is a significant risk of falsely classifying a patient as HER2-negative, which has serious therapy-related implications. Therefore, monitoring sHER2 levels in HER2 tissue-negative patients, including TNBC patients, could be of



substantial benefit to this population of women since their initial tumor HER2 status prevents them from being candidates for HER2-targeted therapies.

Potential Clinical Prognostic Value of Serum HER2 Testing in HER2-Positive Breast Cancer Patients

It has been shown that the prevalence of elevated sHER2 levels ≥ 15 ng/mL is 10–15% in primary breast cancer^{20–26,35,36,43,44} and as high as 90% in HER2-positive MBC patients.^{37,42} Several reports demonstrate that elevated levels of sHER2 can be measured as soon as 3 weeks and up to 24 months before actual clinical signs of recurrence and therefore is an early indicator of progression.^{8,9,35,36,43,44} Persistently high levels^{19,45–48} or a lack of decline⁴⁹ are also associated with progressive disease. Reports have shown that patients with continuously elevated ≥ 15 ng/mL sHER2 levels have a significantly poorer survival after disease recurrence than patients with sHER2 levels continuously < 15 ng/mL. Patients who convert from, < 15 ng/mL to ≥ 15 ng/mL at the time of progression also have decreased survival. In contrast, a decrease in elevated sHER2 levels from ≥ 15 ng/mL to < 15 ng/mL or levels continuously < 15 ng/mL during the course of disease correlated with significantly longer survival.^{19,45,46} Finn et al reported a study of 579 MBC patients in whom changes in sHER2 levels correlated with patient outcome regardless of therapy given. In fact, conversion from less than normal to above normal levels was associated with worse progression free survival (PFS) while conversion from greater than normal to less than normal was associated with better PFS. A consistently low level of sHER2 had better PFS than consistently elevated sHER2 levels.⁴⁷

Collectively, these reports evaluated over 1200 MBC patients and clearly showed that monitoring sHER2 levels is a clinically informative and important tool for detecting early signs of recurrence in the 20–30% of HER2 positive breast cancer patients. These reports as well as several others showed that the sHER2 baseline was a strong prognostic indicator and patients having a baseline value ≥ 15 ng/mL had a worse clinical outcome than patients with a baseline of < 15 ng/mL.

In a report by Moreno-Aspitia et al it was also shown that in early stages, HER2-positive breast cancer patients receiving adjuvant Trastuzumab with sHER2 levels of ≥ 15 ng/mL at the time of recurrence of breast cancer had shorter survival time following recurrence. In patients with elevated levels ≥ 15 ng/mL, there was a 3-year overall survival of 51%. In contrast, there was a 77% overall survival in the group having sHER2 levels < 15 ng/mL (hazard ratio = 2.36; 95% confidence interval: 1.19–4.70, $P = 0.01$). Therefore a high baseline sHER2 level was a prognostic biomarker associated with shorter disease-free survival and a high sHER2 level at recurrence was predictive of shorter survival in early stage breast cancer patients.²⁶

There are an increasing number of reports describing both primary and secondary resistance to Trastuzumab in patients who have already failed hormone and chemotherapy. In fact, reports indicate that approximately 20–30% of patients do not respond to first-time treatment with Trastuzumab and that about 15% will develop resistance during the first year of treatment.^{50,51} Therefore, early identification of these patients could strongly influence their eventual clinical outcome. Since increases in sHER2 reflect disease progression, the sHER2 test can be used routinely to identify patients with progressive disease. Valero and Salmon reported in a group of MBC patients (BCIRG007 study) that approximately 90% (109 out of 123) of patients with HER2 amplification had elevated sHER2 levels (≥ 15 ng/mL) and that 83% of the patients with tumor progression had a significant change in sHER2 levels. The authors also commented that the sHER2 test had good positive predictive value. In fact, when they considered serial measurements, subjects with higher sHER2 levels had a significantly ($P = 0.003$) higher risk of experiencing progressive disease even after adjustment for extent of disease and the presence of visceral disease.⁴² Studies have also shown that in both Trastuzumab-treated patients^{14–17,21,22,25,26,52,53} and Lapatinib-treated patients⁵⁴ that changes in sHER2 levels from pretreatment to post-treatment were associated with clinical outcome. In a multi-site study by Ali et al⁵² there were 307 MBC patients treated with Trastuzumab and the sHER2 levels monitored over a 3-month period. All patients had a baseline sHER2 test with a follow-up



sHER2 test at a median of 30 days after the initiation of treatment. Of the 307 patients, 191 patients (62%) had a significant decline (>20%) in sHER2 levels while 116 patients (38%) did not. The objective response rate was 57% for patients who achieved this decline in sHER2 (>20%) compared with 28% for patients who did not. Patients who achieved a decline of >20% from one blood draw to another had a significantly longer time to disease progression (320 days vs 180 days; $P < 0.0001$), longer duration of response (369 days vs 230 days; $P = 0.008$), and longer overall survival (898 days vs 593 days; $P < 0.018$) than patients who did not decline by >20%. In this pooled analysis of patients from 7 different institutions, patients who did not achieve a significant decline (>20%) in sHER2 levels had decreased benefit from Trastuzumab-based therapy. At the time of the 2008 publication, the authors concluded that such patients should be considered for clinical trials evaluating additional HER2-targeted therapies. Since that publication in 2008, Lapatinib has been approved by the FDA and used for treating HER2-positive breast cancer patients. In 2011, a study was published by Lipton et al at Hershey Medical Center in collaboration with GlaxoSmithKline scientists to evaluate serum HER2 levels in patients receiving Lapatinib monotherapy. Before the study, 79% of the HER2-positive MBC patients (determined by IHC and FISH) had elevated baseline sHER2 levels ≥ 15 ng/mL. The baseline sHER2 level was associated with overall response rate (ORR, $P = 0.043$), but not PFS. Patients with a >20% decrease from baseline of sHER2 at weeks 4, 8, 12, and 16 had a significantly increased ORR and prolonged PFS. Conversely, those with a >20% increase from baseline had a significantly lower ORR and shorter PFS. Significant decreases in sHER2 levels during the first 16 weeks of Lapatinib monotherapy were associated with better clinical outcome (longer PFS and increased ORR) in HER2-positive MBC patients.⁵⁴

In a recent report by Petersen et al,⁵³ 48 HER2 tissue positive patients treated with Trastuzumab for up to six years or until death were monitored with the sHER2 test. A significant decrease in sHER2 of $\geq 20\%$ correlated with no disease progression in 20 out of 21 clinical courses, while a significant increase in sHER2 of $\geq 20\%$ correlated with disease progression

in the disease in 40 out of 44 clinical courses. Patients with no recurrence after Trastuzumab treatment ($n = 18$) had a median sHER2 concentration of 10.5 ng/mL, whereas patients who live with recurrence ($n = 13$) had a median sHER2 of 20.1 ng/mL ($P = 0.002$). Patients who died due to recurrence ($n = 17$) had a median sHER2 of 232.4 ng/mL at the latest measurement before death, ($P \leq 0.0000001$) compared to patients without recurrence. Petersen et al clearly demonstrated the importance of maintaining sHER2 values at the lowest possible levels since there was a significant difference in clinical outcomes and sHER2 levels and a persistently high level of sHER2 was a strong indicator of very poor clinical prognostic outcome. Petersen et al also concluded that decreasing values of sHER2 predicted response to treatment whereas increasing levels predicted drug resistance. Serum HER2 levels above 1000 ng/mL was an indicator that standard doses of Trastuzumab may be insufficient as reflected by low concentrations of serum Trastuzumab.⁵³

Several studies now support the observation that a significant decrease in sHER2 levels from baseline to 30–90 days after initiation of treatment is a predictor of outcome to HER2-targeted therapies. In contrast, patients with increasing sHER2 levels, a persistently high sHER2 level or who do not achieve at least a 20% decline in sHER2 levels in the early weeks and months of HER2 targeted therapies may benefit from treatment with new HER2 inhibitors that are in clinical development.^{52,54}

Since patients can have increases in sHER2 levels that occur earlier than actual clinical signs of recurrence, routine monitoring of sHER2 levels can be an early warning sign for physicians and patients to consider additive or alternative therapy strategies. Earlier detection of cancer progression is an actionable event for the oncologist and one which can trigger intervention with the variety of therapies or combination of therapies that are now available for breast cancer patients. In theory, the sooner one attacks a tumor, particularly while the tumor burden is low, the better the probability of a positive patient outcome. Well-defined clinical studies are needed to test this hypothesis.

Studies conducted thus far have collectively shown that patients treated with hormone therapy,



chemotherapy, Trastuzumab, Lapatinib, or a combination of these therapies have serial changes in sHER2 levels that paralleled changes in the clinical course of disease; therefore, patients with HER2-positive breast cancer can greatly benefit from routine monitoring of the HER2 level.^{14–17,21,22,25,52,54}

Controversy Surrounding Serum HER2 Testing in all Breast Cancer Patients as a Prognostic Biomarker

In contrast to the large number of publications that illustrate the clinical value of sHER2 testing, a handful of publications have reached negative conclusions regarding the clinical value of measuring this specific circulating biomarker for HER2-positive breast cancer. Articles by Lennon et al⁵⁵ Leary et al⁵⁶ and Leyland-Jones et al⁵⁷ are the most notable. All three publications agreed with the 2007 ASCO guidelines and did not recommend sHER2 testing for clinical use. The articles by Leary et al and Leyland-Jones et al primarily reviewed publications that used research use only assays that were not standardized or validated by acceptable diagnostic protocols and procedures. In general, the research use only assays listed in the data tables of these publications did not define the specificity of the capture and detection antibodies in the assay nor was there a definition of what constitutes a statistically significant change between 2 serial blood samples. Therefore, it is difficult to determine how such data could be used to make conclusions about the clinical value of serum HER2 testing. Leyland-Jones et al pointed out that a comparison of results between such studies is problematic because many assays lack diagnostic validation and standardization.

In contrast to these publications, the FDA cleared sHER2 test has defined antibody specificities and a defined a standard cut-off of ≥ 15 ng/mL. In addition, rigorous clinical testing and receiver operator curve analysis is defined as a 20% statistically significant change between 2 serial blood samples. The FDA format is available as an automated test from Siemens or a manual ELISA test from Wilex. Both tests use identical antibodies to the ECD, have been cleared by the FDA, and have shown that the tests results are equivalent. Clinical studies that support this statement have been previously published and have been independently confirmed by others.^{12,20,58}

With respect to the Lennon et al and Leyland-Jones et al the investigators concluded that even after using the FDA cleared sHER2 test, the test was not useful in any situation and that there was insufficient evidence to support the clinical value of the test.⁵⁵ The conclusions from both of these publications were based on sHER2 levels from over 300 MBC patients that used the identical 4 data sets from studies designated BO15935, M77001, WO16229, and M77004. As pointed out by Ali et al⁵⁹ and Tse et al⁶⁰ in subsequent editorials in the Journal of Clinical Oncology, analysis of these 4 studies presented only overall and best tumor response for the MBC patients, but the investigators did not indicate what criteria were employed for analysis of the 4 data sets. In contrast, Ali et al in their 2008 publication utilized World Health Organization or response evaluation criteria in solid tumors and presented data on tumor response, duration of response, time to progression, and overall survival. Ali et al also defined that a significant decline in serum HER2 was a 20% decrease from baseline at a median of 30 days after commencing Trastuzumab. This was obtained by doing the diagnostic receiver operating curve analysis. In sharp contrast, the Lennon et al study presented changes in sHER2 only as a rise, a slight rise, fall, slight fall, and no change, but did not give a definition of a significant change in sHER2.

In the publication by Leyland-Jones et al it was concluded that even using the FDA cleared sHER2 test and the 15 ng/mL standard cutoff, there was still no clinical value to sHER2 testing and that further studies were not recommended. As part of this publication, the authors combined early and late stage breast cancer patients together with non-small cell lung carcinoma (NSCLC) patients to make their conclusions. This was not justified since the test was cleared by FDA for the management and monitoring of MBC patients but not NSCLC patients. Therefore, the ≥ 15 ng/mL standard should not be applied to NSCLC patients without validation studies. In contrast to these reports, more recent publications^{61,62} have concluded that larger sHER2 clinical studies are warranted and recommended because of the many previous positive publications about the clinical utility of sHER2 testing.

The report by Leyland-Jones et al also concluded that there was also a lack of correlation between sHER2 levels in MBC patients and the HER2 status of the primary breast tumor determined by IHC or FISH.



If one considers the well documented issues of at least a 20% discordance in tissue testing together with the conclusions made from non-validated sHER2 assays, it is easy to see why one could conclude that there is a lack of concordance between the HER2 tissue test and the sHER2 test. Many publications that have compared HER2 tissue status in the primary tumor with sHER2 levels of MBC patients using the FDA cleared test have clearly shown a strong correlation between the HER2 status of the primary tumor and an elevated sHER2 level in MBC patients.^{7,15,17,37,63,64} This was also clearly summarized in Table 1 of the Leyland-Jones et al publication.⁵⁷ Although there appears to be a strong correlation between HER2 tissue status in the primary tumor and elevated sHER2 levels in the metastatic setting, additional studies comparing tissue HER2 results with sHER2 levels with the FDA cleared test should bring greater clarification to this matter. Despite the negative conclusions of these 3 publications, there was an acknowledgement from the authors that there is an increasing body of evidence that shows sHER2 levels are closely associated with adverse clinicopathological factors.

Biological Basis and Effects of Shedding of ECD of HER2 and Poor Prognostic Behavior of Breast Cancer

The shedding of the ECD of membrane-bound HER2 molecules is associated with a constitutively active truncated intracellular receptor of 95kDa.⁶⁵ HER2 ECD and p95HER2 are coordinately produced by proteolytic activity involving matrix metalloproteases of the ADAM family.⁶⁶ Though it has still not been established whether p95HER2 levels in breast tumors are directly associated with sHER2 ECD, there is strong circumstantial evidence to suggest that the shedding of ECD may be responsible for the aggressive prognostically poor behavior of HER2 overexpressing breast cancer. Thus, genetically engineered cells with a HER2 gene lacking the ECD gene sequence have been shown to express p95HER2 with significantly increased TK activity and considerably enhanced (10–100-fold greater) transforming potency compared to the full-length receptor.⁶⁷ Furthermore, the expression of p95HER2 is more frequent in node-positive cases compared to node-negative⁶⁸ and appears to be associated with resistance to Trastuzumab treatment.⁶⁹

Summary and Conclusion

It has been clearly shown over several years with numerous clinical studies and thousands of breast cancer patients that the rise and fall of sHER2 parallels the clinical course of disease and provides an effective way to monitor HER2-positive patients and to identify those breast cancer patients incorrectly classified as HER2-negative patients. For HER2-positive patients, increasing sHER2 levels is an early indicator of cancer progression, while significant decreases in the first 12–16 weeks post-therapy is a strong and early indicator for positive clinical outcome. The lack of a significant decrease in those early weeks or persistently high levels is also a strong indicator that patients may benefit from additional HER2 targeted therapies.

MBC patients with sHER2 baseline levels ≥ 15 ng/mL is a strong prognostic indicator for a shorter PFS period; however, conversion from ≥ 15 ng/mL to < 15 ng/mL is a good indicator for better clinical outcomes than patients with continuously elevated levels ≥ 15 ng/mL. In a population of patients with a HER2 tissue-negative primary tumor, periodic testing for elevated sHER2 levels can complement IHC and FISH testing and help identify HER2-positive patients initially classified as HER2-negative or for whom the HER2 status is unknown. Once a breast cancer patient is shown to have an elevated sHER2 ≥ 15 ng/mL, they should be re-evaluated by IHC and FISH to determine their eligibility for HER2-targeted therapies. In conclusion, periodic testing of all breast cancer patients for elevated sHER2 levels can provide valuable information for patient management in both the HER2-positive and HER2-negative groups and be an early warning sign for physicians to consider alternative therapeutic strategies as the number of HER2 targeted drug choices continues to increase.

Author Contributions

Conceived and designed the review: WPC. Analyzed the data: WPC, DB, BJ. Wrote the first draft of the manuscript: WPC. Contributed to the writing of the manuscript: WPC, BJ, DB. Agree with manuscript results and conclusions: WPC, DB, BJ. Jointly developed the structure and arguments for the paper: WPC, BJ. Made critical revisions and approved final version: WPC, BJ. All authors reviewed and approved of the final manuscript.



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WPC holds issued patent number 6,861,511 for the detection and quantification of neu related proteins in the biological fluids of humans. Other authors disclose no competing interests.

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