# Current Detection and Treatment of HER-2/*neu*-positive Breast Cancer

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***Writer's Comment:*** *Though I have always been fascinated with health-related research topics such as cancer, it wasn't until quite recently that breast cancer became more than a topic of interest to me. With the diagnosis and subsequent treatments of my mother's breast cancer in 2005, the concerns that are attached to the horrific disease became a reality for my family. When this review article assignment was introduced in Dr. Pamela Demory's UWP 104E course, I immediately decided to use it as an opportunity to familiarize myself with the current developments in the breast cancer field. After considering several topics I settled upon HER-2/neu-positive breast cancer because of its controversial detection and treatment practices. Countless drafts later, I feel that I have composed a scientifically accurate and complete article on the developments of this aggressive breast cancer. While I thoroughly enjoyed researching my topic, I hope that one day, in the near future, another UCD student will have the opportunity to write about this issue as a problem of the past rather than as a current development. I would like to sincerely thank Dr. Pamela Demory and Dr. Benjamin Edwards for helping me polish this article to perfection!*

*—Shirley Rosenberg*

***INSTRUCTOR'S COMMENT:*** *The scientific review article is the most challenging assignment in my upper division writing courses. Students must read and analyze a number of original research reports in the scientific literature and then organize an article that synthesizes and makes sense of that material for an audience of relative experts in the field. Shirley found a topic that engaged her in a personal way: her mother had been diagnosed and treated for the frighteningly aggressive HER-2/neu-positive form of breast cancer, and so she decided to tackle that as her topic. She did so with*

*remarkable intellectual vigor and attention to detail, going far beyond what was actually required for the class. In this rigorous analysis of the current trends in breast cancer research, Shirley exemplifies the characteristics of a careful researcher and writer. No doubt this paper marks only the beginning of a long career in scientific research and/or medicine.*

*—Pamela Demory, University Writing Program*



According to the National Cancer Institute, breast cancer is the second most frequently diagnosed cancer among American women (1). Though several biological factors play a role in determining the type and severity of each breast cancer case, overexpression of the Human Epidermal Growth Factor Receptor-2 (HER-2/*neu*) proto-oncogene is a factor that touches a large population of breast cancer patients. The overexpression of the HER-2/*neu* proto-oncogene is seen in approximately 25-30% of invasive breast carcinomas and is frequently known as HER-2/*neu*-positive breast cancer (2). Resulting in an increasingly aggressive cancer, HER-2/*neu*-positive tumors are generally less differentiated than HER-2/*neu* negative tumors. In addition, due to the accelerated cell proliferation observed in these aggressive tumors, in the majority of cases the cancer spreads to the lymph-nodes and causes further metastasis (3). As a result of its resistance to traditional chemotherapy regimens, the unique phenotype of HER-2/*neu*-positive breast cancer often restricts the use of traditional chemotherapy treatments, and significantly reduces the longevity of HER-2/*neu*-positive breast cancer patients (3, 4).

Since the discovery of the HER-2/*neu* gene, substantial progress has been made in HER-2/*neu* gene expression assessment and treatment options. The detection and measurement of HER-2/*neu* gene expression, a task that has significantly evolved in its capabilities, is an essential process for accurate diagnosis. From preliminary gene expression measurements, physicians evaluate the intensity and aggressiveness of the cancer and assign treatments accordingly (5). In addition to enhanced detection capabilities, HER-2/*neu*-positive breast cancer treatment options have also advanced. With the development of trastuzumab, an antibody that directly targets the HER-2/*neu* receptor, physicians are successfully reducing the risks of HER-2/*neu*-positive breast cancer among patients (6). Currently, the use of chemotherapy in combination with trastu-

zumab is a standard form of treatment; however, research is still being conducted to determine the most effective dosages and combinations of medicines. This review article will present the recent progress of HER-2/*neu* detection and treatment research in order to shed light on the present capabilities of medical professionals in treating HER-2/*neu* breast cancer patients.

## Mechanism of HER-2/*neu*-positive Breast Cancer

In order to fully evaluate the implications and treatment options of HER-2/*neu*-positive breast cancer it is important to understand its behavior at a cellular level. The HER-2/*neu* proto-oncogene encodes a trans-membrane epidermal growth factor receptor protein (EGF) that plays a significant role in regulating essential cellular mechanisms such as growth and differentiation (3, 7). The receptor, consisting of several structural elements, including an extra-cellular ligand-binding domain, an intracellular tyrosine kinase domain, and a carboxyl tail with tyrosine phosphorylation sites, acts as an initial site for a series of cell signal pathways (7). Upon ligand binding, the receptor is phosphorylated at its tyrosine phosphorylation sites and consequently relays an irreversible signal transduction cascade that initiates the duplication of cellular DNA during the synthesis phase of the cell cycle (7, 8). In cases of HER-2/*neu*-positive breast cancer, the overexpression of the HER-2/*neu* gene results in the increased translation of the trans-membrane growth receptor proteins (7). The tyrosine phosphorylation of these receptors leads to an acceleration of cell signaling and thus to the development of aggressive and resilient tumors. Although much of the HER-2/*neu* signal transduction pathway route presently remains unknown, current evidence supports the correlation between HER-2/*neu* proto-oncogene overexpression, increased tyrosine activity, and the phosphorylation of the trans-membrane growth receptors (8).

## HER-2/*neu* Measurement and Detection Techniques

### *Acceptance of HER-2/*neu *Expression as a Prognostic Indicator*

Initiated in 1978 with the meeting of 79 breast cancer experts in St. Gallen, Switzerland, the annual St. Gallen International Consensus Conference currently houses delegations between internationally distinguished experts of clinical breast cancer (9). In January 2005, 4,166 par-

ticipants from 78 countries attended the Conference in order to modify current guidelines and procedures of breast cancer treatment options (10). Among these, the level of HER-2/*neu* proto-oncogene expression and amplification was accepted as a reliable indicator of high-risk breast cancer (10). Current studies indicate that the detection of accelerated HER-2/*neu* gene amplification and trans-membrane growth receptor overexpression are also strong prognostic indicators of lymph node-positive breast cancer, an additional component that was accepted by the conference panel as a feature of high risk breast cancer (10, 11). Though there are several available HER-2/*neu* expression assays in practice, a standard, error-free method is presently under investigation (12, 13).

### *Common Methods: Immunohistochemistry vs. Fluorescence In-Situ Hybridization*

Immunohistochemical (IHC) staining is presently the most commonly practiced method to assess the location and expression intensity of the trans-membrane growth receptor protein encoded by the HER-2 gene (11, 12). This straightforward procedure uses specifically labeled antibodies to bind to antigens on the trans-membrane growth receptor protein and cause a visible membrane staining effect (12). The results of the assay are generally categorized into four grades (from 0 to +3) according to the intensity of the staining and provide a practical method for HER-2/*neu* diagnosis and treatment assignment (11, 12, 14). Alternately, Fluorescence *in-situ* Hybridization (FISH), though a significantly more costly procedure, is a frequently used technique to detect HER-2/*neu* proto-oncogene amplification (14). In this procedure, researchers hybridize fluorescently labeled DNA probes to tumor cell single-stranded DNA and identify the location of the HER-2/*neu* gene by the fluorescent emissions of the probe (11).

While IHC and FISH are both regularly practiced procedures for HER-2/*neu* gene and protein analysis, medical professionals have questioned the accuracy of and agreement between the two methods. A recent study conducted by Bankfalvi et al. (12) compared the results of IHC and FISH screening on a relatively small number of tumor samples, and contrasted the IHC results of two differing antibodies, A0485 and CB-11. In agreement with the results of other comparable studies, Bankfalvi and colleagues (12) reported a strong correlation between the IHC and

FISH results both in high growth receptor protein overexpression (IHC) to HER-2/*neu* gene amplification (FISH) and in normal growth receptor protein expression (IHC) to the non-amplified HER-2/*neu* gene (FISH). Though this study confirms a general IHC-FISH correlation, it also calls attention to instances when IHC results indicated falsely positive results (corresponding to the use of the A0485 antibody rather than the CB-11 antibody) in comparison to the analogous FISH results (12). This inconsistency, also reported by Sauer et al. (14), identifies an apparent flaw in the IHC method. Originating from the "sensitivity differences between different antibodies" (15) as well as from the sensitivity to procedural variations, the discrepancy ultimately leads to conflicting data that compromises accurate patient diagnosis (12, 14).

In regards to the sensitivity and intensity of IHC staining, several studies, including that of Sauer et al. (14), also mentioned that it is extremely difficult to distinguish between the +2 and +3 IHC grades (15, 16). Their results identified cases in which a negative IHC result was observed in comparison to a corresponding positive FISH result (14). In contrast to the analysis made by Bankfalvi et al. (12), Sauer et al. (14) indicated that if IHC is used as the primary HER-2/*neu* assessment tool, the status of many women will be incorrectly diagnosed and proper treatment may not be implemented (15). Although statistically there appears to be a general correlation between results obtained with IHC and those obtained with FISH, the few cases that are misdiagnosed due to IHC analysis are clinically significant. Results such as those reported in Sauer et al. (14) indicate that although FISH is a more expensive procedure, its increased accuracy in distinguishing between the +2 and +3 IHC grades makes it a more effective method for general HER-2/*neu* gene activity assessment and treatment assignment. Even so, many scientists still believe that IHC is a cost-effective and accurate procedure that should be used as the primary HER-2/*neu* assessment method.

### *CISH: A Possible Alternative to FISH*

Though studies such as the one conducted by Sauer et al. (14) demonstrate a preference for FISH as the "gold standard" for HER-2/*neu* proto-oncogene amplification, further research has been conducted to establish a viable alternative to FISH (14, 16). Chromogenic *in situ* Hybridization (CISH) is a recent HER-2/*neu* assessment technique that uses the chromogenic staining technique of IHC as well as the *in situ*

hybridization technique of FISH (5, 15). Both FISH and CISH are more costly than IHC, but CISH results can be conveniently analyzed using standard bright field microscopy rather than fluorescence microscopy (17). Another significant advantage of CISH over FISH is its extremely stable signal intensity. Although the fluorescent probes used in FISH diminish over time and need to be photographed immediately after hybridization, CISH results are long lasting and may be stored at room temperature for extended periods (17). Several recent studies indicate that CISH is a beneficial method for HER-2/*neu* gene amplification for standard laboratories (5, 15, 17).

In comparing the accuracy between CISH and FISH, a study conducted by Madrid et al. (15) demonstrated that CISH may be an effective replacement of FISH. This study reported an overall 86.25% agreement between IHC (with CB11 antibody) and CISH analysis; however, a meager 45% IHC-CISH agreement was observed in +2 IHC cases (15). In contrast, similar studies detailed a promising 95% IHC-CISH agreement in their +2 IHC cases and an equally high rate of CISH-FISH agreement (5, 17). Differences between the results of these studies may be attributed to the lower sensitivity of CISH in the cases of borderline amplification (+2 IHC grade), as well as to variations in the tumor samples and experimental procedure (5, 17).

Studies demonstrate the apparent benefits of CISH but it is still undecided whether the technique should replace FISH. In comparison to FISH, CISH is a more cost-effective and user-friendly technique for the assessment of HER-2/*neu* gene amplification. Even so, while in most cases CISH results are equally as accurate as FISH, since CISH is a relatively new method, more research must be conducted to further assess its concurrence with +2 grade IHC results.

## Response of HER-2/*neu*-positive Breast Carcinomas to Chemotherapy Regimens

Though there seem to be several possibilities for HER-2/*neu* breast cancer detection, treatment options are quite limited. As previously mentioned, HER-2/*neu*-positive tumors are often resistant to common chemotherapy regimens, a consequence that greatly complicates the treatment of HER-2/*neu*-positive breast cancer patients (3, 4). In the past there has been a lack of conclusive evidence to support the relationship between HER-2/*neu* overexpression and chemotherapy

sensitivity; however, a study conducted in 2001 demonstrated that, *in vitro,* the chemosensitivity to traditional chemotherapy regimens such as cyclophosphamide, methotrexate, and fluorouracil (CMF) is directly related to the level of HER-2/*neu* expression (18). In a more recent study conducted by Pritchard et al. (4), the effectiveness of an alternative chemotherapy regimen, cyclophosphamide, epirubicin, and fluorouracil (CEF) was assessed. Although CEF is costly and may elevate the occurrences of side effects such as alopecia, nausea, vomiting, and stomatitis, its use is extremely beneficial in cases that exhibit the overexpression of the HER-2/*neu* proto-oncogene (4). According to this groundbreaking study, HER-2/*neu*-positive patients treated with CEF rather than with CMF demonstrated a 65% increase in overall survival as well as a 52% increase in relapse-free survival (4).

## Use of Monoclonal Antibody as HER-2/*neu* Treatment

### *Efficiency of Trastuzumab*

Although the administration of chemotherapy regimens such as CEF provides gains for HER-2/*neu* breast cancer patients, the development of the drug trastuzumab has additionally improved longevity statistics. Commonly known as Herceptin, trastuzumab is a recombinant monoclonal antibody that inhibits tumor growth in HER-2/*neu*-positive breast cancer (2, 19). While the exact mechanism of trastuzumab is not fully known, scientists recognize that when trastuzumab binds to HER-2/*neu* trans-membrane growth receptor proteins, it induces apoptosis of the tumor cells and controls the accelerated signal transduction pathway (6, 20). Current studies indicate that trastuzumab used either as a single agent in the treatment of HER-2/*neu*-positive breast carcinomas or in combination with chemotherapy is a very effective treatment option for HER-2/*neu*-positive breast cancer patients. Results of several studies confirm that trastuzumab significantly increases the time to disease progression and the overall longevity of patients while enhancing the effectiveness of chemotherapy regimens (3, 4, 19, 21). Although the administration of trastuzumab therapy has documented benefits, the drug is not an option for all HER-2/*neu*-positive breast cancer patients. Trastuzumab treatments are restricted to HER-2/*neu*-positive breast cancer patients that exhibit a +2 or +3 grade (IHC) staining and positive HER-2/*neu* amplification (FISH) (3, 6 15).

When used as a single treatment agent, trastuzumab therapy increases the median duration of patient survival; however, when administered in combination with chemotherapy treatments, its benefits are even more significant (2, 6, 21). According to a large-scale study conducted by Romond et al. (21), there was a 12% increase of disease-free survival over a three-year period in patients treated with a trastuzumab-chemotherapy combination in comparison to those treated solely with chemotherapy. Additional studies such as those conducted by Slamon et al. (19) and Bartsch et al. (6) reported similar outcomes. Slamon and colleagues (19) also confirmed that over a 30-month follow-up period, patients treated with the trastuzumab-chemotherapy regimen exhibited an astounding 20% reduction in cancer-related death than the corresponding chemotherapy control groups.

It is clear that short-term trastuzumab therapy is an effective form of treatment for HER-2/*neu*-positive breast cancer patients, but scientists are still questioning the benefits of its prolonged use (6). While the small-scale study of Bartsch et al. (6) reported that prolonged trastuzumab treatment beyond the stage of disease progression was effective for some patients, they also speculated that long-term trastuzumab use may result in an acquired resistance to the medication. In addition, though many trastuzumab therapy studies confirm the benefits of trastuzumab-chemotherapy treatments, few studies report the effects of different trastuzumab dosages in treatment assessments. According to the relatively small trial conducted by Vogel et al. (2), an increased 4mg/kg dosage of trastuzumab did not demonstrate any benefits over the standard 2mg/kg regimen. Currently, trastuzumab-chemotherapy treatments are standard for +2 and +3 HER-2/*neu*-positive breast cancer cases but studies are still being conducted to evaluate the most effective dosage combinations and treatment plans for these patients.

### *Cardiac Dysfunction Associated with Trastuzumab*

While the treatment benefits of trastuzumab therapy are very prominent in HER-2/*neu*-positive breast cancer survival statistics, it is important to discuss the risks that are associated with the treatment. The common side effects of trastuzumab are relatively similar to those encountered with traditional chemotherapy; however, trastuzumab therapy may increase the risk of upper respiratory tract infections during treatments (19, 21). More seriously, several scientists assessing the efficacy of trastu-

zumab reported occurrences of cardiac dysfunction among their sample populations (2, 19, 21).

Ranging from a decline in the Left Ventricular Ejection Fraction (LVEF) to more severe instances of congestive heart failure, cardiac dysfunctions appear to persist variably between studies (2, 19, 21). According to Romond et al. (21), in the trastuzumab-chemotherapy population "the cumulative three-year incidence of congestive heart failure increased by about 3 percentage points with the addition of trastuzumab." In contrast, Bartsch et al. (6) noted that while patients in their trial were treated with trastuzumab for more than three years, they did not encounter any cases of congestive heart failure among the population. The direct relationship between trastuzumab therapy and cardiac dysfunction is still undergoing research; however, it is suspected that both age and prior heart conditions are significant risk factors for cardiac dysfunction caused by trastuzumab (2, 19). Though the relationship is not completely understood, all patients that undergo trastuzumab treatment must be initially screened for cardiac irregularities and have their LVEF monitored continuously throughout treatment.

## Conclusion

HER-2/*NEU* BREAST CANCER IS INITIATED by the overexpression and amplification of the HER-2/*neu* proto-oncogene and characteristically demonstrates accelerated tumorigenesis. Patients with HER-2/*neu*-positive breast cancer are traditionally given a poor prognosis because of the aggressiveness of the cancer. Currently, IHC and FISH are the common detection methods of this condition; however, studies are being conducted in order to construct the most effective diagnosis procedure. Although evidence supports an agreement between the two methods, recent studies indicate that IHC screening with the A0485 antibody periodically demonstrates falsely positive results, leading scientists to regard FISH as a standard technique. Nevertheless, though the screening result discrepancy is considered to be an issue in +2 grade IHC readings, IHC is still being used because it is convenient and cost-effective. Recent studies also evaluated the accuracy of CISH, a more convenient method of HER-2/*neu* gene detection. While CISH may prove to be an effective alternative to FISH in the future, further research must be conducted before an official substitution can be made.

Although HER-2/*neu*-positive tumors are resistant to traditional chemotherapy regimens, drugs such as CEF prove to be effective in treatment. Even more so, the combination of trastuzumab and chemotherapy in the treatment of +2 and +3 grade HER2/*neu*-positive cases provides several benefits including a prolonged survival period and an increased probability of disease-free survival. This remarkable treatment demonstrates favorable results, but patients must be aware of its adverse health effects and be monitored continually during treatments. While the correlation between trastuzumab usage and cardiac dysfunction is still being researched, recent studies report substantial evidence suggesting that the relationship is valid. Currently, scientists have successfully reduced the negative outcome that is coupled with HER-2/*neu*-positive prognosis, but more research must be conducted in order to additionally improve the survival rate. With the development of a standard HER-2/*neu* detection method and standard drug-dosage treatments, the population of patients that are diagnosed with HER-2/*neu*-positive breast cancer will not only be able to *hope* for survival, they will be able to *demonstrate* survival.



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