ANALYSIS OF HER2 TESTING IN BREAST CANCER: DISPARITIES, COST-EFFECTIVENESS, AND PATTERNS OF CARE

A Dissertation
Presented to
The Academic Faculty

By

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In Partial Fulfillment
Of the Requirements for the Degree
Doctor of Philosophy in Bioengineering

Georgia Institute of Technology

August, 2009

Analysis of HER2 Testing in Breast Cancer:
Disparities, Cost-effectiveness and Patterns of Care

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For Mom, my Hero, source of all Good things
For Dad, giver of Strength
For Karan, bringer of Joy

Acknowledgements

This dissertation has been made possible because of the gracious support of so many people. Dr. Michael Halpern has been the most consistent and reliable mentor any PhD candidate can hope to have, and for this, I am immensely grateful. This work simply would not have been possible without his guidance. My serendipitous meeting with him several years ago has led me to a career that I find deeply fulfilling, and I cannot thank him enough for his kindness and support through the years.

Dr. Paul Griffin has been a kind and thoughtful advisor, has given me the freedom to develop this work, and has provided critical feedback throughout the process. Through his role as advisor, he has helped me sharpen this work and has greatly supported my research at Tech. I am extremely thankful to him for his immense support during my PhD experience. I am fortunate to have had the opportunity to work with Dr. Griffin.

I met Dr. Richard Nichols on my first day as a PhD student in the department of Biomedical Engineering, and it turned out to be quite lucky for me that I did. Through the years, I have had the honor of taking a directed study course with him, and have we have discussed various topics, from history and politics to research and medicine. His knowledge and wisdom are unparalleled, and I can only hope that some of it has rubbed off on me through these years.

Dr. Robert Butera has been extremely supportive of my work and has been instrumental in giving me an academic home at Georgia Tech in the Bioengineering program. He stepped in and offered his support at challenging times during this research, and this

support has been invaluable to me. I am extremely thankful that he has served on my doctoral committee.

The final member of my doctoral committee, but most certainly not the least, is Dr. Brani Vidakovic, who I am quite certain, is one of the nicest people I have ever had the pleasure of knowing. In addition, Dr. Vidakovic is a remarkable professor, and I have benefited greatly from his immense knowledge. I am very thankful to him for all his support.

Special thanks are due to Ms. Sally Gerrish of the Biomedical Engineering department who has been a great friend and advisor to me through my years in this program. I also thank Erin Hamilton, my colleague in lab who has become a great friend. Erin's friendship has made lab a happy place to be.

I would like to thank the American Cancer Society for the data used in this research.

In addition, there are some very special people I must thank. My dear family; especially my mom, about whom I have only the most wonderful things to say. She is really the most remarkable person I know, and I want to be just like her when I grow up. My amazing Dad: the kindest and smartest person I know – the original Dr. Ashok – whose love and support are immeasurable. There are so many reasons I am glad I came to this place, at this time, for a PhD – and the absolute best thing of all is that it led me to Karan –- who is exceptional and wonderful; who stands with me always, and who has brought into my life infinite and magical happiness.

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List of Abbreviations

HER2	Human Epidermal growth factor Receptor 2
FISH	Fluorescence In Situ Hybridization
IHC	Immunohistochemistry
FDA	Food and Drug Administration
TNM	Solid tumor staging system: T – Tumor Size, N – lymph Node involvement, M – Metastases
NCCN	National Comprehensive Cancer Network
LCIS	Lobular Carcinoma In Situ
DCIS	Ductal Carcinoma In Situ
QALY	Quality Adjusted Life Years
EMR	Electronic Medical Records

Summary

HER2 breast cancer is an aggressive disease that occurs in 20 – 30% of the breast cancer population. Treatment for HER2 breast cancer includes use of an anti-HER2 monoclonal antibody, trastuzumab. Testing for HER2 is of critical importance due to the adverse side effects and substantial costs associated with this anti-HER2 treatment. Currently, two kinds of tests, Fluorescence In Situ Hybridization (FISH) and Immunohistochemistry (IHC), are FDA approved for determination of HER2 status in breast cancers.

Clinical and non clinical factors that affect the choice HER2 test and the use of anti-HER2 therapy in breast cancer were analyzed using a data set containing information from six outpatient oncology clinics in the United States. The analysis showed that geographic location, cancer stage, and diagnosis date (pre- or post-publication of testing guidelines) have significant effects on choice of test. With regard to trastuzumab prescription, geographic location and HER2 status have significant effects on the prescription of trastuzumab. In addition, there was a non-significant trend for certain Medicare patients not to receive trastuzumab therapy. These findings indicate that disparities are present in breast cancer care based on geography and cancer stage, and highlight the importance of testing guidelines.

The cost effectiveness of FISH vs. IHC was determined, by considering the financial and health-related costs associated with testing and subsequent treatment as well as the accuracy of each test. The results show that FISH is the optimal choice for HER2 testing and is more cost-effective than IHC.

Chapter 1

Fluorescence In Situ Hybridization and Immunohistochemistry for HER2 Status Detection in Breast Cancer

The American Cancer Society estimates that in the United States alone, over 182,000 women will be diagnosed with invasive breast cancer and over 40,000 women will die of this disease in 2008. [1] However, rates of death from breast cancer are declining, and the American Cancer Society attributes this to earlier detection and better treatment methods. Optimal breast cancer treatment involves determining the stage of disease at diagnosis as well as characteristics of the tumor, including estrogen and progesterone receptor status and HER2 overexpression.

The human epidermal growth factor receptor 2, called HER2 or c-*erb*B-2, encodes a tyrosine kinase. The HER2 gene belongs to a family of genes that encode transmembrane receptors for growth factors. [2] Gene amplification of HER2 with subsequent protein overexpression has been shown to exist in 20 % to 25% of invasive breast cancers. [2-7] HER2 amplification and overexpression are associated with increased tumor aggressiveness and poor prognosis. [2, 3]

Trastuzumab (Herceptin®) is a humanized monoclonal antibody indicated for treatment of HER2 positive breast cancer. Trastuzumab is an anti-HER2 antibody that works synergistically with chemotherapy in HER2 overexpressing cancer cells (7). This drug has significant benefits for HER2 positive breast cancer patients; however, it is associated with elevated risks of serious adverse events (most significantly, cardio

toxicity) and with substantial costs. Thus, to ensure appropriate use of this medication for breast cancer treatment, accurate testing for HER2 is of critical importance.

There are currently two main types of tests used to assess levels of HER2 in breast cancer: Fluorescence in Situ Hybridization (FISH) and Immunohistochemistry (IHC). FISH measures HER2 gene amplification while IHC measures levels of the HER2 protein. The first practical application of the FISH technique was in 1980 and the technology has evolved in the 20 years since that time. [8] FISH uses fluorescent DNA probes to identify specific parts of a chromosome; in this case the HER2 gene. The probe is made complementary to the sequence that is being detected and is labeled with a fluorescent marker to facilitate visualization using a fluorescent microscope. Immunohistochemistry, which has been used since the 1940s, consists of using an antibody to link a desired antigen to a detectable marker, such as a stain, enzyme, or radioactive element. This makes it easier to visualize the antigen under a microscope or with appropriate detectors. In the case of HER2 detection, monoclonal or polyclonal anti-HER2 antibodies are used. There are currently six FDA-approved FISH and IHC tests for HER2 diagnosis in the United States: PathVysion (FISH, Vysis Inc.), INFORM (FISH, Ventana Medical Systems), FISH PharmDx (FISH, Dako), InSite (IHC, BioGenex Laboratories, Inc), HercepTest (IHC, Dako), and Pathway (IHC, Ventana).

A number of published reviews have evaluated literature associated with HER2 testing. In particular, a joint report from the American Society of Clinical Oncology and the College of American Pathologists [9] and a report from the National Comprehensive Cancer Network [10] provide detailed summaries of the literature associated with HER2 testing and include recommended guidelines for HER2 testing. This chapter synthesizes key findings regarding differences, sources of variability, and costs for IHC and FISH

testing for HER2, and concludes by discussing needed areas for additional research. As such, this literature synthesis will be useful in identifying current issues related to IHC vs. FISH testing for HER2, and in deciding upon specific areas in which research is needed for future clinical and policy development.

1. 1 Methods and Materials

Literature for this synthesis was identified from searches of the National Library of Medicine's MEDLINE database. Only studies published in English over the past 10 years (1999-2008) were considered for inclusion. Articles with the terms "Receptor, erbB-2" and "Breast Neoplasms" and either "Immunohistochemistry" or "In Situ Hybridization, Fluorescence" were identified. Titles of articles were reviewed to identify literature relevant to: variability in HER2 testing; comparisons between IHC and FISH; costs time, and cost-effectiveness of HER2 testing; and testing algorithms. References cited in identified articles were also reviewed.

1.2 Results

1.2.1 Variability in HER2 IHC Results based on Laboratory Characteristics and Scoring

IHC is a relatively simple diagnostic procedure, requiring less time, expertise, and cost than many other molecular diagnostic tests (such as FISH). However, while the ease with which IHC can be performed and its widespread use in a majority of laboratories performing HER2 testing are two of its advantages [(11)], IHC test results can exhibit substantial variability. Variability in scoring of IHC results can occur between laboratories and between personnel in the same laboratory due to several factors. Irrespective of the choice of test method, laboratory experience is vital in order to obtain accurate and reliable results. [(12)] Laboratories that handle high volumes of tests show higher quality

and more standardized IHC testing results as measured by higher levels of IHC and FISH concordance. [13] Quality assurance methods in smaller laboratories have been reported to be more problematic than those of larger laboratories regarding testing for HER2 status. [14]

Poor standardization of immunohistochemical protocols has been blamed for the variability in IHC results. Using test results from 12 French laboratories, Vincent-Salomon et al. evaluated calibration of IHC procedures in those laboratories where poor concordance between IHC and FISH results were noted. They demonstrated the importance of calibration of immunohistochemical procedures using antibody retrieval and antigen dilution in order to improve accuracy and reliability of the process. [7] Related to this, Sauer reported that the intensity of IHC staining is dependent on formalin fixation, which can vary from laboratory to laboratory, and even within the same laboratory. [15] While fixation is an issue with FISH also, the DNA is a more stable target for testing. [15] Automated image analysis systems attempt to reduce variability between observers by quantifying staining intensity. However, such systems cannot eliminate inherent variation until standardization of immunohistochemical processes between and within laboratories occurs. [16]

Variability between testing laboratories may be more pronounced for samples that show low to intermediate positive results using IHC. Results from a survey by the American College of Pathologists showed excellent agreement between laboratories for cancer samples with no HER2 protein overexpression or high levels of overexpression, but considerable variation among those with low levels. [17]

1.2.2 Variability in IHC Results among Test Antibodies

The use of different antibodies (with differing levels of sensitivity and specificity) can contribute to the variability of results in IHC. [18-21] Tubbs et al. analyzed 400 infiltrating ductal breast carcinoma samples with two antibodies against the HER2 protein: the Dako polyclonal antibody (HercepTest) and the monoclonal antibody CB11. Their analysis compared IHC results against the gold standards of gene amplification and mRNA profiling. Although the same scoring criteria were used (based on the manufacturer's guidelines for HercepTest), the HercepTest resulted in 23% false positive results while the CB11 monoclonal antibody resulted in 17% false positives. [18]

Lebeau et al. evaluated IHC results using five different monoclonal antibodies and two polyclonal antibodies. [22] The monoclonal antibodies tended to identify the same tumor samples as HER2 positive while the polyclonal antibodies showed less agreement. Further, the monoclonal antibodies identified fewer samples as being HER2 positive (26% to 27% of samples) compared to the two polyclonal antibodies (33% and 42%). Among 57 samples with no HER2 gene amplification as measured by FISH, positive results were seen with IHC using monoclonal antibodies in 1% to 3% of samples. For IHC using the two polyclonal antibodies on these samples, positive results were seen in 7% and 13% of samples. Among 20 samples with high levels of gene amplification based on FISH, four of the five monoclonal antibodies and both polyclonal antibodies indicated positive IHC results for 100% of the samples (one monoclonal antibody indicated positive results from only 18 of the 20 samples). [22] Therefore, the monoclonal antibodies had greater specificity compared to the polyclonal antibodies, while both antibody sets (with one monoclonal antibody exception) had comparable sensitivity.

1.2.3 FISH vs. IHC

1.2.3.1 Concordance

As prescription of trastuzumab therapy should rely on the test that is used to determine HER2 status, the accuracy and reliability of the HER2 test used can seriously affect patient outcomes. The FDA approved HercepTest, InSite and Pathway and their corresponding scoring systems (0, 1+, 2+, 3+) are of particular interest due to their clinical relevance and widespread utilization with regard to trastuzumab therapy. Discordance between IHC and FISH has been reported particularly for intermediate (2+) IHC results. Currently, for HercepTest, a score of 3+ is classified as strongly positive and a score of 2+ is classified as weakly positive for HER2 overexpression. For InSite and Pathway, both 2+ and 3+ scores are classified as positive. All three tests use similar categorization to classify tissues into the 2+ and 3+ scores (Table 1.1).

Several studies have analyzed the validity of IHC testing using FISH as the gold standard. A number of these studies have indicated issues with IHC testing, particularly with respect to false positive results. [3, 15, 16, 18, 23-26] Wang et al. used a polyclonal antibody to perform IHC on 52 infiltrating breast cancer tissues and scored them as "high positive", "medium positive", "low positive" and "negative." Their results showed that 9 out of 13 specimens evaluated as "medium positive" by IHC failed to show any gene amplification using FISH. [3] Jacobs et al. reanalyzed 48 invasive breast cancer samples that had previously been determined to be HER2 negative by two IHC assays as well as FISH. [27] Jacobs et al. found that using the HercepTest test, 58.4% of these samples were scored as HER2 positive, presumably false positive results.

Table 1.1: Positive Scores in FDA Approved IHC Assays[1]

Staining Pattern	Score	Interpretation
A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells	2+	1. HercepTest: Weakly Positive 2. InSite: Positive 3. Pathway: Positive (membrane staining ring is seen to be thin)
Strong, complete membrane staining is observed in more than 10% of the tumor cells	3+	1. HercepTest: Strongly Positive 2. InSite: Positive 3. Pathway: Positive (membrane staining ring is seen to be thick)

1. Sources: www.dakousa.com, www.ventanamed.com, and www.fda.gov

A majority of the false positive IHC cases (using FISH as a gold standard) are found among 2+ results. [18] This suggests that IHC 2+ results cannot reliably be considered HER2 positive, as follow up tests using FISH and other IHC protocols on the same cancer samples can result in negative HER2 classification. [6, 18, 24, 28-30] A number of studies have reported that a quarter or less of tissue specimens that scored IHC 2+ demonstrate corresponding gene amplification by FISH. [6, 22, 28] In a study by Jimenez et al., all five 2+ results in an assay performed with the Zymed antibody showed negative gene amplification using FISH. [29]

Lebeau et al. reported that of seven antibodies tested (five monoclonal and two polyclonal), HercepTest resulted in the greatest number of samples being scored as HER2 positive, with a majority of the 2+ results ultimately showing no gene amplification by FISH. [22] Tubbs et al. reported that HercepTest showed greater discordance with FISH than did the monoclonal antibody CB11 from Ventana. [18] Considering the impact of 2+ false positives on patient outcomes and costs, most of these studies recommend not using IHC 2+ results as a sole criterion for trastuzumab therapy.

A number of other studies also report that IHC 0, 1+ and 3+ scores show better levels of agreement with FISH results than did 2+ scores. For instance, Kakar et al. reported that samples with 3+ IHC staining showed 88% concordance with FISH and 0/1+ staining showed 99% concordance with FISH, while 2+ results showed only 35% concordance.[21] Thompson et al. reported that only 0 and 3+ results from IHC show substantially concordant results with FISH, and that 1+ and 2+ were not clearly predictive of gene amplification status. [23] Jimenez et al. found that all cases scored as 3+ using IHC, with 3 different antibodies (Zymed, clone 31G7; Ventana, clone CB11; Dako, polyclonal), showed gene amplification by FISH. [29] Lebeau et al also reported that despite the use of different antibodies, IHC 3+ results consistently showed gene amplification by FISH. [22] It is important to note that even though discordance between 0/1+ IHC results and FISH results (i.e., negative IHC and positive FISH results for the same sample) has been reported to be as low as less than 1% (19, 21, 31), these discrepancies are important due to their impact on the correct (or potentially incorrect) HER2 diagnosis, treatment and survival.

Since IHC and FISH do not detect the same biological alterations in cancerous cells (i.e., IHC measures overexpression of the HER2 protein while FISH measures gene amplification), absolute concordance may never be achieved between these two methods. [32, 33] Samples with negative results from IHC testing (scored as 0 or 1+ under the HercepTest, Insite and Pathway scoring systems) do not typically show gene amplification under FISH analysis. [19, 24, 28, 29, 31, 34] While some studies have reported on samples that show gene amplification (i.e., positive FISH results) when IHC results are negative [19, 21, 25, 31] ,the proportion of these IHC negative/FISH positive cases is usually very small. For example, Ridolfi et al found one IHC negative sample out of 750 invasive breast carcinomas showed gene amplification when tested with FISH. Ridolfi et al.

suggest that samples may be classified as IHC negative/FISH positive (and thus potentially IHC false-negative) due to loss of epitope (i.e., an antibody binding site) during the fixation process. [19, 31] Another possible explanation for this discordance is amplification in the absence of overexpression. [19] Similarly, while most studies report good agreement between IHC strongly positive (3+) results and positive FISH results, there are 3+ IHC cases that are negative for HER2 amplification based on FISH. [31] Dowsett et al found that such FISH negative/IHC positive cases typically have better prognosis than FISH positive cases, which suggests that these cases may be IHC false positives. [31] In some cases, IHC positive/FISH negative cancers have been attributed to protein overexpression in the absence in gene amplification. [21, 35] Also, dual-probe FISH (which quantifies the number of HER2 genes relative to the number of chromosomes) would classify as negative those cases that overexpress HER2 due to polysomy of chromosome 17 rather than multiple copies of the HER gene on a single chromosome.[28] While polysomy has an additive effect with gene amplification, polysomy without gene amplification can also cause an increase in HER2 protein production.

1.2.3.2 Costs, Time, and Cost-Effectiveness of FISH and IHC

It is clear that FISH costs more and requires more time to complete than IHC. The average cost per test has been reported to be approximately $482 for FISH versus $89 for IHC. [36] Based on Medicare reimbursements, Elkin et al. valued FISH at $381 while HercepTest was valued at $85. [37]

In addition, FISH testing is a longer and more labor-intensive procedure than IHC. FISH requires more time for both sample preparation and analysis compared to IHC. [14] In a study comparing IHC with the monoclonal antibody CB11, the HercepTest IHC kit, and

the Oncor Ventana INFORM FISH kit, CB11 IHC and HercepTest were found to take 1 day each while FISH required 2 days, with 4 hours on the first day and 7 hours on the second. [38] Kakar et al reported that each IHC assay, with a maximum of 40 slides, took 105 minutes while each FISH test, with a maximum of 20 slides, required 173 minutes divided over two days. [21] In addition, laboratory personnel required 3 minutes to analyze IHC results but needed over 15 minutes to analyze FISH results. [21]

Few cost effectiveness studies comparing IHC and FISH for HER2 testing have been performed. Studies that have compared cost-effectiveness of these two tests are not in agreement regarding which test should be used for HER2 testing. [37-38]

1.2.3.3 Algorithms for HER2 Testing

The extensive discussion in the literature on FISH vs. IHC for HER2 testing informs recommendations for how testing should be performed. These recommendations vary from advocating the use of IHC as the primary test, using both IHC and FISH, and using only FISH. Wolff et al. [9] and Carlson et al. [10] provide algorithms both for initial IHC testing and for initial FISH testing, and stress the importance of good laboratory practices in order to accurately assess HER2 status.

While FISH is widely considered the more accurate assay, some studies that take into account cost, laboratory experience, and expertise required to perform FISH recommend IHC as a reliable, accurate and cost effective primary method for HER2 testing. [39] Standardized IHC procedures have been recommended in some cases for use as the primary method for HER2 detection except when protein expression levels are very low.[40] However, this recommendation often comes with the caveat that IHC testing may need to be followed up with FISH analysis particularly in cases with IHC 2+ results. [21]

In contrast, Sauer et al. recommended that FISH should be used as the primary testing method for HER2 status, even though it is more complex and more expensive than IHC.[15] Their analysis of HER2 status in 215 breast carcinomas led them to conclude that determining gene amplification is more important than determining overexpression. Specifically, Sauer et al pointed out that overexpression without gene amplification occurs in cancers that belong to a group with better prognosis than those cancers that have gene amplification. [15] Bartlett et al. also recommend the widespread implementation of FISH for HER2 testing; they further indicate that IHC could become the test of choice in the future if improved test calibration and standardization occur. [41]

Considering the adverse effects of trastuzumab treatment without true positive HER2 status, retesting of even those cases that show IHC 3+ status with FISH has also been recommended. [25] If effective quality control methods and standardization techniques are available, the use of IHC as the initial method and FISH to test only equivocal cases may be reliable, but concerns exist about this algorithm considering the present state of IHC testing. [41] Variations of this algorithm exist, such as the one proposed by Falo et al. that suggests the use of IHC with the CB11 antibody as the first step to diagnose positive cases. This is followed by reanalyzing all negative cases with HercepTest and then utilizing FISH only with those samples that are CB11 negative and HercepTest positive.[38] Another variation is suggested by Torrisi et al who recommend using FISH to retest all cancers which show complete immunostaining in less than 50% of neoplastic cells. [42]

A number of studies, such as that by Arnould et al. [43], have reported that FISH amplification status is highly correlated with patient response to trastuzumab therapy. In contrast, other published literature, much of which is summarized by Carlson et al. (10),

and a recent letter by Paik et al. [44] indicate equivalent benefits in terms of disease progression for women with breast cancer treated with trastuzumab, regardless of whether they were HER2 positive or negative (assessed by both IHC and FISH). Results of HER2 testing may also be influenced by HER2 expression heterogeneity within a tumor; as reported by Striebel et al. [45], repeat testing of tumors with equivocal HER2 results changed the HER2 status classification in more than half of 17 biopsy specimens. In developing appropriate testing and treatment recommendation, tumor sampling procedures and the potential benefit of trastuzumab among patients with equivocal or even negative HER2 results will need further study.

1.2.3.4 Biological Differences between FISH and IHC

FISH and IHC detect different biological components; FISH detects sequences of DNA while IHC detects protein overexpression. Apparent discrepancies in test results may either be due to artifacts (false positive or false negative test results) or true biological differences (overexpression in the absence of amplification or amplification in the absence of overexpression). With respect to biological phenomena, HER2 protein overexpression may either be the result of HER2 gene amplification or increased levels of HER2 transcription caused by the preferential binding of the HER2 transcription factor OB2-1. [46] In some cases, HER2 gene amplification may be present without corresponding HER2 protein overexpression when the amplified genes do not actively produce their protein products.

FISH positive/IHC negative results and FISH negative/IHC positive results have been reported in various studies. [21, 31, 35] Most of these cases behave in a way that is aligned with the expected behavior associated with the corresponding FISH result. For example,

a majority of cases that are positive for HER2 overexpression by IHC but negative for gene amplification by FISH have better prognosis than cases that are HER2 positive by both tests. [15,31] With regard to FISH positive/IHC negative cases, a majority of them behave as true HER2 positive cases; studies have shown these tumors have the same survival rates as tumors with positive results on both tests. [15] In addition, clinical response to trastuzumab is strongly correlated with gene amplification (as detected by FISH). [47]

1.3 Discussion

We have summarized and synthesized literature on Fluorescence In Situ Hybridization and Immunohistochemistry testing protocols for HER2 gene amplification and protein overexpression, respectively, in breast cancer. Many published studies show high levels of variability with IHC results, while FISH appears to be more reliable and serves as a gold standard for testing. IHC protocols vary in terms of the laboratory experience, score interpretations, and antibodies used for testing. When IHC results are intermediate, literature shows that they cannot be reliably used to determine a cancer's HER2 status. This problem with interpretation of IHC results has been illustrated in several studies, particularly with respect to 2+ results, using the FDA approved HercepTest. The strengths of IHC are that it is less expensive than FISH and it requires less laboratory personnel time and expertise.

While there is a sizeable body of literature comparing different aspects of FISH and IHC, we found certain limitations in existing literature. These gaps in research include: potential performance characteristics of IHC testing with improved standardization; correlations between treatment outcome and test(s) used for HER2 detection; disparities in HER2 testing and in subsequent anti-HER2 treatment, and cost-effectiveness of IHC

vs. FISH testing. For this first area, a number of studies indicate that calibration and standardization of IHC protocols could result in increased accuracy and reliability for this test. Dowsett et al. concluded that standardization and quality control methods are imperative to improve both FISH and IHC, particularly in laboratories that are less experienced or not centralized. [48] O'Malley et al. indicated that participation in quality assurance programs was an effective way to improve the accuracy of IHC test results.[49] Despite studies on standardization, the question of whether calibration and standardization could result in IHC becoming equal to (or more effective than) FISH in terms of accuracy, reliability, and other factors is not clear. It appears that with available technology and methods, while IHC shows improved performance when standardized, IHC results still may have to be validated by FISH, particularly for ambiguous cases (such as with 2+ staining for HercepTest). More studies evaluating whether improved IHC methods (in addition to studies quantifying the level of improvement required for IHC) could effectively replace FISH are required to fully assess the appropriate roles of these two tests for HER2 detection.

In addition, more research is needed with respect to outcomes for breast cancer patients based on variations in HER2 testing patterns and treatment. Particularly in light of studies reporting benefits from trastuzumab therapy among HER2 negative patients [10, 44], additional studies are needed to determine the associations among types of HER2 test(s) used for women with breast cancer, treatment (or not) with trastuzumab therapy, and patient outcomes. Given the potential uncertain in identifying appropriate patients for trastuzumab therapy, collection of outcomes data associated with test type/results and specific treatment received is crucial. Examination of the role of clinical and non-clinical factors in the choice of test and treatment for breast cancer is required.

Related to evaluation of patient outcomes, studies on cost-effectiveness of HER2 testing patterns are much needed. Several existing studies compare the costs of the tests only and do not account for costs associated with outcomes of potentially incorrect treatment. For example, cost-effectiveness studies which include the adverse health outcomes as well as the loss of time and money involved in treating a patient falsely diagnosed as HER2-positive by IHC could result in FISH being classified as being cost-effective (or even costs-saving) as compared to IHC. In the published literature, there is little disagreement that FISH is more accurate than IHC. By performing IHC first, followed by FISH for intermediate case, the best possible outcomes would be to classify patients as well as would be the case with performing FISH first for everyone. The argument for performing IHC first is therefore largely economic. Given the potentially serious consequences of incorrect trastuzumab therapy (either not treating someone who is HER2 positive or treating someone who is HER2 negative), strong evidence is needed regarding cost-savings resulting from initial IHC testing or lack of cost-effectiveness for initial FISH testing. Further, such evidence needs to come from "real world" settings rather than from studies of IHC vs. FISH that are performed under ideal conditions such as select, high quality laboratories.

In this dissertation, we attempt to address some of these areas which have not been investigated in detail. Specifically, we examine clinical and non-clinical factors and their role in HER2 testing and trastuzumab treatment. In addition, we also determine the cost-effectiveness of FISH vs. IHC as it pertains to HER2 testing in breast cancer.

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Chapter 2

Impact of Clinical and Non-clinical Factors on the Choice of HER2 Test for Breast Cancer

2.1 Introduction

Mortality rates associated with breast cancer have declined in recent years due to earlier detection and better treatment methods. [1] Determining cancer stage as well as biological characteristics of the tumor such as hormone status and HER2 status is critical to providing appropriate treatment. HER2 positive breast cancer is a type of breast cancer characterized by amplification of the HER2 gene with subsequent protein overexpression. The HER2 gene, the human epidermal growth factor receptor 2, encodes a tyrosine kinase and belongs to a family of genes that encode transmembrane receptors for growth factors. [2] Gene amplification of HER2 with subsequent protein overexpression has been shown to exist in 20 % to 30% of invasive breast cancers. [2-7] HER2 amplification (i.e., the presence of an increased number of gene copies per cell) and overexpression (i.e., the increased production of protein by a gene) are associated with increased tumor aggressiveness and poor prognosis. [2, 3]

Treatment for HER2 breast cancer includes use of a targeted therapy: a humanized monoclonal antibody, trastuzumab (Herceptin®) which works against HER2 overexpressing cells. This drug has significant benefits for HER2 positive breast cancer patients in terms of improving clinical outcomes; however, it is associated with elevated risks of cardio-toxicity and with substantial financial costs of over $120,000 for treatment. Thus, to ensure appropriate use of this medication for breast cancer treatment, accurate testing for HER2 is of critical importance.

There are currently two main types of tests used to assess levels of HER2 in breast cancer: Fluorescence in Situ Hybridization (FISH) and Immunohistochemistry (IHC). FISH measures HER2 gene amplification while IHC measures levels of the HER2 protein. The following FISH and IHC tests are FDA-approved for HER2 diagnosis in the United States: PathVysion (FISH, Vysis Inc.), INFORM (FISH, Ventana Medical Systems), FISH PharmDx (FISH, Dako), InSite (IHC, BioGenex Laboratories, Inc), HercepTest (IHC, Dako), and Pathway (IHC, Ventana).

A number of published reviews that have evaluated HER2 testing methods show high levels of variability within IHC results, while FISH is more reliable and generally serves as a gold standard for testing. [6-20] IHC protocols vary in terms of laboratory experience, score interpretations, and the kinds of antibodies used for testing. [7-16] IHC results for HER2 testing are scored form 0 (negative result) to 3+ (positive). When IHC HER2 test results are intermediate (2+), literature shows that they cannot be reliably used to determine a cancer's HER2 status. [6, 12, 17-20] The strengths of IHC are that it is less expensive than FISH and it requires less laboratory personnel time and expertise. [8, 16, 22-23]

FISH testing for HER2, which is more expensive and requires greater laboratory expertise and more time than does IHC, is regarded as more accurate as it is associated with better response rates to anti-HER2 therapy [24-27]. That is, HER2 status determined by FISH correlates better with response to anti-HER2 therapy than does HER2 status determined by IHC. Despite the superiority of FISH in terms of accuracy and reproducibility, both IHC and FISH continue to be used for HER2 testing. It is therefore important to assess the factors that influence which of these tests is selected for use. Previous studies have examined race, insurance status, and other socioeconomic

factors that are associated with disparities in breast cancer diagnosis, treatment and survival. [28 – 34] Socioeconomic status is associated with breast cancer stage at diagnosis, treatment type, and likelihood of mortality [31]; for example, advanced stage tumors are found in greater proportions in minorities when compared to white women [32] and uninsured patients and those covered by Medicaid are more likely to have advanced stage breast cancer at the time of diagnosis when compared with patients who are privately insured. [34] While these studies have considered the impact of various sociodemographic factors on diagnosis and treatment of breast cancer, the role of such factors on the choice of HER2 test has not been examined. The goal of this study is to determine whether certain clinical and non-clinical factors affect the choice of HER2 test among women who are tested for HER2.

2.2 Materials and Methods

The data utilized for this research was provided by The American Cancer Society, which purchased the data set from Rabbit Healthcare Systems. Rabbit Healthcare Systems is located in Austin, TX, and primarily develops electronic medical records for oncology practices. The data set used for this study consists of information from the Rabbit Healthcare Systems electronic medical records from six private practice oncology practices. The data-set is unique in that it includes information on HER2 status that is not currently available from other large cancer data-sets, such as SEER (Surveillance Epidemiology and End Results, from the National Cancer Institute) and the National Cancer Database. [35-36] The Rabbit Health Systems data-set includes the type of HER2 test performed (IHC, FISH, or neither) and the results of the HER2 test, which is information not generally available in medical claims data-sets. Further, while most

medical claims data-sets include patients with one type of insurance (e.g. Medicare or private insurance), the Rabbit data-set includes patients with several types of insurance.

The entire data set contains information on 3348 breast cancer patients diagnosed from 1972 to 2008. Table 2.1 summarizes the information available in the data-set. Table 2.2 gives details about the six outpatient oncology practices that contributed to this data-set.

Table 2.1: Details of Data

Description	Fields
Demographics	1. Unique Patient ID 2. Date of birth 3. Zip code (first 3 numbers) 4. Diagnosis date 5. ICD9 Code 6. Diagnosis description 7. Primary insurance 8. Secondary insurance 9. Date of death (if applicable)
Diagnosis	1. Unique Patient ID 2. ICD9 Code 3. Date of diagnosis 4. Description of diagnosis 5. Level of diagnosis (primary, secondary)
Tumor Markers	1. Unique Patient ID 2. Date of test 3. Name of marker (ER, PR, HER2) 4. Status (Positive/Negative) 5. Test
Staging	1. Unique patient ID 2. ICD9 Code 3. Date of staging 4. Tumor Size (T) 5. Node (N) 6. Metastasis (M) 7. Tumor Grade
Targeted Therapy & Chemotherapy	1. Unique patient ID 2. Date of treatment 3. Drug name 4. Generic drug name 5. Dose 6. Units of dosage
Hormone Therapy	1. Unique patient ID 2. Medication name 3. Dose 4. Date of treatment
Vitals	1. Unique patient ID 2. Date of visit 3. Weight 4. Height 5. BP systolic 6. BP diastolic 7. Pulse 8. Respiratory rate 9. Temperature
Office Visit Information	1. Unique patient ID 2. Date of visit 3. Visit type (New patient, follow up) 4. ICD9 code and diagnosis description

Table 2.2: Details of Clinical Practices that Contributed to Data-set

Practice Code	Region	Number of Physicians
A	Los Angeles	2
B	West Texas	3
C	Washington State	6
D	South Dakota	1
E	Central Texas	3
F	South Texas	1

This study investigated whether certain factors are associated with the choice of test used for HER2 testing. Therefore, the study population included patients whose data had information on the type of HER2 test performed (IHC or FISH). Patients were excluded from analysis if the HER2 test field did not have any information or was indeterminate. Patients were also excluded from the analysis if values for any of the independent variables were missing or indeterminate. Based on this exclusion method, 1338 patients met the criteria for this study. Analyses were performed using multivariate logistic regression to model the impact of location, stage, date of diagnosis, insurance coverage, hormone status and age on the choice of HER2 test type. Multivariate logistic regression was selected due to its ability to serve as an effective technique in the analysis of the effect of covariates on a dichotomous dependent. [(23)] The dependent variable was dichotomous, coded as FISH HER test (=1) versus IHC HER2 test (=0). All analyses were performed using SPSS statistical software version 16.0 for Windows, Release 16.0.1.

2.3 Analysis

2.3.1 Preliminary Models

In preliminary models, the following independent variables were included:

- Location: coded as five nominal dichotomous variables, one variable each for Washington, Los Angeles, South Texas, Central Texas, and West Texas with the South Dakota location serving as the reference. South Dakota is selected as the reference since it is well defined (i.e., not defined as "other" or a miscellaneous category; a criterion which is satisfied by all location variables) and does not have the lowest number of cases (Los Angeles has the lowest number of patients with 50). Leaving out Los Angeles (the smallest location) and the West Texas (the largest location with 457 patients) to avoid comparisons with either extreme, any of the remaining three locations could have been used as the reference.
- Diagnosis Date: coded as a dichotomous variable which takes one of two values depending on whether a patient was diagnosed before or after publication of HER2 testing guidelines in 2001. Patients diagnosed in 2001 fell into the category of those diagnosed after publication of guidelines since these guidelines were published in early 2001 and were officially considered the 2000 update to previous guidelines.
- Age (continuous variable)
- Complete TNM staging (T variables – Tumor Size, N variables – lymph node invasion level, M variables– presence of distant metastasis, each coded as a set of ordinal dichotomies; for

example T is coded with 3 separate dichotomous variables T0, T1, T2 with T3/T4 serving as a reference)

- Insurance (Medicare, Medicaid, Medicare with supplemental Private insurance, Private or Uninsured). Insurance was divided into 4 separate nominal (dichotomous) variables with uninsured status being the reference group: Medicaid, Medicare (with or without secondary Medicaid), Medicare with supplemental private insurance, and Private Insurance.
- Estrogen Receptor (ER) status (nominal dichotomous variable), and Progesterone Receptor (PR) status (nominal dichotomous variable).

Age, insurance, N and M staging, and hormone status (which includes both ER and PR variables) were eventually removed from the model when no statistically significant association with the dependent variable was observed.

2.3.2 Final Model

A total of 1338 patients were included for the study (table 2.3). The final model consists of nine independent variables: five location variables, three T stage variables, and a variable which indicates whether initial breast cancer diagnosis was before or after publication of testing guidelines in 2001. Almost one-third (31.84%, or 426 out of 1338) of breast cancer patients in the data set were tested with FISH, over two thirds were tested with IHC (68.16%, or 912 out of 1338), and 26.31 % of patients (352 out of 1338) were HER2 positive. The independent variables in the final model (table 2.4) are *Loc ALosAngele*s, *LocBWestTexas, LocCWashington, LocECentralTexas, LocFSouthTexas*,

T0, T1, T2 and *DiagnosisDate*. With respect to the dependent variable, all interpretations are with respect to the higher category (HER2 Test = 1 = FISH).

Table 2.3: Final Model

Number of Patients	**Tested w/FISH**	**Tested w/IHC**	**From Location**	**Stage**	**Diagnosis Date**
1338	426 (31.84 %)	912 (68.16%)	A (Los Angeles) 50 (3.74%) B (West Texas) 457 (34.16%) C (Washington) 95 (7.10%) D (South Dakota) 137 (10.24%) E (Central Texas) 381 (38.48%) F (South Texas) 218 (16.29 %)	T0 65 (4.86%) T1 754 (56.35%) T2 407 (30.42%) T3/T4 112 (8.37%)	Before 2001 142 (10.61%) After 2001 1196 (89.34%)

Table 2.4: Variables in Final Model – Factors that Affect Choice of HER2 Test

Variable Name	Description	Type	Possible Values
HER2 Test	FISH or IHC (HER2 testing method)	Dependent Nominal Dichotomous Categorical	0 – IHC 1 – FISH
LocALosAngeles	Location of oncology practice	Independent Nominal Dichotomous (Reference: Loc D – S. Dakota)	0 – Not Location A 1 – Location A
LocBWestTexas	Location of oncology practice	Independent Nominal Dichotomous (Reference: Loc D – Midwest, S. Dakota)	0 – Not Location B 1 – Location B
LocCWashington	Location of oncology practice	Independent Nominal Dichotomous (Reference: Loc D – S. Dakota)	0 – Not Location C 1 – Location C
LocECentralTexas	Location of oncology practice	Independent Nominal Dichotomous (Reference: Loc D – S. Dakota)	0 – Not Location E 1 – Location E
LocFSouthTexas	Location of oncology practice	Independent Nominal Dichotomous (Reference: Loc D – S. Dakota)	0 – Not Location F 1 – Location F
T0	Indicator of whether tumor is in T0/Tis stage (TNM staging) Reference: T3/T4 stage	Independent Ordinal	0 – Not Tis/T0 1 – Tis/T0
T1	Indicator of whether tumor is in T1 (TNM staging) Reference: T3/T4 stage	Independent Ordinal	0 – Not T1 1 – T1
T2	Indicator of whether tumor is in T2 stage (TNM staging) Reference: T3/T4 stage	Independent Ordinal	0 – Not T2 1 – T2
DiagnosisDate	Indicator of whether disease diagnosis was before or after 2001	Independent Nominal Categorical	0 – Before 2001 1 – After 2001

2.4 Results

The results of the final model demonstrate that diagnosis date (before or after 2001), location and T value have a statistically-significant effect on the choice of HER2 test (see table 2.5 for output for logistic regression). The model is well-fitted with a Hosmer-Lemeshow Goodness of Fit statistic of 0.462 (table 2.6). This indicates that the null hypothesis (which states that there is no difference between observed values of the dependent variable and those values of the dependent variable that are predicted by the model) should not be rejected. In other words, the Goodness of Fit statistic indicates that the model's estimates fit the data at an acceptable level. [37] The Wald statistic for each of the significant effects (Location A – Los Angeles, Loc E – Central Texas, Loc F – South Texas, Stage T0, Diagnosis date) is large, showing that these variables are statistically significant predictors of the dependent variable (table 2.5).

Table 2.5: Logistic Regression Output

Variable	B	S.E.	Wald	Df	Sig.	Exp(B)	95.0% C.I.for EXP(B)	
	Logistic Coefficient					Odds Ratio	Lower	Upper
Loc A: Los Angeles	1.101	.396	7.721	1	.005	3.007	1.383	6.538
Loc B: West Texas	-.137	.298	.211	1	.646	.872	.486	1.564
Loc C: Washington	.476	.361	1.735	1	.188	1.609	.793	3.267
LocE: CentralTexas	3.023	.287	110.611	1	<.001	20.543	11.696	36.082
LocF: SouthTexas	.854	.298	8.219	1	.004	2.350	1.310	4.213
Stage T0	-1.373	.660	4.331	1	.037	.253	.070	.923
Stage T1	-.057	.255	.050	1	.823	.945	.573	1.557
Stage T2	.094	.269	.124	1	.725	1.099	.649	1.860
Diagnosed Before 2001	-1.154	.242	22.676	1	<.001	.315	.196	.507
Constant	-1.846	.344	28.808	1	<.001	.158		

Table 2.6: Goodness of Fit of the Model

Hosmer and Lemeshow Test

	Chi-square	df	Sig.
	7.708	8	.462

Patients in Loc E (Central Texas), Loc F (South Texas), and Loc A (Los Angeles) are significantly more likely to be tested with FISH than those in the reference location (D). The odds that a person in Central Texas (Location E) is tested with FISH are 20.543 times the odds that a person at the South Dakota site (Location D) is tested with FISH. The odds that a person in South Texas (Location F) is tested with FISH are 2.35 times the odds that a person in South Dakota (Location D) is tested with FISH. The odds that a person in Loc A (Los Angeles) is tested with FISH are also greater than the odds that a person in South Dakota is tested with FISH, by a factor of 3.007.

Patients who have Tis/T0 breast cancer are significantly less likely to be tested with FISH than those who are in the T3/T4 stages. The odds of a person in stage Tis/T0 breast cancer being tested with FISH are 0.253 times the odds of that of a person who is in stage T3/T4 being tested with FISH. The likelihood of being tested with FISH for patients with T1 and T2 disease are not significantly different from the likelihood among T3/T4 patients.

Patients diagnosed prior to the publication of HER2 guidelines in 2001 are less likely to be tested with FISH than those diagnosed after 2001. The odds of a person diagnosed before 2001 being tested with FISH is 0.315 times the odds that a person diagnosed after 2001 is tested with FISH.

These results indicate that location, diagnosis date, and T stage have an effect in the choice of HER2 test. Specifically, for patients considered in this study, those tested in Central Texas, South Texas, and Los Angeles were more likely to be tested with FISH than those in the Midwest. Also, with regard to diagnosis date, patients diagnosed after 2001 are more likely to be tested with FISH than those who were diagnosed before 2001. Patients tested for HER2 during the Tis/T0 stage of breast cancer are less likely to be tested with FISH than those in T3/T4 stage.

2.5 Discussion

Based on the statistical analysis performed, it is seen that location, diagnosis date and stage have significant effects on the choice of HER2 test. With diagnosis date, it is seen that those tested after 2001 were more likely to be tested with FISH than those tested prior to 2001, a result that that can be explained by the publication of major HER2 testing guidelines in 2001. The case with location and stage is more complex. Each effect is now considered in detail.

2.5.1 Location

With respect to location, patients tested in Central Texas, South Texas, and Los Angeles were more likely to be tested with FISH than those in Location D (South Dakota). Previous studies have explored the relationships between physician specialty, race/ethnicity, insurance and quality of care for women with breast cancer. [38-42] These studies suggest that African American women may not receive the same standard of care as do White women [40], although breast cancers in African American women tend to be more aggressive than those in White women. [41] No published studies identified to date have indicated differences in HER2 testing pattern based on the race/ethnicity of

patients with breast cancer, and information on race/ethnicity was not available in the data set for the current study. While it is likely that the proportions of African American women among the breast cancer patient populations in the Los Angeles and two Texas sites were greater than the proportion in the South Dakota site, it is difficult to speculate whether this contributed to the observed differences in type of HER2 test used. Further investigation of the racial and ethnic makeup of the various locations considered in this study could lead to more insights in this respect.

In a 2004 retrospective study of variables affecting HER2 testing, Stark et al. found that surgical oncologists were more likely to test for HER2. [38] This correlates with greater survival of breast cancer patients when treated by surgical oncologists. [42] While the data set used in this research contained information on the number of physicians in each practice, the specialty practice area of each physician was not provided. The specialty area and associated training of the physicians in the clinics considered could have influenced the choice of HER2 test and is worth examining in future studies.

2.5.2 Diagnosis Date

HER2 testing patterns during and after 2001 are significantly different than prior to 2001; this effect can be explained by the publication of major HER2 testing guidelines in 2001.[43] The relevant segment from the 1997 guideline and the 2001 update is reproduced below:

"1997 Recommendation: Present data are insufficient to recommend the use of c-*erb*B-2 (*HER-2*/*neu*) gene amplification or overexpression for management of patients with breast cancer.

2000 Recommendation: c-*erb*B-2 overexpression should be evaluated on every primary breast cancer either at the time of diagnosis or at the time of recurrence. Measures of c-*erb*B-2 amplification may also be of value." [43]

This recommendation unequivocally confirms that all primary breast cancer patients must be tested for HER2. Around the time of this publication in early 2001, several studies appeared regarding the superiority of FISH in terms of accuracy and correlation with clinical outcomes. [6, 11-12, 15, 19-20, 24] When the recommendation of the 2001 guidelines is considered along with the observation of various major publications regarding FISH being the better test, it appears likely that a shift towards using FISH began to occur – an effect we see in the present analysis.

2.5.3 Stage

TNM staging (Tumor size, Node and Metastasis) information was provided in the data set used for this research. In preliminary models it was seen that N and M values were not significantly associated with the choice of HER2 test; these factors were subsequently removed from the model. The T stage, however, has a significant association, with patients with lower T stage (i.e., small tumors) being less likely to receive FISH testing. Tis/T0 tumors maybe Lobular Carcinoma In Situ (LCIS, also known as Lobular Neoplasia; technically a non-cancerous and non-invasive condition which increases the risk of developing cancer in the future), Ductal Carcinoma In Situ (DCIS) or Stage IIA invasive breast cancer, depending on certain factors, including T, N and M values of the tumor [44]. For LCIS and DCIS tumors, the T, N and M values are Tis, N0 and M0 respectively, while for stage IIA these values are T0, N1 and M0. In the present study, all Tis/T0 tumors included in the analysis except for one (which was stage IIA breast cancer), were LCIS or DCIS. Currently, NCCN guidelines do not specify

assessment of HER2 status among LCIS or DCIS patients. Furthermore, the value of HER2 testing in DCIS cases remains uncertain, with some studies finding a link between disease progression and/or recurrence and HER2 status in DCIS, while other studies showing no such associations [45 - 48]. For example, one particular study reported an association between HER2 gene amplification in DCIS tumors and the expression of specific genes linked with suppression of apoptosis, another study implicated HER2 signaling in early breast tumorigenesis, while a third study found that HER2 does not play a major role in transformation of DCIS to invasive ductal carcinoma [46-48] Further examination regarding differences in outcomes for women with DCIS who are tested and treated (if appropriate) for HER2 positive breast cancer is required but at this time, the value of HER2 testing (with FISH or IHC) for DCIS remains largely unknown. The same is true for LCIS. At this time, with the value of HER2 testing for these In Situ tumors not having been clearly established, the results of this study show that physicians choose to test DCIS and LCIS cases with the older, quicker and less expensive test (IHC).

2.6 Conclusion

Analysis of factors associated with choice of HER2 test shows that location, diagnosis date (before or after 2001) and T-stage have significant effects on whether FISH or IHC is selected. Patients receiving treatment at the Central Texas, South Texas, and Los Angeles study sites are more likely to be tested with FISH than patients treated at the South Dakota site. Patients tested after the 2001 publication of updated guidelines were more likely to be tested with FISH – an effect explained by the emphasis of the guidelines that all breast cancer patients must be tested for HER2 and the publication since of numerous studies advocating the use of FISH over IHC. Finally, patients with Carcinoma In Situ (T0/Tis) of the breast are less likely to be tested with FISH, probably

to due the lack of evidence available regarding the value of HER2 testing in In Situ tumors. Further studies on the relationships between race/ethnicity of patients, physician specialties and HER2 testing sites, In Situ tumors and the benefit (if any) of HER2 testing and suitable follow-up treatment, are needed to provide more clarity regarding decision making in HER2 testing.

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Chapter 3

Factors that Affect Treatment with Trastuzumab in Breast Cancer Patients

3.1 Introduction

HER2 breast cancer is an aggressive form of the disease associated with increased tumor aggressiveness and poor prognosis. [1-2] Gene amplification of HER2 with subsequent protein overexpression has been shown to exist in 20 % to 30% of invasive breast cancers. [1-6] While HER2 breast cancers are prevalent in one fourth of the breast cancer population, the availability of targeted therapy for these cancers has provided significant benefit in terms of health outcomes. Trastuzumab (Herceptin®) is a humanized monoclonal antibody specifically indicated for treatment of HER2 positive breast cancer. HER2 status is the only factor currently specified by the FDA as being relevant in the decision regarding whether trastuzumab is prescribed or not. This study determines whether other factors, clinical and non-clinical, influence the prescription of this drug.

The American Cancer Society estimated that in 2008 over 180,000 women would be diagnosed with breast cancer, but also found that the rates of death from breast cancer have declined due to earlier detection and better treatment. [7] In addition to determining tumor stage and grade, optimal treatment involves determination of tumor characteristics such as estrogen and progesterone receptor status as well as HER2 status. Trastuzumab may be prescribed as adjuvant treatment for node-negative or node-positive breast cancer that has been determined to be HER2 positive. [8] It may also be

prescribed as primary single agent treatment or in combination with taxol (for patients who have previously received chemotherapy regimens) for metastatic breast cancer. [8]

Trastuzumab works synergistically with chemotherapy in HER2 overexpressing cancer cells by selectively binding to the extra-cellular domain of the HER2. [7, 9] Through this binding, trastuzumab stops the HER2+ cancer cell from continuing its proliferation. [10] In addition, the human immune response recognizes and destroys trastuzumab coated cells. [10] While trastuzumab has significant benefits for HER2 positive breast cancer patients it is associated with elevated risks of serious adverse events and with substantial monetary costs. Trastuzumab increases the risk of developing ventricular dysfunction and congestive heart failure. [11-12] Thus, to ensure appropriate use of this medication for breast cancer treatment, testing for HER2 is of critical importance. There are currently two main types of tests used to assess levels of HER2 in breast cancer: Fluorescence in Situ Hybridization (FISH) and Immunohistochemistry (IHC).

A study by UnitedHealth Group, a large private insurance organization, found that 12% of patients treated with anti-HER2 therapy were not positive for HER2 or were not tested for HER2 at any point. [13] This led to UnitedHealth Group requiring that HER2 status be reported in trastuzumab claims. Such a discovery opened the door to an important question: Do other factors, including non-clinical issues such as insurance status and/or clinical markers, play a role with regard to prescription of trastuzumab? How do these factors play a role, and what is their relative importance? Understanding the relationship between these factors and the prescription of trastuzumab therapy will serve as a foundation in improving quality of care given to breast cancer patients, by ensuring that anti-HER2 therapy is provided appropriately, as indicated.

3.2 Materials and Methods

The data utilized for this research was provided by The American Cancer Society. The data was collected by Rabbit Healthcare Systems (Austin, TX). The data includes information on patients diagnosed from 1972 to 2008 in 6 outpatient oncology practices in the United States. The entire data set contains information on 3348 invasive breast cancer patients collected from each visit made by patients to the physician's office during this time. The subset of data used for this particular study consists of 4 files, each containing categorical data. A detailed view of the content of the relevant files utilized for this study is provided in Table 3.1. In addition, Table 3.2 gives details about the six outpatient oncology practices that contributed to this data-set.

The study population included those patients who received some form of chemotherapy (such as taxol or carboplatin) and/or targeted therapy (trastuzumab). Patients were excluded from the analysis if the values for any of the independent variables were missing or indeterminate. Patients who were diagnosed before the first FDA approval of trastuzumab in September 1998 were also excluded from the analysis since the drug was not available outside the clinical trial setting before this time. Based on these criteria, 683 cases were included for the study. Multivariate logistic regression was used to model the impact of location, stage, diagnosis date, insurance coverage, hormone status, HER2 status, triple negative status, HER2 test and age on whether anti-HER2 therapy (trastuzumab) is given or not . Logistic regression is selected since it serves as an efficient technique when dealing with a dichotomous dependent variable. [(14)] SPSS statistical software version 16.0 was used for analysis.

Table 3.1: Details of Data Files

File Number	Description	Fields
1	Demographics	10. Unique Patient ID 11. Date of birth 12. Zip code (first 3 numbers) 13. Diagnosis date 14. ICD9 Code 15. Diagnosis description 16. Primary insurance 17. Secondary insurance 18. Date of death (if applicable)
2	Tumor Markers	6. Unique Patient ID 7. Date of test 8. Name of marker (ER, PR, HER2) 9. Status (Positive/Negative) 10. Test
3	Staging	10. Unique patient ID 11. ICD9 Code 12. Date of staging 13. Tumor Size (T) 14. Node (N) 15. Metastasis (M) 16. Tumor Grade
4	Targeted Therapy & Chemotherapy	7. Unique patient ID 8. Date of treatment 9. Drug name 10. Generic drug name 11. Dose 12. Units of dosage

Table 3.2: Details of Clinical Practices that Contributed to Data-set

Practice Code	Region	Number of Doctors
A	Los Angeles	2
B	West Texas	3
C	Washington State	6
D	South Dakota	1
E	Central Texas	3
F	South Texas	1

3.2.1 Preliminary Models

The dichotomous dependent variable in this study is *Trastuzumab*, which takes on a value of 1 if trastuzumab is given and 0 if trastuzumab is not given. In preliminary models, the following independent variables were included for the analysis:

- *HER2 Test:* FISH or IHC, coded as a nominal dichotomous variable.
- Hormone Status: two variables - Estrogen Receptor (*ER*) status (nominal dichotomous variable), and Progesterone Receptor (*PR*) status (nominal dichotomous variable).
- *Triple Negative Status*: indicates if a patient is ER-, PR- and HER2-, coded as a nominal dichotomous variable.
- TNM Stage Variables: specifies complete TNM staging : *T* – Tumor Size, *N* variables – lymph node invasion level, *M* variables– presence of distant metastasis, each coded as a set of ordinal dichotomies; for example T is coded with 3 separate dichotomous variables T0, T1, T2 with T3/T4 serving as a reference.
- *DiagnosisDate:* dichotomous variable which indicates whether patients were diagnosed before or after publication of major HER2 testing guidelines in 2001.[15]
- *Age* (continuous variable)
- *Location:* coded as five nominal dichotomous variables, one variable each for Washington State, Los Angeles, South Texas, Central Texas, and West Texas with the South Dakota site serving as the reference. Any of the location variables would have served as a suitable reference since all are well defined (i.e. not defined as “other”) and comparisons with any of them would be meaningful (as opposed to comparing with a miscellaneous category). South Dakota was

selected since geographically, it is the most different from the remainder of the categories (the others all being on the West coast or in Texas) which serves as a concrete reference.

- *Insurance*: divided into the following 4 separate nominal dichotomous variables with Private insurance status serving as the reference: Medicaid, Medicare (with or without secondary Medicare), Medicare with supplemental private insurance, and Uninsured. Private insurance status is chosen as the reference since it is well defined and is not the smallest insurance category.
- *HER2 Status*: A nominal variable which takes on one of three values depending on whether the HER2 status of the patient is unknown, negative or positive. Unknown HER2 status indicates that HER2 status information was not obtained for the patient, i.e., the patient was not tested for HER2.

HER2 test, ER status, PR status, Triple Negative Status, TNM staging variables, DiagnosisDate and Age were removed from the model when no statistically significant associations with the dependent variable was observed.

3.2.2 Final Model

The final model included 683 patients (table 3.3) and consists of the following 10 dependent variables:

- *LocALosAngeles*
- *LocBWestTexas*
- *LocCWashington*
- *LocECentralTexas*
- *LocFSouthTexas*

- *NoInsurance*
- *Medicaid*
- *Medicare* (Alone or with secondary Medicaid)
- *MedicarewithPriv*
- *HER2 Status*

As in preliminary models, with respect to location, Location D (South Dakota) is treated as the reference and with respect to insurance, Private insurance is treated as the reference.

From the 683 included patients, 23.69% were given trastuzumab while 27.38% were determined as HER2 positive (by either FISH or IHC). 19.18% of the 683 patients were HER2 positive and received trastuzumab, while 2.05% of patients were HER2 negative and treated with trastuzumab. Of the 683, 1.46% had unknown HER2 status and were given trastuzumab.

Table 3.3: Final Model

Number of Patients	Treated with trastuzumab	Not treated with trastuzumab	From Location	Insurance	HER2 Status
683	155 (22.69%)	528 (77.31%)	A (Los Angeles) 76 (11.13 %) B (West Texas) 187 (27.38 %) C (Washington) 99 (14.49 %) D (South Dakota) 67 (9.81%) E (Central Texas) 130 (19.03 %) F (South Texas) 124 (18.16 %)	Uninsured 14 (2.05%) Medicaid 72 (10.54 Medicare *(alone or w/Medicaid)* 47 (6.88 %) Medicare *(w/Private)* 144 (21.08 %) Private 406 (59.74%)	Unknown 138 (15.52.%) Negative 390 (57.10%) Positive 187 (27.38%)

3.3 Results

The final model shows that location and HER2 status have significant effects on whether trastuzumab therapy is given or not (see table 3.4). In addition to these two factors, we see a non-significant trend of patients with Medicare and supplemental private insurance not receiving trastuzumab.

The Hosmer-Lemeshow Goodness of Fit test (see table 3.5) for the model has a p-value of 0.291. Since this value is greater than 0.05, it indicates that the null hypothesis (which states that there is no difference between observed values and those values that are predicted by the model) should not be rejected and that the model is well fitted.

Table 3.4: Logistic Regression Output

Variable	B Logistic Coefficient	S.E.	Wald	Df	Sig.	Exp(B) Odds Ratio	95.0% C.I.for EXP(B)	
							Lower	Upper
LocALosAngeles	-.200	.544	.136	1	.713	.818	.282	2.377
LocBWestTexas	-.742	.484	2.349	1	.125	.476	.184	1.230
LocCWashington	-.444	.526	.715	1	.398	.641	.229	1.796
LocECentralTexas	-.674	.511	1.739	1	.187	.510	.187	1.388
LocationFSouthTexas	-1.354	.550	6.062	1	.014	.258	.088	.759
NoInsurance	-20.545	9427.791	.000	1	.998	.000	.000	.
Medicaid	-.462	.465	.984	1	.321	.630	.253	1.569
Medicare	-.849	.599	2.006	1	.157	.428	.132	1.385
MedicarewithPriv	-.693	.368	3.543	1	.060	.500	.243	1.029
HER2Status			190.562	2	.000			
HER2Status Unknown	-2.937	.393	55.853	1	.000	.053	.025	.115
HER2Status Negative	-4.228	.325	168.952	1	.000	.015	.008	.028
Constant	1.751	.446	15.451	1	.000	5.763		

Table 3.5: Hosmer and Lemeshow Test

Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	9.641	8	.291

In addition, the Wald statistic for each of the significant covariates is large, confirming that these variables indeed have significant effects on the dependent variable (see table 3.4).

Examination of the odds ratio in Table 3.4 shows that patients treated in Location F (South Texas) are less likely to be given trastuzumab than those treated in Location D (South Dakota). Specifically, the odds that a person at the South Texas site (Location F) is given trastuzumab are 0.258 times the odds that a person at the South Dakota site (Location D; reference) is given trastuzumab. With respect to HER2 status, patients who have unknown or negative HER2 status are less likely to be given trastuzumab than those who are HER2 positive. The odds that a HER2 unknown patient is given trastuzumab are 0.053 times the odds that a HER2 positive patient is given trastuzumab. Similarly, the odds that a HER2 negative patient is given trastuzumab are 0.015 times the odds that a HER2 positive patient is given trastuzumab. Alternatively, this may be stated as follows: Being HER2 positive significantly increases a patient's odds of receiving trastuzumab. From the output of the logistic regression, it is also seen that there is a trend of patients with Medicare and supplemental private insurance not receiving trastuzumab, but since the p value is greater than 0.05, this represents a non-significant trend. The cause for this trend being non-significant may be due to limited statistical power.

3.4 Discussion

Treatment for HER2 breast cancer should follow confirmation of positive HER2 status using Florescence In Situ Hybridization or Immunohistochemistry. Official guidelines provided by Genentech, the manufacturer of Herceptin, state that this drug should only

be used in patients who have demonstrated HER2 overexpression. [8, 16] Determination of HER2 status prior to prescription of trastuzumab is critically important not only due to the benefit of this therapy for HER2 positive tumors, but also due to the potential for serious side effects of this therapy for all those who receive it. The risk of side effects of trastuzumab, which include cardiac dysfunction, pulmonary toxicity (such as pleural effusions and pulmonary edema), infection, infusion reactions (which may be mild or life-threatening depending on the patient), neutropenia, and anemia remain for all patients who receive trastuzumab, irrespective of their HER2 status. [8] A retrospective analysis that assessed cardiac dysfunction rates due to trastuzumab treatment from records of patients enrolled in any one of seven phase II and phase III trials for trastuzumab conclusively determined that the drug is associated with an increased risk of cardio-toxicity. [11] The risk for cardiac dysfunction, as assessed from these seven clinical trials, was as follows: 27% of patients receiving trastuzumab with anthracycline and cyclophosphamide, 13% of patients receiving taxol and trastuzumab and 3% to 7% of patients receiving trastuzumab as a single agent experienced cardiac dysfunction. [11] In comparison, 1% of patients receiving taxol as a single agent experienced cardiac dysfunction. [11] The severity and frequency of serious side effects underscores the importance of adhering to prescription guidelines for trastuzumab.

HER2 positive status is currently the only requirement for receipt of trastuzumab therapy. The results of this study show that patients who are HER2 positive are significantly more likely to receive trastuzumab than those who are HER2 negative or whose HER2 status is unknown. Although this analysis shows that trastuzumab is widely prescribed only for HER2 positive tumors, further analysis is needed to determine why it is prescribed (even in small numbers) to those who are HER2 negative (or whose HER2 status is unknown). In all locations, not all patients who received trastuzumab were

HER2 positive (table 3.6). Follow up is required with non-HER2 positive patients who have been given trastuzumab particularly in light of studies reporting benefit from trastuzumab therapy among HER2 negative patients. [17-18] If the clinical benefit of trastuzumab is established for HER2 negative tumors, re-assessment of trastuzumab prescription protocols would be required.

Table 3.6: Trastuzumab and HER2 Information by Location

Note: All percentages are expressed in terms of percent of total number of patients in that location.

Location	**Total Number**	**HER2+**	**Total Given Trastuzumab per location**	**HER2 + & Given Trastuzumab per location**
Location A (Los Angeles)	76	23 (30.26%)	23 (30.26%)	20 (26.31%)
Location B (West Texas)	187	50 (26.73%)	36 (19.25%)	29 (15.96%)
Location C (West Texas)	99	29 (29.29%)	25 (25.25%)	23 (23.23%)
Location D (South Dakota)	67	21 (31.34%)	21 (31.34%)	17 (25.37%)
Location E (Central Texas)	130	45 (34.62%)	37 (28.46%)	36 (27.69%)
Location F (South Texas)	124	19 (15.32%)	13 (10.48%)	6 (4.8%)

From the present analysis, it is seen that patients treated in South Texas (Location F) are less likely to receive trastuzumab than those treated at the South Dakota site (Location D; reference location). The current data set does not have race/ethnicity or socioeconomic information, which may be some of the underlying reasons behind the location effect. It is likely that the Texas and Los Angeles location have greater Black and Non-Hispanic White population but it is difficult to speculate further about what this result means. However, it does lead us to conclude that there is the possible existence of disparities based on location. Further studies are required to conclusively determine the cause of these differences. From the available data, it is clear that fewer patients presented with HER2 positive status in Location F than in any of the other sites, a factor that influences trastuzumab prescription (table 3.6). However, not all HER2 positive patients in Location F (or in any of the locations) were given trastuzumab. This could be due to one or more of the following: medical reasons (such as infusion reactions at the time of initial administration of the drug or pre-treatment cardiac illness which would preclude patients from receipt of trastuzumab therapy) or patient refusal. Patient refusal to begin or continue trastuzumab therapy has been documented [18, 19] and could be due to financial reasons. While private insurance plans will cover trastuzumab for HER2 positive breast cancer patients in node negative and node positive cancers or in metastatic breast cancer [20], the high cost of trastuzumab (over $3000 per month) may push the yearly coverage maximum for some patients above their insurance plans. The cost of trastuzumab treatment is a serious of an issue for all patients. However, some relief is provided by Genentech, which pays costs towards trastuzumab for uninsured and underinsured patients in the United States in certain cases (after ineligibility for all forms of public insurance are confirmed by the company) through the Genentech Access to Care Foundation. [21-22] If Genentech will pay for trastuzumab treatment in the United States, the question arises as to why a non-significant trend is seen with regard to

uninsured patients being less likely to receive the drug. It has been shown that patients covered by Medicare do not routinely undergo HER2 testing that and clinical judgment may replace histological findings when it comes to trastuzumab use among Medicare patients and this scenario may be extendable to uninsured patients. [23] In addition, Medicare covers costs of trastuzumab only for metastatic breast cancers and not for adjuvant treatment of node-negative or node-positive non-metastatic breast cancer. [23-24] This could be the reason we see a non-significant trend for some Medicare patients (those with supplemental private insurance). It is likely that a similar effect is not seen for other non-private insurance groups due to lack of power.

To further understand why all HER2 positive patients are not given trastuzumab and why geographic location has a significant effect on the prescription of this drug, more extensive analysis of insurance coverage and detailed analysis of patient adherence/refusal patterns with regard to treatment are required. In addition, analysis of race, ethnicity and socioeconomic status of patients could provide insight into whether these factors play a causal role with regard to the effect of location and insurance on trastuzumab prescription. Currently, oncology clinics and hospitals need to evaluate their prescription patterns of anti-HER2 therapy to ensure that they are adhering to guidelines. In particular, if anti-HER2 therapy is prescribed for non-HER2 positive patients (or if anti-HER2 therapy is not prescribed for HER2 positive patients), follow-up with the prescribing provider should be required to determine rationale.

3.5 References

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Chapter 4

Cost-Effectiveness Analysis of Fluorescence in Situ Hybridization and Immunohistochemistry for HER2 Testing in Adjuvant Treatment of Breast Cancer

4.1 Introduction

Breast cancer is a leading cause of cancer-related deaths worldwide. However, rates of death from breast cancer are declining due to earlier detection and better treatment methods. [1] One such treatment is trastuzumab (Herceptin®). It is a targeted therapy indicated for the treatment of breast cancers that exhibit HER2 amplification and/or protein overexpression. Accurate detection of HER2 status is critical for appropriate planning of breast cancer therapy since 20 to 30% of breast cancers are HER2 positive, and this is associated with increased tumor aggressiveness and poor prognosis [2 - 7]. Testing for HER2 status is a topic of debate due to the issue of accuracy of available HER2 tests as well as the high cost of subsequent anti-HER2 therapy. In this study, we examine the cost effectiveness of FDA approved HER2 testing methodologies by considering test accuracy, health outcomes and financial costs.

Trastuzumab has been shown to be cost-effective in the adjuvant treatment of HER2 breast cancer. [8] Trastuzumab has significant benefits for HER2 positive breast cancer patients; however, it is associated with elevated risks of serious adverse events and with substantial costs. While recent studies have determined some benefit of trastuzumab for HER2 negative patients, this requires further examination. [9] Current guidelines for treatment specify that tumor samples must be HER2 positive to receive benefit from trastuzumab. Therefore, to ensure appropriate use of this medication for breast cancer treatment, testing for HER2 is of critical importance.

There are currently two main types of tests used to assess levels of HER2 in breast cancer: Fluorescence in Situ Hybridization (FISH) and Immunohistochemistry (IHC). FISH measures HER2 gene amplification while IHC measures levels of HER2 protein to determine if overexpression is present. There are six FDA-approved FISH and IHC tests for HER2 diagnosis in the United States: PathVysion (FISH, Vysis Inc.), INFORM (FISH, Ventana Medical Systems), FISH PharmDx (FISH, Dako), InSite (IHC, BioGenex Laboratories, Inc), HercepTest (IHC, Dako), and Pathway (IHC, Ventana).

IHC and FISH vary in terms of cost and accuracy. A number of published reviews have evaluated HER2 testing. [10-27] These studies show high levels of variability with IHC results, while FISH is more reliable and serves as a gold standard for testing. [18-27] IHC protocols between laboratories vary in terms of the laboratory experience, score interpretations, and antibodies used for testing. [14-23] IHC for HER2 testing is scored on a scale from 0 (negative result) to 3+ (positive result). When IHC results are intermediate (indicated by a score of 2+), literature shows that they cannot be reliably used to determine a cancer's HER2 status. [13, 19, 24-27] The strengths of IHC are that it is less expensive than FISH and it requires less laboratory personnel time and expertise. [15, 23] With regard to clinical outcomes, FISH testing is associated with better response rates with anti-HER2 therapy. [28-31]

FISH is a more expensive test then IHC. The average cost per test has been reported to be approximately $482 for FISH versus $89 for IHC. [8] The costs of performing the tests relative to effectiveness of the tests (e.g., the accuracy and value of results for IHC vs. FISH) determine the cost-effectiveness of HER2 testing. However, few cost-effectiveness analyses of HER2 testing have been performed, and the results of these

analyses are not in agreement. Based on their cost effectiveness analysis, Elkin et al. concluded that FISH should be the primary method for HER2 testing [32]. In contrast, Falo et al. concluded that the ideal testing algorithm would involve using FISH only in those cases where FISH analysis is absolutely necessary and to use IHC (with both a monoclonal and polyclonal antibody) as the primary method for HER2 detection. [33] In a study comparing FISH (using the Oncor/Ventana INFORM kit) and IHC (using a polyclonal antibody for HER2 detection) Jacobs et al. concluded that based on the high level of correlation (91%) between the two assays, the higher cost of FISH, and the longer times needed for this test, routine use of FISH is not justifiable. [34] Cost-effectiveness studies which include the adverse health outcomes as well as financial costs involved in treating a patient falsely diagnosed as HER2-positive are needed in order to obtain a complete understanding of the two testing methods. The present study compares IHC and FISH while accounting for health and monetary costs associated with outcomes of unnecessary and potentially incorrect treatment. In addition, a no-test scenario where, hypothetically, all breast cancer patients are treated with trastuzumab is also presented for comparison.

4.2 Materials and Methods

Models for IHC, FISH, and No Test scenarios, complete with relevant testing costs and details of subsequent treatment and related health outcomes are modeled using TreeAge Pro Suite 2009 decision analysis software (figures 4.1-4.3). Deterministic analysis using point estimates and probabilistic sensitivity analysis using Monte Carlo simulations are performed in TreeAge. Costs of treatment and testing as well as probabilities for use in the models are obtained from literature. [8, 32, 35 - 38] We utilized Quality Adjusted Life Years (QALYs) in the model. QALYs are a measure of the quality

and quantity of life years gained for a specific intervention. [39] They are widely used in health economics research because they provide a way to compare interventions across diseases; that is, this measure is not disease specific. There are two main components to QALYS: a measure of quality of life (known as health utility) and a measure of life years gained (measured in years). A year in perfect health has a health utility value of 1, with other states having utility values between 0 and 1. [39] Death is considered to have a value of 0 for utility. [39] This utility value is multiplied by the number of years gained by the intervention in order to obtain a QALY value. We assume that the quality of life of patients on trastuzumab therapy does not vary significantly from the quality of life for patients on chemotherapy. We made this assumption since in addition to responses from patients in the trastuzumab randomized trial, which found no significant differences between quality of life on chemotherapy and on trastuzumab therapy, an earlier study has also made this assumption. [32, 40-41] With this assumption and QALY estimates from previous studies, QALY values are assigned to the various branches in the models. We compared QALY values gained in each of the scenarios (No Test, IHC, FISH) in order to arrive at a conclusion regarding which strategy is most cost effective.

The No Test model considers the scenario wherein neither FISH nor IHC are used for HER2 testing and all breast cancer patients are treated with adjuvant trastuzumab without HER2 testing (figure 4.1). The IHC and FISH models consider a newly diagnosed breast cancer patient being tested for HER2 (with either IHC or FISH; figures 2 and 3). Numerous studies have established that 20 – 30% of breast cancers are HER2 positive, and this is reflected in both models as the Actual HER2+ rate. [2-7] The sensitivity and specificity of the tests are reflected as true positive and true negative rates in the figures. Depending on the outcome of the test, the patient is offered adjuvant treatment with trastuzumab. A majority of patients with HER2 positive test

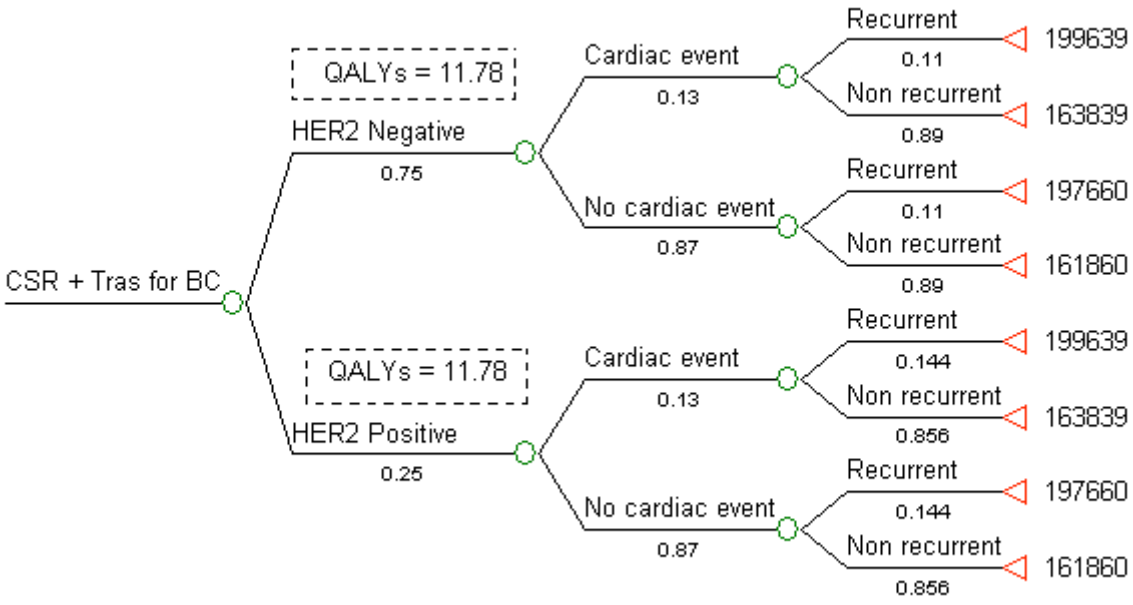
results (true positives and false positives) undergo anti-HER2 treatment with trastuzumab in addition to chemotherapy, but some do not, as shown in the model. The probability of receiving trastuzumab therapy based on HER2 test results was computed using a data-set containing information on breast cancer patients, testing, and treatment (provided by the American Cancer Society; collected by Rabbit Health Systems, Austin, TX). Data was collected from six outpatient oncology clinics located across the United States. Out of 746 patients considered from this data-set, 199 were HER2 positive and 140 were HER2 positive and treated with trastuzumab. Therefore, patients who are found to be HER2+ have a probability of 0.7 of being treated with trastuzumab. This is shown in figures 4.2 and 4.3 under the "Trastuzumab" treatment branches for true and false positives. Treatment with trastuzumab carries risk of adverse cardiac-related side effects which is also incorporated into the model. Recurrence rates for each branch of treatment are shown in the model as obtained from literature. [8, 35]

Costs associated with each stage of testing, treatment and management of disease are incorporated into the model. With respect to HER2 testing, the cost of FISH is $482 per test while IHC costs $89 per test. [8] With regard to treatment, the cost of trastuzumab therapy is $126511, in addition to the cost of chemotherapy. All patients are assumed to receive some form of therapy (i.e., chemotherapy, radiation or surgery) either alone or in addition to trastuzumab. Treatment with chemotherapy, radiation or surgery alone is estimated at $31,149. [8, 36] The cost of treatment for recurrent and non-recurrent disease as well as the cost of management for cardiac dysfunction associated with trastuzumab are included in the model and are based on estimates from previous analyses. [8, 37]

Monte Carlo simulations are used to analyze the effect of uncertain parameters in the models. Specifically, in this study, Monte Carlo simulations are used to determine the

effect of varying false positive and false negative probabilities on the overall model. Numerous studies have established that IHC false positives are a major source of variability when using this test for HER2 and that these rates vary with the particular IHC test that is selected for use. [3, 16, 18-19, 24, 42-44] Therefore, understanding how the IHC model performs under varying false positive probability is of interest. The Beta distribution, a continuous probability distribution, lends itself well to modeling probabilities as it is defined in the interval [0, 1]. In this analysis, for both the FISH and IHC trees, the false positive probability is varied using a Beta distribution to model the probability values, and the associated α and β values for this distribution are assigned using the expected value for the variable obtained from literature. [32]

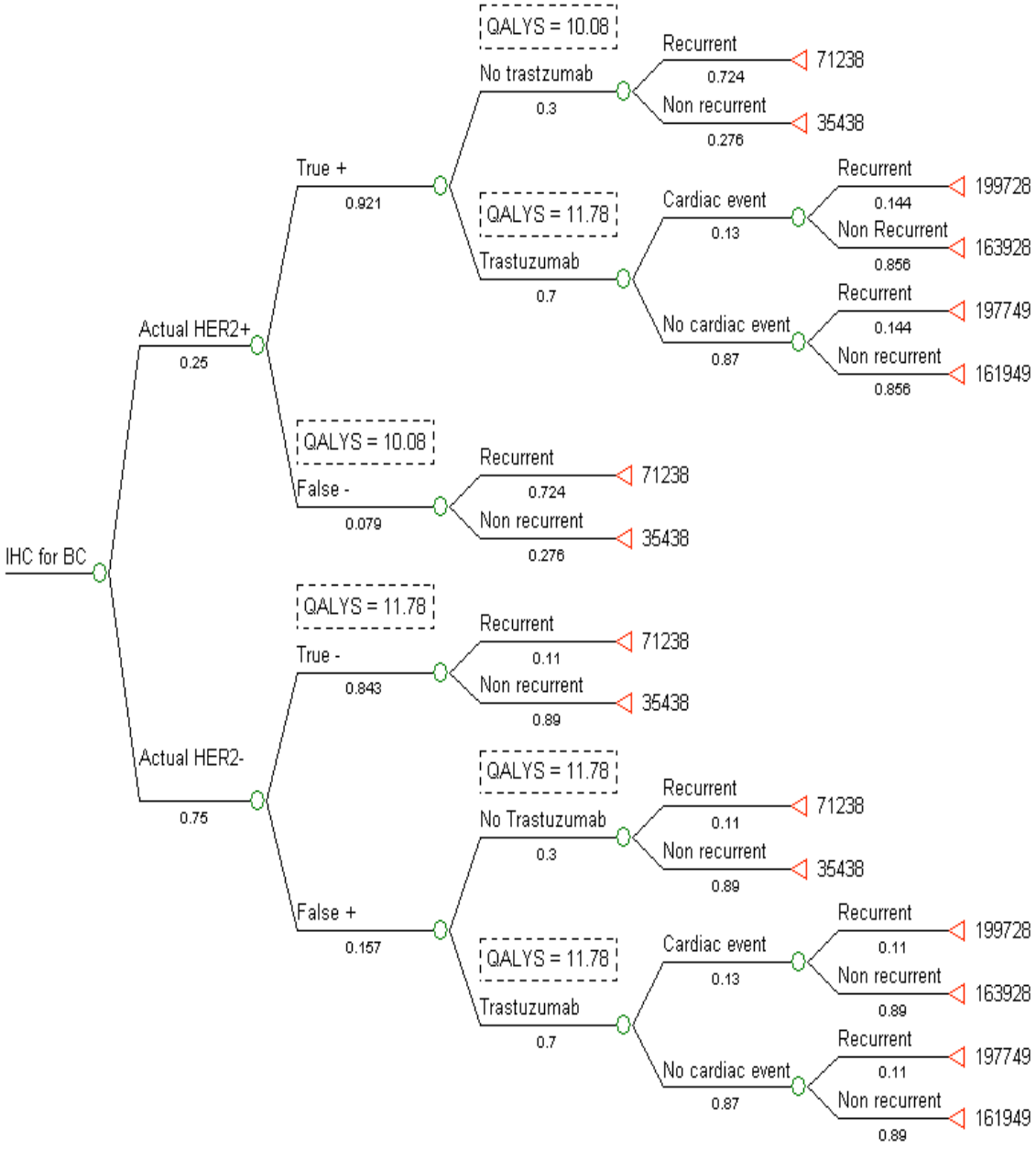
Figure 4.1: No HER2 Test Scenario



Key:

- CSR + TRAS for BC = Chemotherapy/Surgery/Radiation with Trastuzumab for Breast Cancer
- QALYs = Quality Adjusted Life Years associated with the nearest node to the right of box
- Payoff values at terminal nodes are in dollars
- Values below branches are probabilities

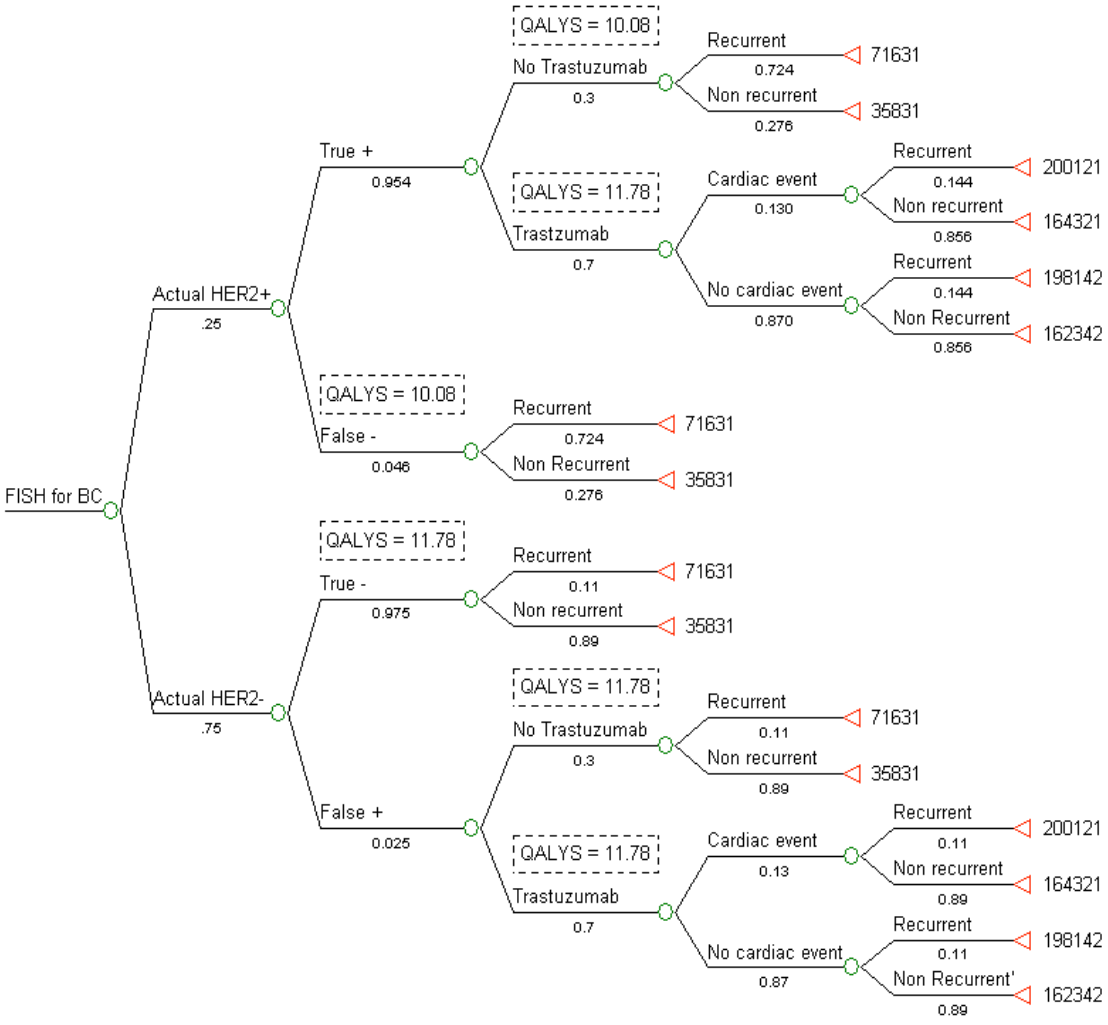
Figure 4.2: IHC for HER2 Testing in Breast Cancer – A Model



Key:

- IHC for BC = Immunohistochemistry for Breast Cancer
- QALYs = Quality Adjusted Life Years associated with the nearest node to the right of box
- Payoff values at terminal nodes are in dollars
- Values below branches are probabilities

Figure 4.3: FISH for HER2 Testing in Breast Cancer – A Model



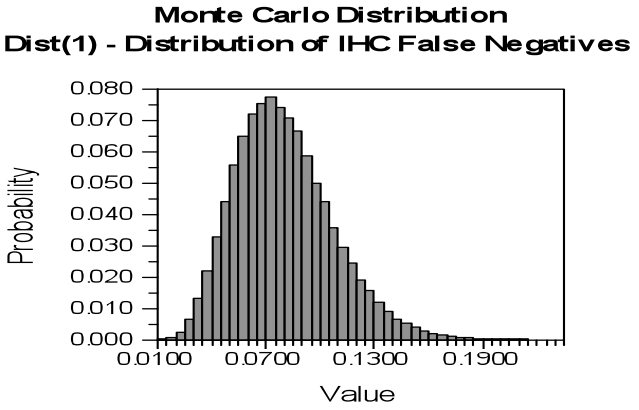
Key:

- FISH for BC = Fluorescence In Situ Hybridization for Breast Cancer
- QALYs = Quality Adjusted Life Years associated with the nearest node to the right of box
- Payoff values at terminal nodes are in dollars
- Values below branches are probabilities

Similarly, the false negative probability is modeled using a Beta distribution.

The input distributions are shown in figures 4.4-4.5. Monte Carlo probabilistic sensitivity analysis is applied in order to determine the behavior of the model under these parameter uncertainties.

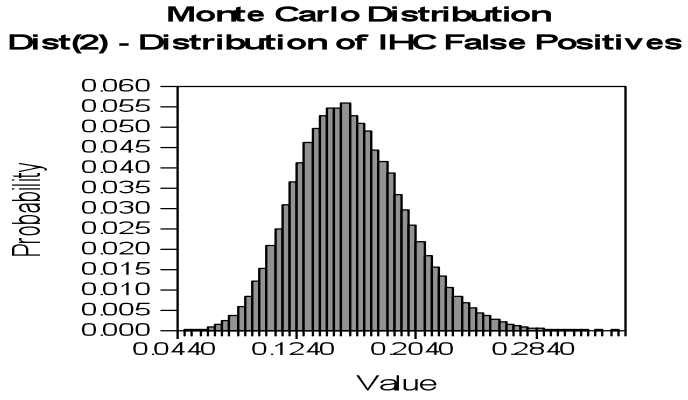
Figure 4.4a: Input Beta Distribution for IHC False Negatives



Key:

- Beta distribution: α = 8, β = 92, Expected Value = 0.08

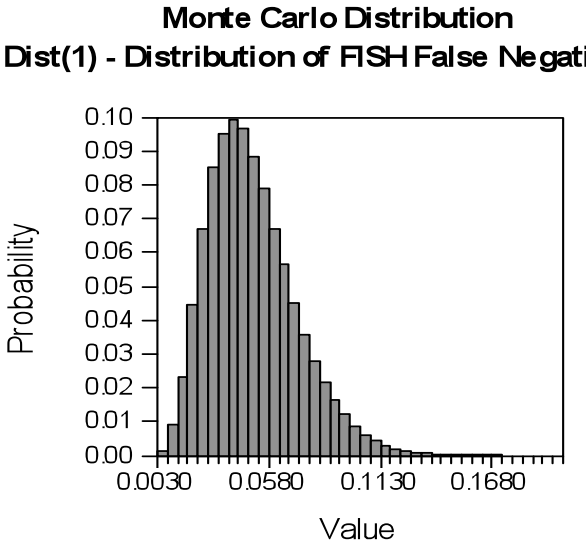
Figure 4.4b: Input Beta Distribution for IHC False Positives



Key:

- Beta distribution: α = 16, β = 84, Expected Value = 0.16

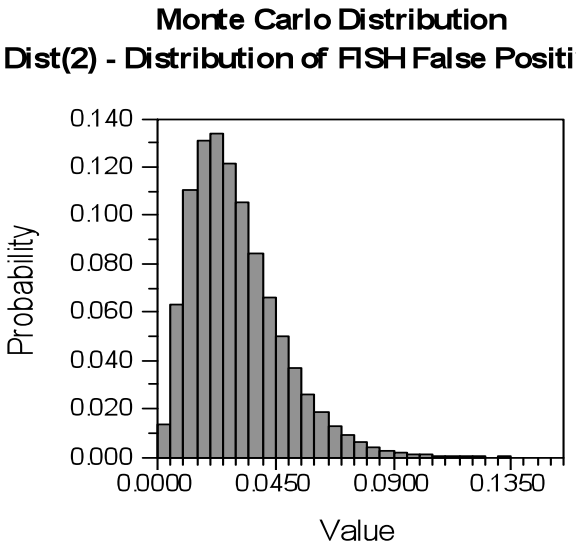
Figure 4.5a: Input Beta Distribution for FISH False Negatives



Key:

- Beta distribution: α = 5, β = 95, Expected Value = 0.05

Figure 5b: Input Beta Distribution for FISH False Positives

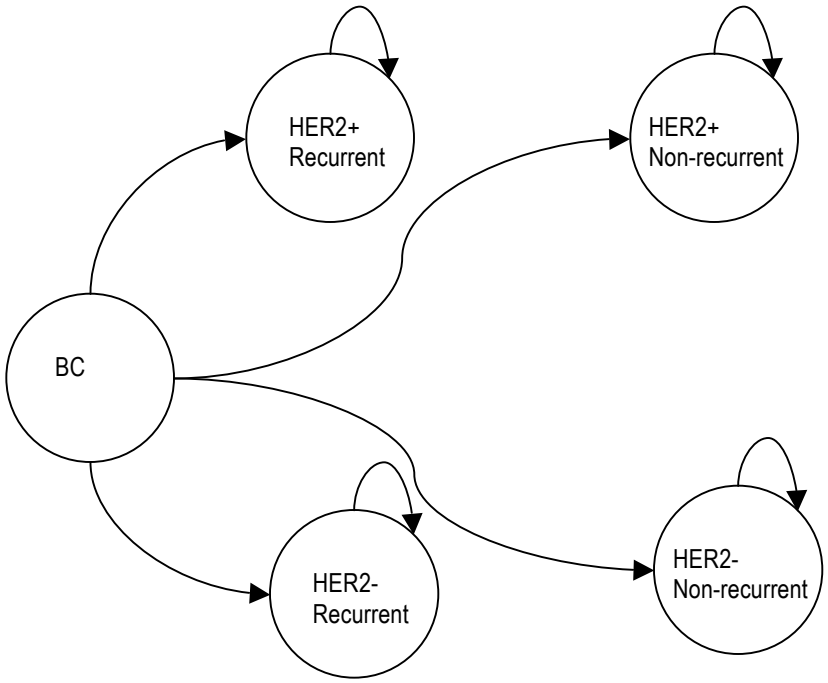


Key:

- Beta distribution: α = 3, β = 97, Expected Value = 0.03

The disease states described in the decision trees above may be modeled using a Markov model (figure 4.6). Such a model shows the various possible states that a patient with an initial breast cancer diagnosis may enter, depending on the course of testing and treatment that is undertaken. Patients may move in allowed paths between certain states based on transition probabilities which are specified in the decision trees.

Figure 4.6: State Transition Model for Patients Diagnosed with Breast Cancer



Key:

- BC = Breast Cancer
- HER2+ Recurrent = Actual HER2+ breast cancer that recurs
- HER2+ Non-recurrent = Actual HER2+ breast cancer that does not recur
- HER2- Recurrent = Actual HER2- breast cancer that recurs
- HER2- Non-recurrent = Actual HER2- breast cancer that does not recur

4.3 Results

The No Test, IHC, and FISH models are shown in figures 4.1 – 4.3. A description of the results using probabilistic sensitivity analysis follows that using point estimates. The analyses show that the FISH model dominates both the IHC and No Test models and is the cost effective technique for HER2 testing in breast cancer.

4.3.1 Point Estimate Analysis

The expected cost of the No-test model based on the specified costs and probabilities is $166,360 (figure 4.7). The expected cost of HER2 testing and treatment per patient with IHC, taking into account the probabilities of various scenarios and costs associated with trastuzumab therapy, chemotherapy, radiation and surgery is $72,405 (figure 4.8). A similar rollback for FISH shows that the expected cost of testing and treatment when FISH is used for HER2 testing in breast cancer is $64,626 per case (see figure 4.9). Considering QALYs, the expected value of total QALYs gained from IHC testing (and subsequent treatment) and FISH testing (and subsequent treatment) are 11.63 and 11.64 respectively. While the expected cost of FISH testing and treatment is lower than IHC testing and treatment, the expected QALYs are higher with FISH (by 0.01 QALY). Therefore, with FISH, there is a gain in QALYs for less cost than with IHC. From a cost effectiveness perspective, FISH testing clearly dominates IHC since it costs less money and provides more health benefit than IHC. The No Test scenario is ruled out by extended dominance, since the incremental cost effectiveness ratio of this strategy (when compared to FISH) is $726,671, well above all accepted thresholds of cost-effectiveness. [45 - 46] A commonly used threshold is $50,000: Interventions that cost less than $50,000 per QALY gained are considered cost effective. This number comes from the annual Medicare cost of caring for a dialysis patient in the US. [45] A limitation of

QALYS is that this threshold value has not changed and has been arbitrarily assigned as a determination of society's willingness to pay for interventions. [46] Though many believe it should be higher than $50,000, the No Test scenario would fall well above all proposed thresholds.

Assuming that that knowledge of disease state does not detract from QALYs, the ideal scenario for HER2 positive patients would be a true positive result on the HER2 test since this would enable appropriate treatment planning and minimize chance of recurrence. Being in this state provides the opportunity for appropriate treatment with trastuzumab and the highest QALY-payoff (11.78, which is the same number of QALYs if the patient was actual HER2 negative). The probability of being true positive is 0.033 greater if tested with FISH; i.e. there is a 3.58% percentage probability increase that a patient who is true HER2 positive will be diagnosed as positive if tested with FISH rather than IHC. Put another way, considering the entire path to reach trastuzumab treatment, the probability of reaching this node (*Actual HER2+* **>** *True +* **>** *Trastuzumab*) is 0.16695 with FISH (0.25 * 0.954 * 0.3) while it is 0.161175 for IHC (0.25 * 0.921 * 0.7). Therefore, the probability increase that a person with breast cancer will arrive at this node is 0.006 greater if tested with FISH rather than IHC, which represents a 3.58 percentage probability increase. The expected value of cost at the true positive node for IHC is $135560 while for FISH it is $135943, a difference of $383.

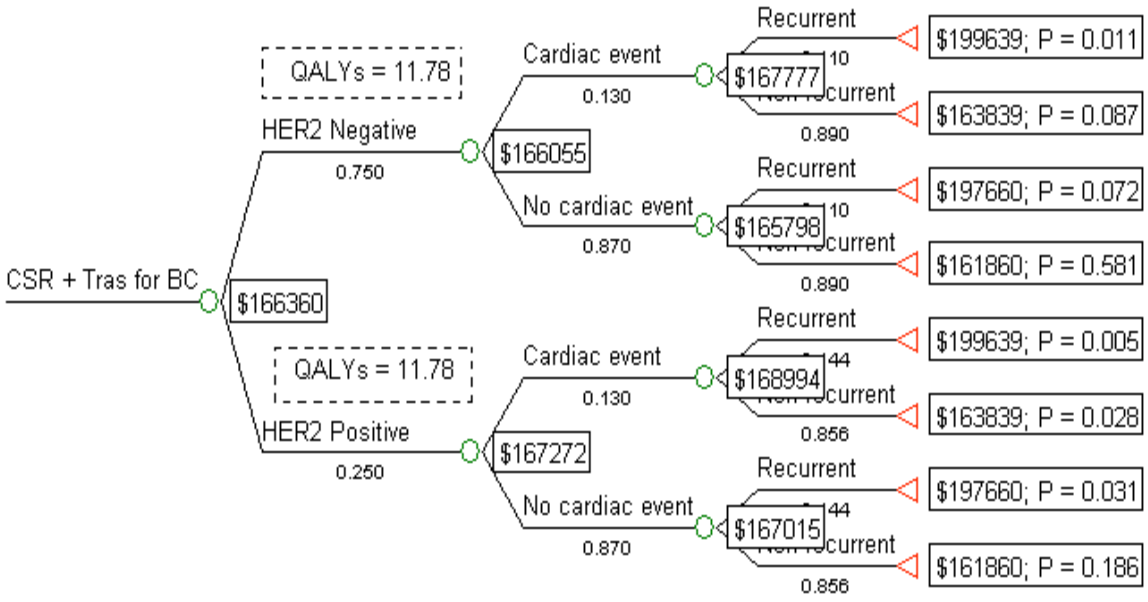
For HER2 negative patients, the ideal scenario would be a true negative result where the QALYS are 11.78. While QALYS remain the same for all HER2 negative patients, whether or not they are treated with trastuzumab, the cost of treatment varies significantly depending on whether trastuzumab is prescribed or not. With FISH, the

probability of being true negative is higher than with IHC, this time with a larger margin (0.132). There is a 15.66 percentage probability increase that a patient who is HER2 negative will be diagnosed as negative if tested with FISH rather than IHC.

From a patient perspective, the worst case scenario is being HER2 positive and being diagnosed as HER2 negative since this reduces the likelihood of being treated with trastuzumab significantly and increases the possibility of recurrence. This in turn increases the risk of having lower QALYs than if correctly diagnosed. The likelihood of this situation is greater with IHC, with HER2 positive patients having 0.079 probability of being tested as negative, while the same probability for FISH cases is a lower 0.046.

From a cost-perspective, the worst case scenario is treatment with trastuzumab when there is no established benefit from this treatment. QALYS remain at 11.78 years for all patients who are HER2 negative, but there is a significant increase in cost for those who are treated with trastuzumab. This particular scenario occurs when HER2 negative patients are diagnosed as positive (False positives).

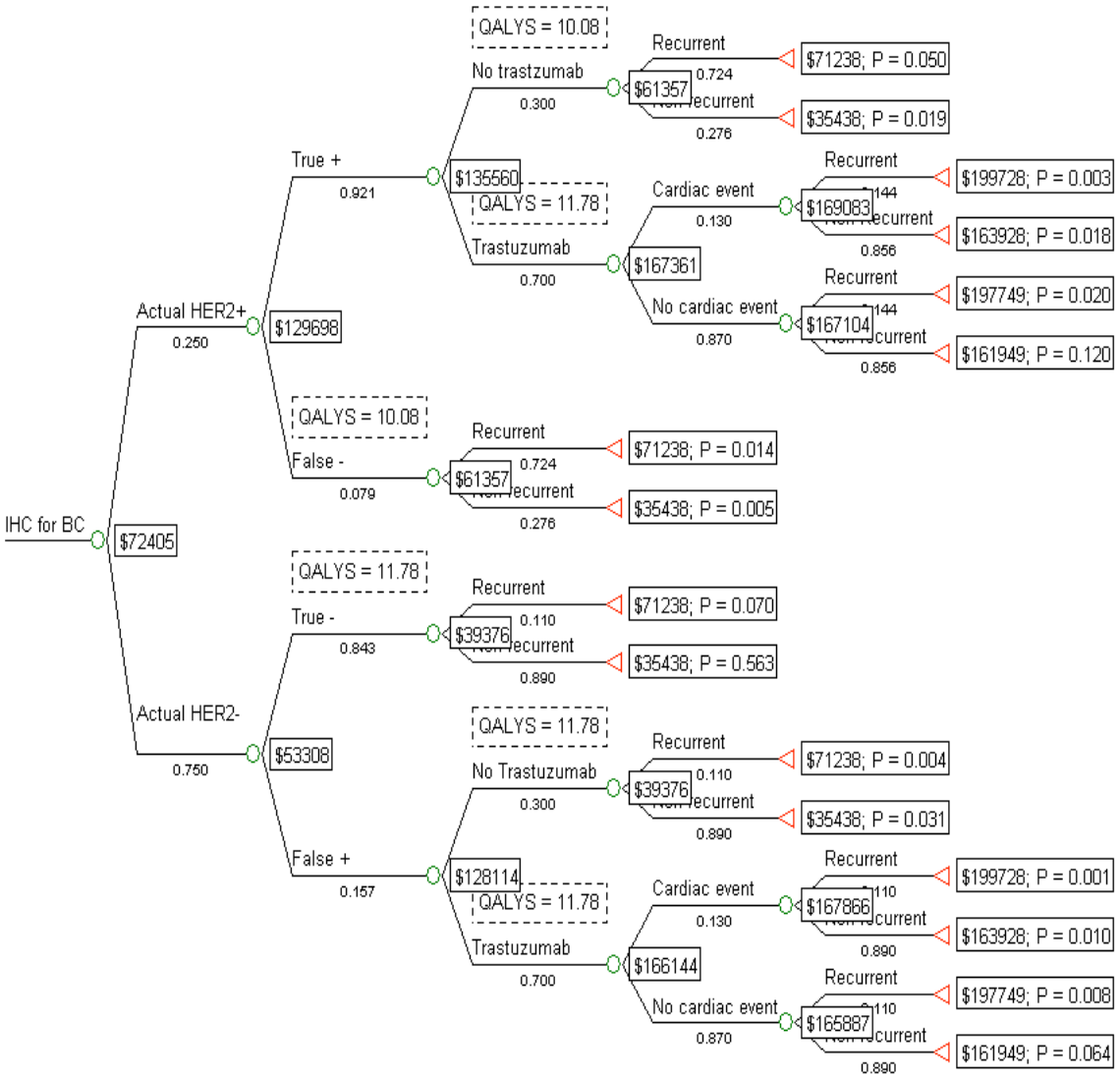
Figure 4.7: No Test Scenario – Expected Values



Key:

- CSR + TRAS for BC = Chemotherapy/Surgery/Radiation with Trastuzumab for Breast Cancer
- QALYs = Quality Adjusted Life Years associated with the nearest node to the right of box
- Payoff values at terminal nodes are in dollars
- Values below branches are probabilities
- Expected values for each node in dollars shown at node

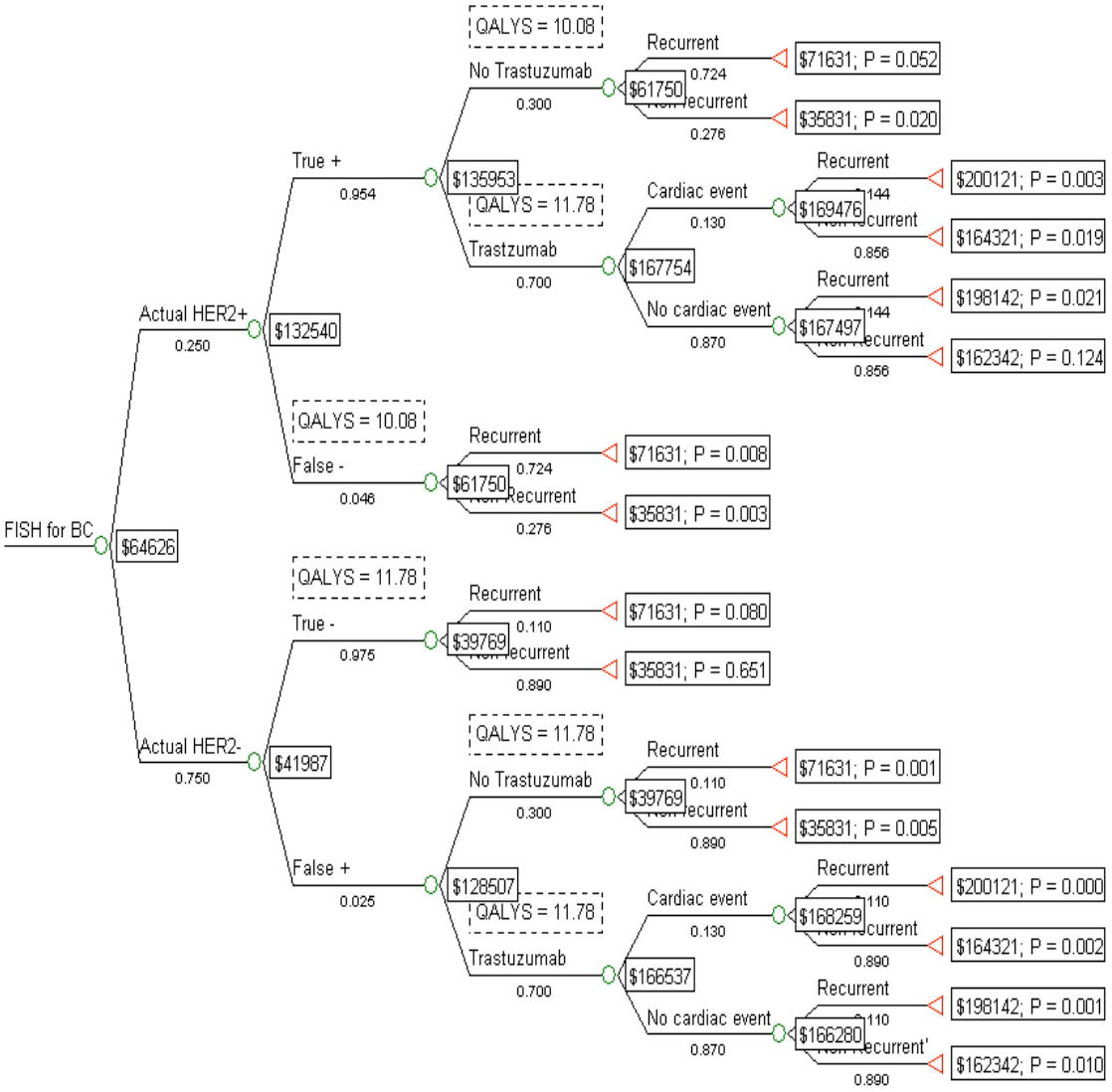
Figure 4.8: IHC for HER2 Testing in Breast Cancer – Expected Values



Key:

- IHC for BC = Immunohistochemistry for Breast Cancer
- QALYs = Quality Adjusted Life Years associated with the nearest node to the right of box
- Payoff values at terminal nodes are in dollars
- Values below branches are probabilities
- Expected values for each node in dollars shown at node

Figure 4.9: FISH for HER2 Testing in Breast Cancer – Expected Values



Key:

- FISH for BC = Fluorescence In Situ Hybridization for Breast Cancer
- QALYs = Quality Adjusted Life Years associated with the nearest node to the right of box
- Payoff values at terminal nodes are in dollars
- Values below branches are probabilities
- Expected value for each node shown in dollars at node

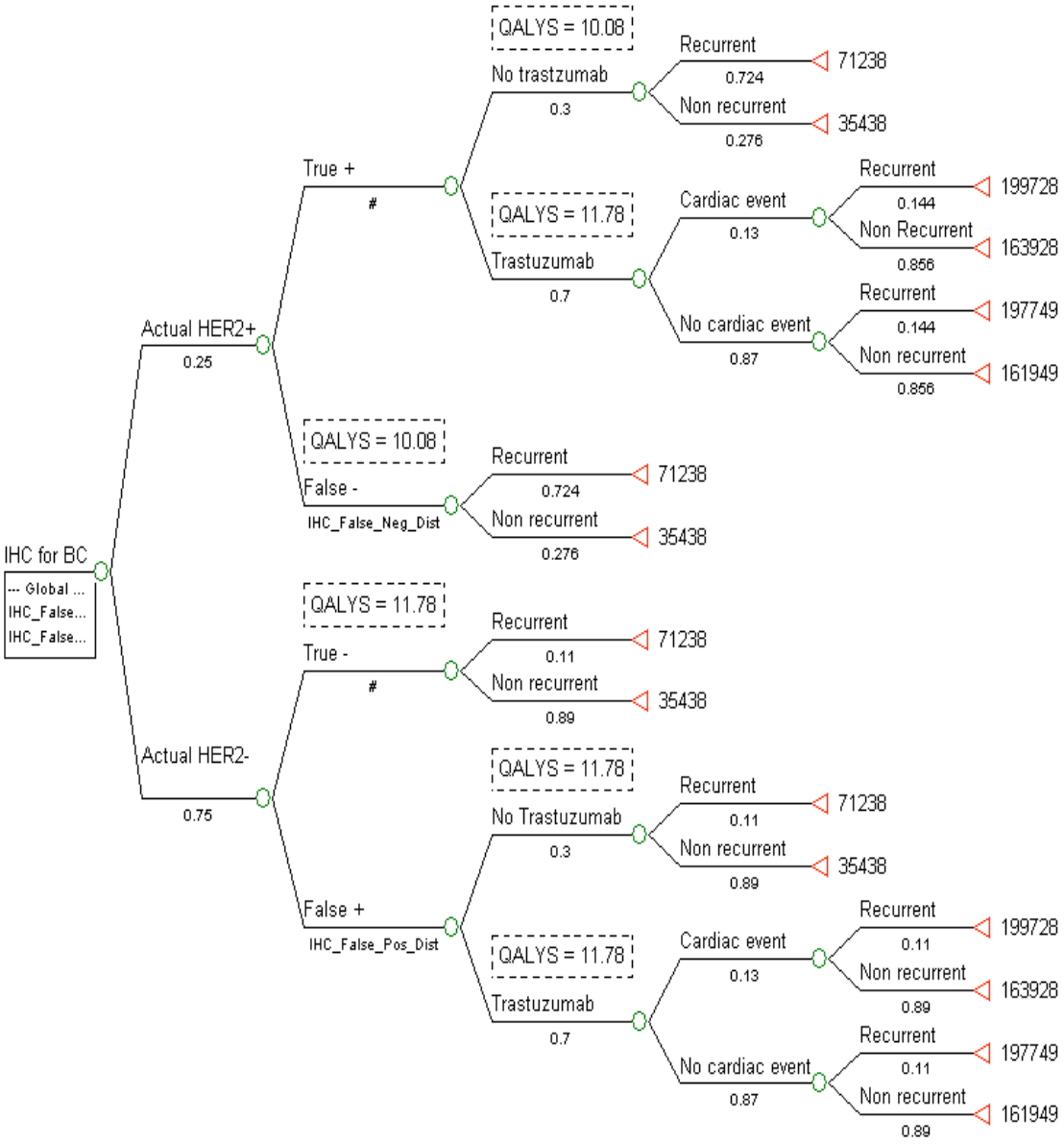
The false positive probability of IHC is significantly higher than that of FISH (0.157 for IHC vs. 0.025 for FISH); i.e., with respect to IHC, there is an 84.07 % decrease that a patient who is HER2 negative will be diagnosed as positive if tested with FISH.

Overall, the expected value of the *Actual HER2 Negative* node is less for FISH than IHC (by a value of $11321), due to the increased probability of being incorrectly treated with trastuzumab with IHC. The expected value of the *Actual HER2 Positive* node is higher for FISH (by $2842) due to higher likelihood of correct trastuzumab treatment (which is significantly more expensive than chemotherapy, radiation or surgery). Combining both situations, and their associated probabilities, the total expected value of using FISH for HER2 testing in breast cancer is $64,626 and the total expected value for IHC is higher at $72,405.

4.3.2 Probabilistic Sensitivity Analysis

The point estimate analysis described previously considers the IHC and FISH models under deterministic conditions where all parameters were assigned specified point values that were not varied. In this part of the analysis, we consider the effect of uncertainty in the model, and present results if certain parameters, specifically the false positive and false negative probabilities, behaved as probabilistic elements. Monte Carlo simulation is utilized to compute expected values using these uncertainties. The modified models are shown in figures 4.10 and 4.11.

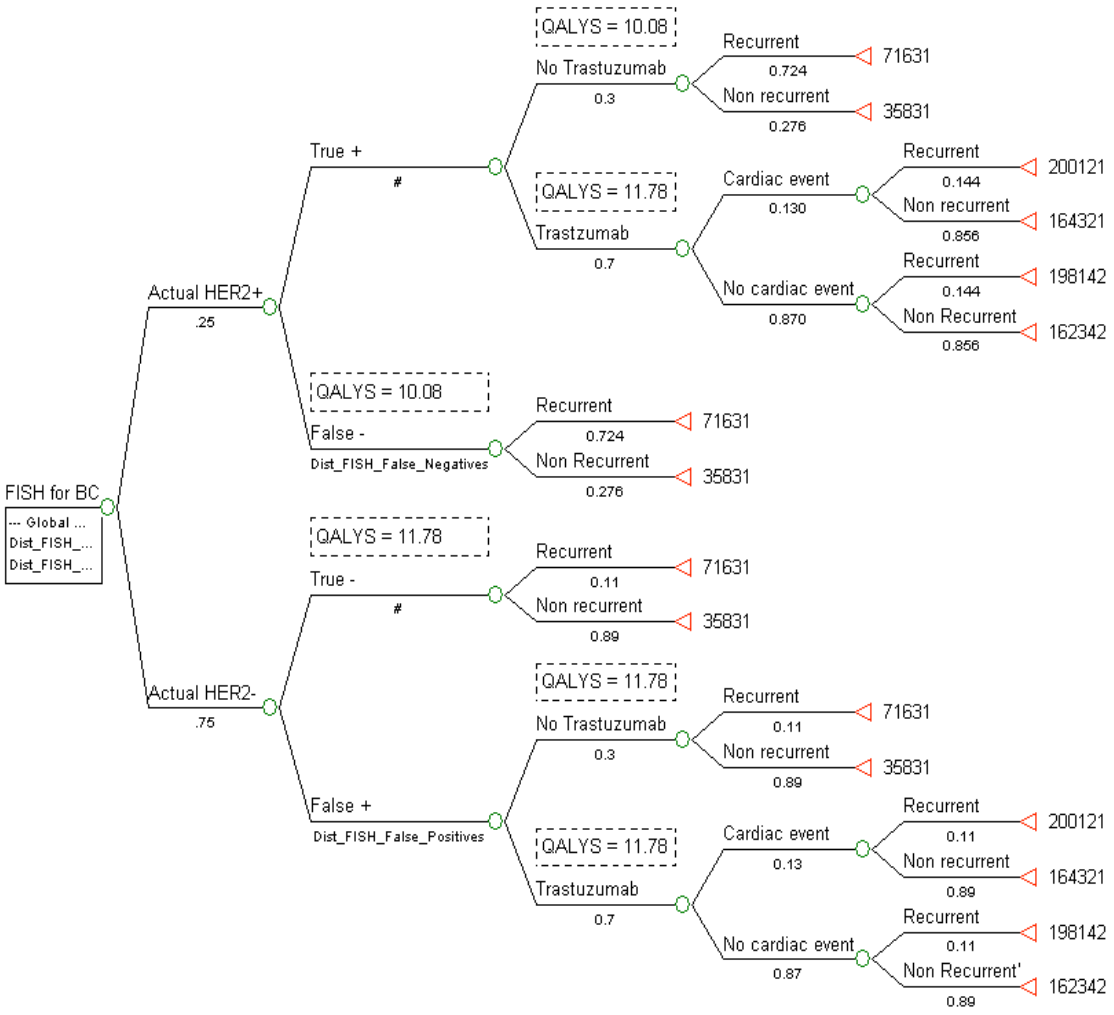
Figure 4.10: IHC Model for Probabilistic Sensitivity Analysis



Key:

- IHC for BC = Immunohistochemistry for Breast Cancer
- QALYs = Quality Adjusted Life Years associated with the nearest node to the right of box
- Payoff values at terminal nodes are in dollars
- Values below branches are probabilities
- # indicates probability of branch determined by distribution value assigned to complementary branch
- Global distribution declarations shown at root node:
  1. IHC_False_Neg_Dist: Beta distribution, $\alpha = 8$, $\beta = 92$, Expected Value = 0.08
  2. IHC_False_Pos_Dist: Beta distribution, $\alpha = 16$, $\beta = 84$, Expected Value = 0.16

Figure 4.11: FISH Model for Probabilistic Sensitivity Analysis



Key:

- FISH for BC = Fluorescence In Situ Hybridization for Breast Cancer
- QALYs = Quality Adjusted Life Years associated with the nearest node to the right of box
- Payoff values at terminal nodes are in dollars
- Values below branches are probabilities
- # indicates probability of branch determined by distribution value assigned to complementary branch
- Global distribution declarations shown at root node:
  1. Dist_FISH_False_Negatives: Beta distribution, $\alpha = 5$, $\beta = 95$, Expected Value = 0.05
  2. Dist_FISH_False_Positives: Beta distribution, $\alpha = 3$, $\beta = 97$, Expected Value = 0.03

The Monte Carlo simulation is run through 100000 iterations of the model (for both the IHC and FISH models). The simulation is run at the root node (IHC for BC and FISH for BC in figures 4.10 and 4.11). The comparative summary of Monte Carlo simulation results for IHC and FISH is shown in Table 4.1. In addition to one Monte Carlo run with 100000 iterations, 30 additional runs were completed to provide a better estimate of the values in table 4.1.

Table 4.1: Monte Carlo Probabilistic Sensitivity Analysis Results for IHC and FISH

Statistic	**IHC Single Run Value**	**IHC Multi Run Average**	**FISH Single Run Value**	**FISH Multi Run Average**
Mean	$72591	$72587	$64881	$64884
Std Dev	$2474	$2478	$1195	$1199
Minimum	$64093	$64240	$61173	$60991
2.5%	$68156	$68151	$63026	$63021
10%	$69526	$69499	$63529	$63534
Median	$72449	$71480	$64710	$64709
90%	$75848	$75816	$66468	$66476
97.5%	$77820	$78190	$67680	$67698
Maximum	$84327	$85482	$73093	$73428
Standard Error	$7.82	$7.84	$3.79	$3.79

The distribution of outcomes for 100000 iterations of the Monte Carlo simulation is shown in Figure 4.12 for IHC and Figure 4.13 for FISH.

Figure 4.12: Distribution of Outcomes for IHC Simulation (at Root Node)

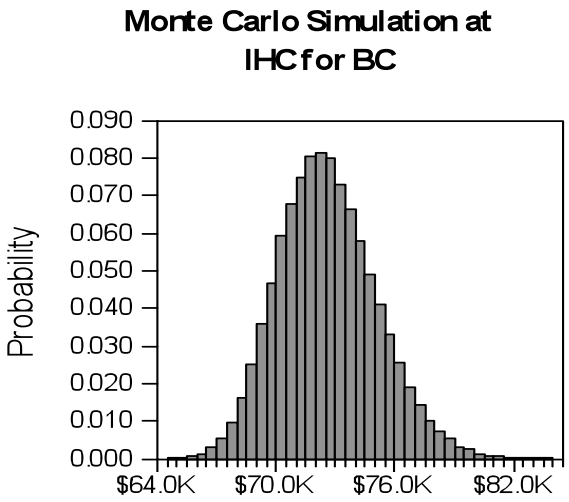
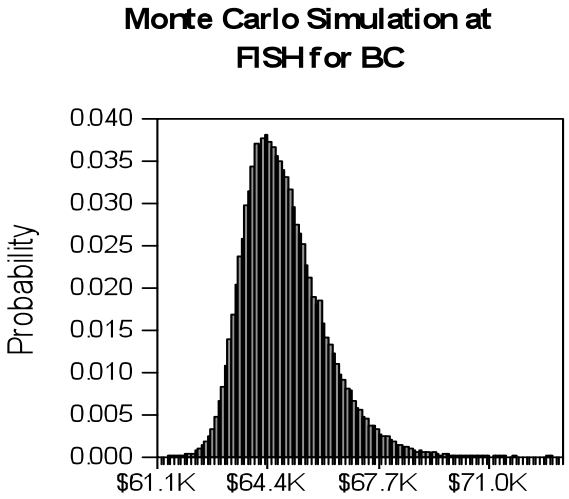


Figure 4.13: Distribution of Outcomes for FISH Simulation (at Root Node)



From Table 4.1, we see that the cost of testing and treatment with IHC is higher than FISH at all major statistical sampling levels. For example, the minimum value of the cost of testing and treatment with IHC testing for HER2 is greater than corresponding scenario with FISH by $2920 (table 4.1, single run comparison). When probabilistic sensitivity analysis is performed at the *Actual HER2-* node of the IHC model, the value is $53,577 as compared with $42,429 for the same node on the FISH tree. The higher expected value of false positives for IHC increases the cost of treatment of actual HER2 negative patients, which drives up the overall cost with IHC. On an average, the cost of testing and treatment with IHC is $7710 greater than the cost of testing and treatment with FISH. The range of outcomes is also wider for IHC (figures 4.12 and 4.13) indicating that the costs of FISH are more concentrated around the expected mean value.

The cost of IHC approaches the mean cost of FISH at the minimum value on the IHC cost curve (table 4.1, figures 4.12 and 4.13). At that point, the values of the false positive probability and false negative probability for IHC are 0.046 and 0.011 respectively. In order for IHC to become competitive with FISH, the false positive probability would have to decrease by over 80%. Similarly, the false negative probability of would have to decrease by 37.5%.

4.4 Discussion

FISH dominates IHC and No Test in terms of cost effectiveness. From the point estimate and the probabilistic sensitivity analyses discussed above, it is clear that although FISH by itself is a more expensive test, the overall cost of FISH testing and subsequent treatment is typically lower than the overall cost of IHC testing and subsequent treatment. FISH increases the likelihood of being diagnosed and treated correctly with

trastuzumab, which thereby leads to gain in QALYs. A detailed look at each model shows that FISH is more advantageous to use than IHC in all scenarios. At every single chance node, FISH testing provides a higher probability of being detected and treated correctly. In certain nodes (as with false positive cases), using IHC provides a distinct cost disadvantage that is several thousand dollars large, and ending up on this route is much more likely using IHC. In other words, when the FISH route costs more by a few hundred dollars (at maximum), it always results gain of QALYs, while when the IHC route costs more (at several thousand dollars on average) there is no health benefit associated with the cost. With regard to adverse health outcomes, the probability of not receiving trastuzumab for HER2 breast cancer as indicated by the FDA is decreased if tested with IHC. This situation is of particular concern due to the reduced quality of life and increased chance of recurrent disease with HER2 breast cancer that is not treated with anti-HER2 therapy. In addition, the high false positive probability of IHC puts patients at a higher risk of being diagnosed as HER2 positive (when actual HER2 negative). This in turn increases the risk of these patients developing trastuzumab-induced cardiac dysfunction.

In this study, we did not evaluate the cost-effectiveness or clinical efficacy of trastuzumab treatment, as this has been established by previous studies. [8, 47-48] This analysis focused on the cost effectiveness of FISH and IHC testing methods as ways to detect HER2 positive breast cancers, and subsequent treatment with trastuzumab, chemotherapy, radiation or surgery. From the findings in this study, we believe that if trastuzumab therapy is cost-effective and clinically beneficial to patients in terms of health outcomes, then FISH should be the only method of HER2 testing used. The benefits of using FISH overwhelmingly outweigh the $393 monetary benefit of IHC being the cheaper test.

The reasons for FISH being the better alternative for HER2 testing are two-fold:

1. Higher costs of FISH are outweighed by the significantly higher accuracy rates of FISH testing, especially considering the enormous cost of trastuzumab treatment. That is, FISH testing increases the likelihood that trastuzumab is given to patients who will receive QALY benefit from it.
2. The high false positive probability of IHC testing dilute the cost advantage of this test since incorrect treatment with trastuzumab is an additional cost of $126511 per patient when compared to the $393 savings from using IHC instead of FISH. Treating false positive patients with trastuzumab provides no QALY gain.

When the sensitivity and specificity rates are varied and costs are computed, the financial advantages of using FISH become clearer. From Table 4.1, it is evident that the cost of FISH and associated treatment for breast cancer is lower than IHC in all sampled scenarios at varying levels of specificity and sensitivity. In fact, even if IHC had no monetary cost (i.e., if IHC cost = $0), FISH would still be the more cost-effective. This is because the cost of IHC and FISH are small when compared to the cost of treatment with trastuzumab. FISH enables appropriate treatment with this drug, and being tested with FISH decreases the likelihood that persons who do not require the drug receive it. Therefore, we see significant cost savings with FISH despite the test itself being a few hundred dollars more expensive than IHC. For IHC to be competitive with FISH, the test would have to have become more accurate by improving specificity and sensitivity. The required improvement in false positive and false negative probabilities for IHC is 80% and 37% respectively. While it is possible that with automated image analysis, better quality assurance techniques in laboratories and increased standardization of procedures IHC could achieve higher accuracy rates, it is doubtful if false positive and

false negative probabilities could decrease as significantly as required or if the cost and time associated with making these improvements is worth the effort.

Considering health outcomes and financial costs, FISH is a more cost-effective method than using IHC for HER2 testing in breast cancer. We recommend the use of FISH for all breast cancer cases in order to improve clinical outcomes as well as reduce overall costs of testing and treatment.

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Chapter 5

Conclusion

Knowledge of the existence of differences in the care of HER2 breast cancer is a fundamental step toward understanding if these differences are based on sound clinical reasoning or if there is a need to reduce and ultimately eliminate these differences. In this research, we found that geographic location, cancer stage (specifically, the T stage) and time of diagnosis (before or after 2001) have significant effects on the choice of HER2 test. With respect to location, we found patients in Central Texas, South Texas and Los Angeles sites are significantly more likely to be tested with FISH than those in South Dakota. Our analysis also showed that patients who have in situ tumors are significantly less likely to be tested with FISH than those with advanced T stage disease. Finally, with respect to HER2 testing, we found that patients tested before publication of the 2001 testing guidelines were less likely to be tested with FISH than those diagnosed after guideline publication. These results highlight the importance of testing guidelines, show that physicians currently test conservatively, with the older and less expensive test, and reveal the existence of possible disparities in HER2 testing based on location.

We also analyzed the effects of clinical and non-clinical factors on the prescription of anti-HER2 therapy with trastuzumab. We found that patients treated in the South Texas site were less likely to receive trastuzumab treatment when compared to patients in the South Dakota site. We also found that HER2 positive patients are significantly more likely to receive trastuzumab than HER2 negative or HER2 unknown patients. These results reveal the existence of possible disparities due to location in the use of trastuzumab. In addition, while it appears that trastuzumab is largely prescribed for

HER2 positive patients (as currently required for receipt of trastuzumab), patients with other HER2 statuses (negative/unknown) also receive this drug even though the clinical benefit for this group of patients using trastuzumab has not been established.

Cost of testing and treatment for HER2 breast cancer is a topic of significant debate. The choice of test plays a critical role in determining suitable candidates for anti-HER2 therapy. Since much of the debate in this field is due to the difference in price between the two methods used for HER2 testing and the high cost of trastuzumab treatment, we analyzed the overall cost effectiveness of IHC and FISH by including costs of subsequent treatment. We showed that FISH is the best choice for HER2 testing since it is both lower in cost per QALY gained (when testing and subsequent treatment are considered together) and is associated with better response to trastuzumab in terms of clinical outcomes. The high false positive rate of IHC drives down the cost effectiveness of this test by over-selecting patients for trastuzumab treatment, and makes it unsuitable for use in HER2 testing. Although IHC is less expensive than FISH and takes less time to complete, these advantages are outweighed by the lower accuracy levels of the test. The improvements required to make IHC competitive with FISH are significant and unlikely to occur in the short term.

Certain limitations of this study may have potentially prevented other significant effects from being reported, specifically with regard to the analyses dealing with factors influencing HER2 testing and treatment. For example, the effect of insurance status on trastuzumab prescription was seen as a non-significant trend, likely due to the small sample size included for analysis. Also, certain kinds of data were unavailable for analysis. For example, socio-economic and race data were unavailable and therefore were not considered during the analysis. Information regarding physician specialty in

each outpatient clinic was also not provided in the data-set. It is likely that inclusion of such factors into this analysis will add to our knowledge regarding the cause of existing disparities.

The next steps in this research area involve gaining a deeper understanding of the clinical and non-clinical factors that affect HER2 testing and treatment. Specifically with regard to HER2 testing, the value (if any) of HER2 testing for in situ tumors needs to be determined, in addition to the role of socioeconomic and race issues in this medical decision making process. With regard to anti-HER2 therapy, the role of patient behavior in terms of refusal to begin therapy or failure to adhere to therapy could provide insight into why trastuzumab is not prescribed for all HER2 positive patients. In addition, the interaction of treatment patterns with age, race, and socio-economic factors needs to be considered and analyzed. The value (if any) of trastuzumab therapy for HER2 negative patients requires further examination and if clinical benefit is established, an overhaul of current testing and treatment patterns would be required.

In summary, this research utilized Electronic Medical Record (EMR) data from patients diagnosed with breast cancer in the United States to determine if any significant associations exist between various factors and HER2 testing and treatment. In addition, we also analyzed the cost effectiveness of FISH vs. IHC testing in HER2 breast cancer. Our results show that disparities exist in the care of HER2 breast cancer and that FISH is the best choice for HER2 testing among currently approved FDA tests.