National Surgical Adjuvant Breast and Bowel Project (NSABP) Foundation

Annual Progress Report: 2005 Formula Grant

Reporting Period

July 1, 2009 – December 31, 2009

Formula Grant Overview

The NSABP Foundation, Inc. received $1,588,633 in formula funds for the grant award period January 1, 2006 through December 31, 2009. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Gene Expression Profiling of BCPT Cancer Events for NSABP Genomic Database - Sharing of the NSABP clinical trials data has contributed significantly in improving the outcome of breast and colon cancer. Effective sharing of more complex data from genomics studies requires development of a new mechanism (the NSABP Genomic Database). The purpose of this project is to perform gene expression profiling of tamoxifen-resistant tumors procured in the NSABP Breast Cancer Prevention Trial in order to initiate the NSABP Genomic Database. Description of molecular differences between tamoxifen-resistant versus naturally occurring breast cancer will provide unique scientific resources for a better understanding of the mechanism of tamoxifen resistance.

Duration of Project

1/1/2006 – 6/30/2008

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 2: Project Title and Purpose

Prediction of Trastuzumab Response in Breast Cancer - This project is aimed at validating a set of genes that are found to be predictive of the degree of benefit from trastuzumab added to adjuvant chemotherapy in the treatment of breast cancer. Trastuzumab was originally developed to target HER2 protein over-expressed on the cancer cell surface. However, only half of HER2-positive tumors respond to trastuzumab. Furthermore, trastuzumab, when added to chemotherapy reduced the recurrence rate of even HER2-negative tumors in NSABP clinical

trial B-31. These results suggest that there are molecular determinants of tumor response to trastuzumab other than HER2. Whole-genome expression analyses of 400 tumors from NSABP B-31 identified many candidate genes that predict benefit from trastuzumab. If validated, the predictive test will help many women who have breast cancer to get the right treatment.

Duration of Project

2/15/2008 – 12/31/2009

Project Overview

The NSABP trial B-31 tested the worth of adding trastuzumab, a humanized monoclonal antibody against HER2 protein, to adjuvant chemotherapy in the treatment of HER2-positive breast cancer. In the B-31 trial, HER2 positivity was defined as either immunohistochemistry 3+ or HER2-gene amplified. Overall, there was an approximately 50% reduction of recurrence rate, 3 years after randomization. However, the benefit from trastuzumab was also observed in about 10% of the study cohort who had HER2-negative tumors. Based on this surprising clinical finding, it was hypothesized that there must be other molecular determinants of trastuzumab response. Microarray gene-expression analyses (with Affymetrix® and Agilent technologies platforms) were performed on 400 randomly selected cases from the NSABP B-31 trial. From this effort, many genes that may be predictive of the degree of benefit from trastuzumab were identified. Since it is expected that many of the identified predictive genes are false discoveries due to the problem of multiple comparisons, microarray analysis of an additional 400 cases from the B-31 trial is now being conducted in order to refine the list of predictive genes and to build a predictive model. This project is a final validation study of the predictive algorithm using the rest of the B-31 cohort (N=800). The predictive test is expected to have a very significant clinical impact.

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Expected Research Outcomes and Benefits

Trastuzumab is a very effective but expensive drug commonly used in the treatment of HER2-positive breast cancer. Currently, all patients with HER2-positive tumors are eligible to receive trastuzumab. However, the testing for use of HER2 is not optimized. Only about one half of HER2-positive tumors respond to the drug. Furthermore, even patients who have HER2-

negative tumors may gain benefit from adjuvant trastuzumab. Development of a predictive test that improves on current HER2 testing may completely change the way trastuzumab is used in the clinic and potentially help save many lives.

Summary of Research Completed

The specific aim of the project was to develop and validate a predictive algorithm for trastuzumab benefit using gene-expression profiling methods.

In order to make sure our approach of predictive model building was sound, we initiated a collaboration with Dr. Richard Simon, chief of the Biometrics Branch of the National Cancer Institute. Agilent microarray data were anonymized after combining them with clinical follow-up information and were sent to Dr. Simon.

Dr. Simon thought the number of clinical events for distant recurrence was too low to have confident statistical analyses, and, therefore, it was decided that updated clinical follow-up data would be used: Data were updated as of September 2009 with more than 100 additional events compared to the data used in our original analyses reported in the background section. Due to the cross over of patients in the control arm to receive trastuzumab after clinical results were unblinded, any cases that crossed over were censored at the time of cross over.

Agilent microarray data were available from 753 of 800 cases assigned to the discovery set. Missing cases were due to inadequate amounts of tumor tissue in the blocks or to assay failures.

We investigated genome-wide interaction with the treatment effect of trastuzumab on hazard of distant disease recurrence. There are more than 40,000 probes in the Agilent array, and we first filtered the genes for absolute expression levels to eliminate genes that are not technically reproducible because of low hybridization intensity. This filtering resulted in having 8428 genes for analyses.

Initial analysis was done using distant-disease free survival as a clinical end point. For formal statistical tests for gene-by-trastuzumab interactions of all 8428 genes, we fitted Cox proportional hazards regression models of log2 gene expression and treatment adjusted for er, pnod and tsize for each of the 8428 genes (probes) in the array:
log hazard ratio~$\beta 1_{\text{treatment}}+\beta 2_{\text{tumor size}}+\beta 3_{\text{number of positive nodes}}+\beta 4_{\text{gene}}+f(\text{gene})*\text{treatment}$.
The patient-specific treatment effect is the change of log hazard ratio due to treatment, which is beta1+f(gene). Although there are 189 genes whose p-value is less than 0.05, if we control the false discovery rate at 0.05 or 0.1 by the Benjamini-Hochberg method, no genes are detected to have significant interaction with the treatment effect. Of note is the fact that two HER2 probes represented in the Agilent array both showed statistically significant interaction (at p=0.0157 and 0.0171 for time to distant disease recurrence) with the extended follow-up data. There were more than 100 significantly predictive genes, and HER2 was not the top-most predictive gene (ranking at 38th). However, based on the a priori hypothesis and known biology, it is reasonable to conclude that HER2 mRNA is predictive of the degree of benefit from trastuzumab—while the other genes wait for validation in the independent dataset. To confirm this interaction from the discovery set, now with extended follow-up data, we were able to correlate six different

measures of HER2 mRNA levels with trastuzumab benefit (3 probes from the Affymetrix® GeneChip®, 2 probes from the Agilent array, and 1 from the NanoString assay). In the following analyses, 633 cases from the discovery set for which data for all six measures of HER2 mRNA levels were available are included. For this analysis, we used disease-free survival as the clinical end point since this was the primary end point in the B-31 trial. We performed interaction tests by fitting a Cox proportional hazard model; log hazard ratio~$\beta 1_{treatment}+\beta 2_{tumor\ size}+\beta 3_{number\ of\ positive\ nodes}+\beta 4_{gene}$+f(HER2 gene probe)*treatment. The patient-specific treatment effect is the change of log hazard ratio due to treatment, which is beta1+f(HER2 gene probe). Negative values of the treatment effect mean that the hazard of distant recurrence is decreased by the treatment. We drew linear fit of gene vs. the treatment effect, β1+f(HER2 gene probe) with 95% confidence bands for the treatment effect. Table 1 shows the result of this analysis. Table 1 shows that for the disease-free survival endpoint, all six measures of HER2 mRNA show statistically significant interaction with varying cut-offs for trastuzumab benefit. The reason for variance is due to differences in hybridization efficiency and in behavior of each probe. NanoString measurement correlated very well with both Agilent HER2 probes with a Spearman Rank Correlation of 0.9 (Figure 1). Figure 2 shows linear fit of the Cox model for all six measures of HER2 mRNA. It is remarkable that all of them show linear prediction of trastuzumab benefit. Depending on which measure was used, the proportion of clinical HER2-negative cases (defined by immunohistochemistry [IHC] and fluorescence in situ hybridization [FISH]) varies from 29.2% to 100% (with a wide confidence interval).

Technical reproducibility of the NanoString assay.

In order to examine the presence of heterogeneity of HER2 mRNA expression within each tumor and also to address technical reproducibility of the NanoString assay, we examined cases from NSABP trial B-16 which had more than two tumor blocks available. Figure 3 shows the scatter plot of HER2 mRNA normalized to internal control genes from each tumor plotted against paired tumors from the same patient. There was excellent reproducibility of the assay and lack of heterogeneity between two blocks from the same patient. This result shows the potential of the NanoString assay as a reproducible clinical assay for HER2 mRNA which we can pursue for FDA approval.

Analyses of the entire available B-31 cohort with the NanoString assay

We then assayed all remaining cases from NSABP B-31 using the NanoString assay with a total N of 1553. Linear fit of the Cox model adjusted for clinical variables resulted in Figure 4. The figure shows that there is a linear correlation between the level of expression of HER2 mRNA measured by the NanoString assay and degree of benefit from trastuzumab. Of note is the fact that most of the cases in B-31 are below the cut-off of trastuzumab benefit according to this model. To examine how mRNA expression levels measured by the NanoString assay and the cut-off from the Cox model correlate with clinical HER2 status defined by FISH and IHC assays, we plotted HER2 mRNA levels of each tumor and categorized them according to each possible combination of HER2 FISH and IHC assay results as shown in Figure 5 (data from only 1471 cases from which both FISH and IHC data available are included in the plot). The figure indicates that the HER2-negative tumors express significantly lower levels of HER2 mRNA but most of them still express levels above the cut-off for trastuzumab benefit.

The data described in this report were presented in detail to the officials of the Cancer Treatment Evaluation Program, Cancer Diagnosis and Treatment Program, National Cancer Institute (NCI) during a face-to-face meeting held on February 23, 2010. NCI officials agreed that the new proposed trial NSABP B-47 is now fully justified to move forward with the proviso that the study drug trastuzumab would be provided by the drug company. In addition, the NCI recommended that the path for FDA approval of a NanoString-based HER2 assay used in this study be included in the B-47 protocol.

We are in the process of analyzing NanoString data for other predictive genes and for potential development of a predictive algorithm in collaboration with Dr. Richard Simon.
A manuscript is being prepared for submission to The New England Journal of Medicine.

Clinical Significance

We have demonstrated that HER2 mRNA measurement using the new NanoString assay provides a linear prediction of the degree of benefit from trastuzumab added to adjuvant chemotherapy in the treatment of HER2-positive and HER2-negative breast cancers. We also established a cut-off for trastuzumab benefit using this assay. Most of the HER2-negative tumors defined by current clinical HER2 assays (FISH and IHC) are expected to derive benefit from trastuzumab although to a lesser degree when compared to HER2-positive tumors. A significant proportion of HER2-positive tumors may derive a lesser degree of benefit than what is known today (an approximate 50% reduction). The HER2 NanoString assay could help medical oncologists identify such patients, so that these patients can be candidates for trials testing the addition of other targeted agents to the trastuzumab regimen. The study provides further justification for a new NCI-funded clinical trial, NSABP B-47.

Table 1. Results of interaction tests for HER2 by trastuzumab interaction in the discovery cohort using six different measures of HER2 mRNA

Platform	Gene probe representing HER2 mRNA	Interaction p-value	Cut-off point for trastuzumab effect	Proportion of HER2 negative cases that may benefit from trastuzumab
NanoString	ERBB2	0.0033	10.17	45.8%
Agilent	A_24_p284420	0.00092	8.78	37.5%
Agilent	A_23_p89249	0.00063	11.43	29.2%
Affymetrix	234354_x_at	0.0013	None (all benefit)	100%
Affymetrix	216836_s_at	0.00036	3.81	29.2%
Affymetrix	210930_s_at	0.0075	4.45	72.9%

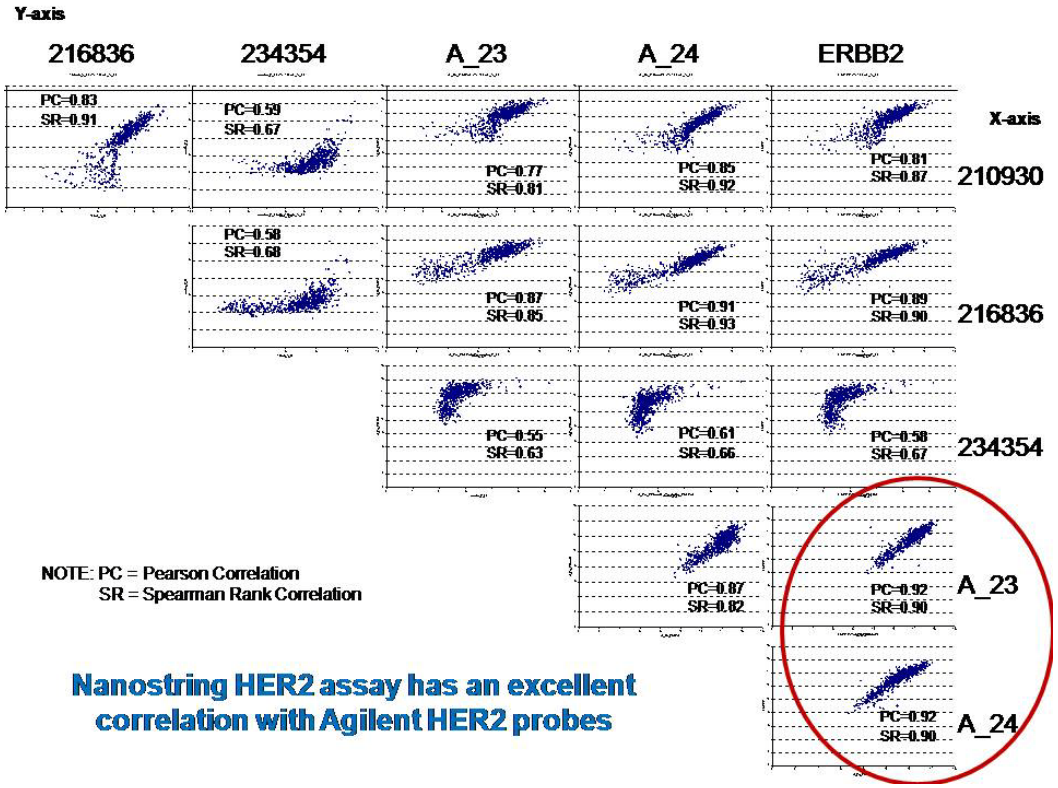

Figure 1. Correlation among HER2 mRNA expression measurements by three different technical platforms (Agilent, Affymetrix, and NanoString)

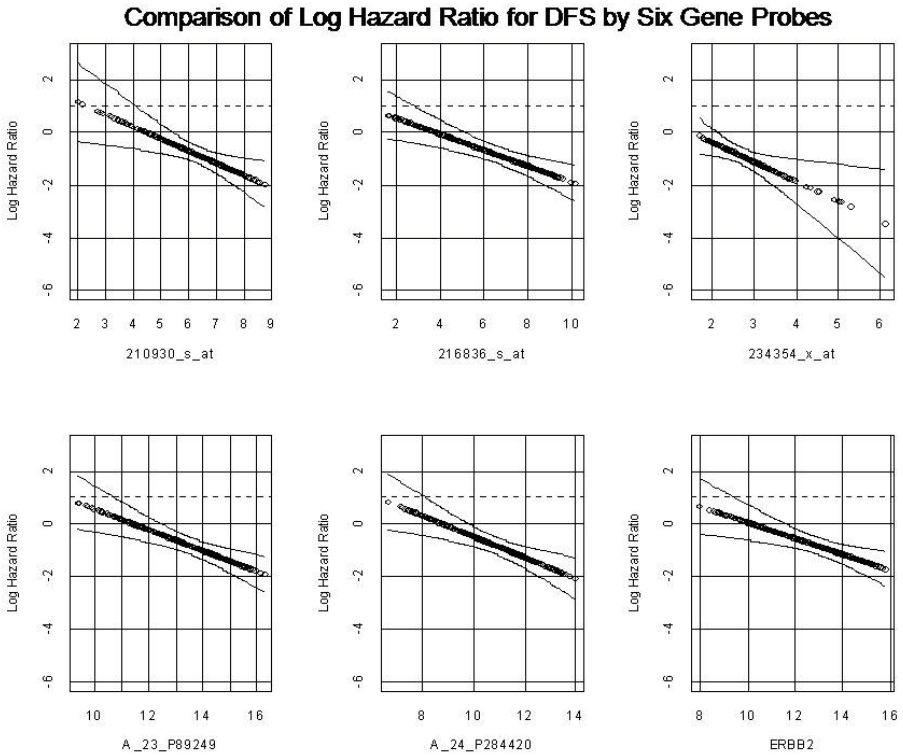

Figure 2. Log hazard ratio of trastuzumab treatment compared to chemotherapy-alone arm according to expression levels of HER2 mRNA measured by three different technical platforms (Agilent, Affymetrix, and NanoString)

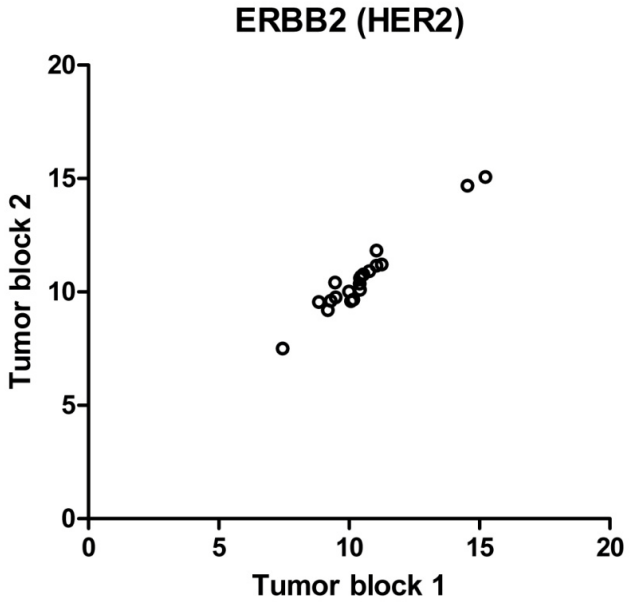

Figure 3. Reproducibility of HER2 mRNA measured by the NanoString assay

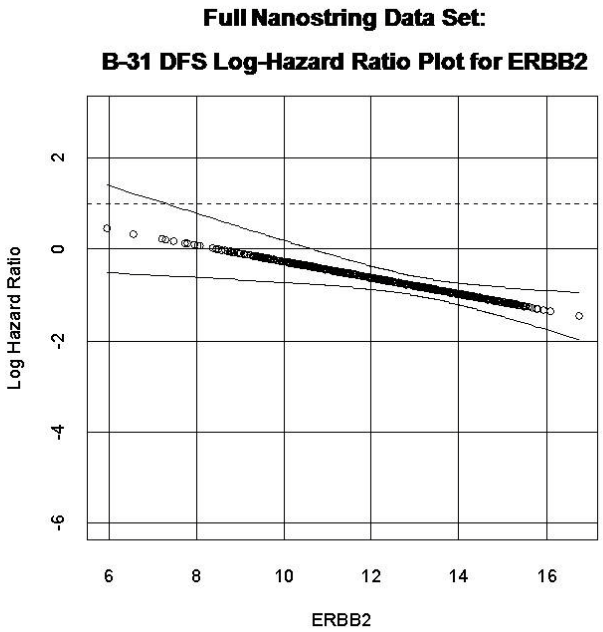

Figure 4. Log hazard ratio of trastuzumab treatment according to levels of expression of HER2 (ERBB2) mRNA measured by the NanoString assay in 1553 patients from NSABP trial B-31

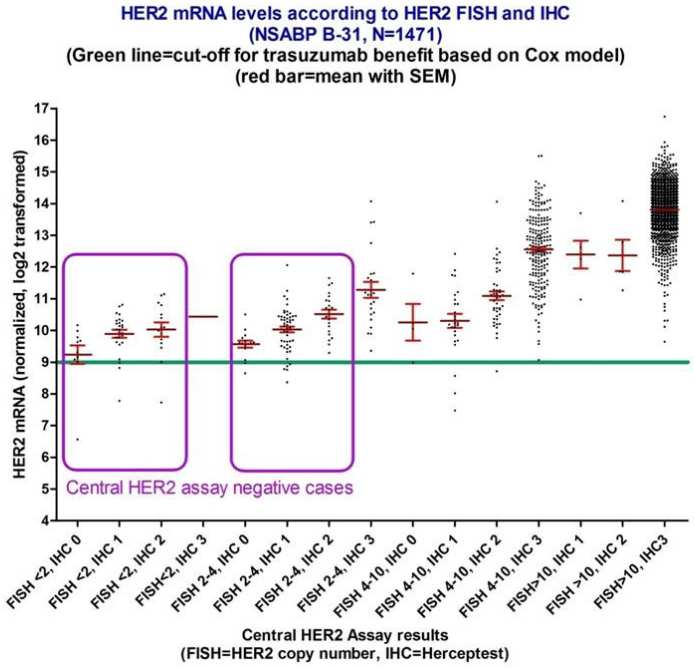

Figure 5. HER2 mRNA expression levels measured by the NanoString assay according to clinical HER2 assay categories