National Surgical Adjuvant Breast and Bowel Project (NSABP) Foundation

Annual Progress Report: 2008 Formula Grant

Reporting Period

July 1, 2010 – June 30, 2011

Formula Grant Overview

The National Surgical Adjuvant Breast and Bowel Project (NSABP) Foundation, Inc. received $1,288,794 in formula funds for the grant award period January 1, 2009 through December 31, 2011. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Development of Prognostic Index for Colon Cancer Patients Using Gene Expression Profiling – Currently about a half of patients with a colon cancer diagnosis receive very toxic chemotherapy regimens containing oxaliplatin. However, we know that much milder chemotherapy that does not contain oxaliplatin may be effective in preventing recurrence in a considerable proportion of colon cancer patients. A test that can identify those who do not need oxaliplatin will help many patients avoid unnecessary, toxic therapy. We will utilize a whole genome expression analyses method to screen tumor specimens collected from already finished, large clinical trials conducted by the National Surgical Adjuvant Breast and Bowel Project to develop a prognostic test to achieve this goal. Statistical rigor will be exercised in the project to assure that the test we develop will be highly reliable for actual clinical use.

Anticipated Duration of Project

1/1/2009 - 12/31/2011

Project Overview

The broad research objective of the project is to improve the clinical care of patients with colon cancer diagnoses. The specific aim is to develop a prognostic test using gene expression profiling to identify patients who may not require more than 5-fluorouracil plus leucovorin (FULV) adjuvant chemotherapy. The current standard of care for Stage II or III colon cancer is adjuvant chemotherapy with an oxaliplatin-containing regimen (FULV plus oxaliplatin [FLOX or FOLFOX, based on schedule]). This standard was established by the NSABP trial C-07, which showed the superiority of FLOX over FULV. However, the FOLFOX regimen is very toxic, with numerous side effects, including neurotoxicity. Data from NSABP C-07 and preceding trials conducted by our group and others make it clear that many patients may not require oxaliplatin. Yet, there are no reliable prognostic markers in clinical use to identify patients whose prognoses are good enough after treatment with only FULV such that oxaliplatin

is not required. Developing such a prognostic test will relieve suffering from unnecessary toxic therapy and lead the way to personalized care of colon cancer patients.

Our goal is achievable because 1) we have collected tumor biopsy specimens in paraffin blocks from a majority of patients enrolled in the NSABP C-07 trial that tested oxaliplatin, and 2) we have methods to use these blocks for whole genome expression profiling at relatively low cost to allow screening of a large number of cases with statistical rigor.

We will use the Whole Genome DASL assay (Illumina) to examine expression levels of 25,000 genes in tumor biopsy samples to identify genes that are prognostic for FULV-treated patients in NSABP C-07 (N=1,100). The cohort will be divided into two subcohorts of 550 each; one will be used for gene discovery and the other for refinement of discovered genes in order to minimize false discovery. Linearity and dynamic range of the expression data for each prognostic gene and correlation between the genes will be used to further select candidate genes. We will then develop the nCounter assay (NanoString) to rescreen the selected prognostic genes in the same samples to develop a prognostic algorithm based on the nCounter assay. The algorithm will be prospectively tested in completely independent FULV-treated cohorts from the NSABP C-05 and C-06 trials (N=1000) not used for building of the prognostic algorithm. The statistical rigor built into the project will ensure that the developed prognostic algorithm will be a reliable clinical test for use in routine clinical practice.

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Other Participating Researchers

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Expected Research Outcomes and Benefits

Treatment of colon cancer is evolving rapidly and many new agents are being added to what used to be considered a standard chemotherapy (FULV). However, the addition of new agents comes at the expense of increased toxicity to patients and increased health care costs. Since not all patients need such toxic treatments, identifying those who do not need such treatments is a top clinical research priority.

The prognostic index for colon cancer treated with an FULV adjuvant chemotherapy regimen developed from this project is expected to help identify patients with stage 2 or 3 colon cancer diagnoses, who may not need more than FULV treatment and still enjoy good long-term survival. This prognostic test will result in sparing these patients from unnecessary treatment

and toxicity associated with additional therapy such as oxaliplatin, bevacizumab, or cetuximab. It will also reduce the social burden resulting from less discriminate use of the expensive agents.

Summary of Research Completed

I. Additional Expression Data Collected

Expression profiling of C-08 cases with whole genome expression DASL arrays.
We completed expression profiling of 466 cases from NSABP clinical trial C-08 with Illumina DASL arrays. The purpose of this profiling was several-fold and does have project overlap with another State project, but there is no budgetary overlap. The purpose of the profiling for this project was to serve as a validation cohort for prognostic models obtained from analysis of the whole genome expression profiling of the C-07 discovery cohort.

II. Data Analysis of Expression Data

A. *Improvements Made in Data Analysis of C-07*

A variety of factors have necessitated that we repeat the analysis of the C-07 gene expression data, which included whole genome DASL data from 966 cases and were discussed in our last report. Due to some errors that were made in our last analysis of the C-07 DASL data, it was necessary to repeat the analysis of C-07 DASL data with a better-defined discovery cohort. We have also taken several steps that will improve the data analysis process for all of our projects, including the analysis of the C-07 and C-08 DASL data. These steps are detailed below.

The first step was to employ a Biostatistician who would work at the NSABP Division of Pathology Laboratory. We have hired a dedicated Biostatistician, Noriko Yamaguchi-Tanaka, who has extensive knowledge in the analyses of molecular data as it relates to clinical data. Dr. Yamaguchi's extensive statistical expertise with molecular data, her location in the NSABP Pathology Laboratory, and the fact that her only responsibility is to work with correlative science studies has and will vastly improve the quality and the speed with which we are able analyze the high-volume gene expression data that we have gathered for C-07.

Secondly, we have improved the data analysis process by mandating the development of a written, detailed, biostatistical protocol for our data analysis before any analyses are started. We found this to be necessary so that all of the molecular and statistical steps are clearly outlined in order that both the molecular and biostatistical personnel understand how the analyses will proceed. This protocol is written and approved by all relevant personnel: our Correlative Science Biostatistician, the main Clinical Trial Biostatistician, the Director of the NSABP Division of Pathology Laboratory and the Assistant Director of Molecular Profiling, so that biological, regulatory, and statistical matters can be addressed from the beginning. We have outlined and completed the primary, secondary and exploratory goals, which are detailed below.

B. Model Building with Whole Genome Expression DASL Data

To correct the problems associated with our previous analysis, we have identified a new cohort as the discovery cohort consisting of 866 cases from NSABP C-07 and for which we have whole genome DASL data.

Prognostic models built with C-07 DASL whole genome expression data. Prognostic models were constructed using the oxaliplatin arm, the control arm, and both arms (FULV and oxaliplatin +FULV) of the C-07 trial.

Prognostic models built in the FULV and oxaliplatin arms of C-07. We initially attempted to build a model using the oxaliplatin arm of the trial because we wanted to validate the model with cases from the control arm of NSABP C-08 (modified FOLFOX6 regimen). In this way, both the discovery and validation data sets would consist of patients that were both treated with oxaliplatin and FULV. Therefore, we attempted to build models in the oxaliplatin arm of the C-07, but the number of prognostic genes in this arm of the trial was limited and the genes which were used to build models to distinguish high- and low-risk patients were less satisfactory than models in which only the clinical covariates were used in the model. Building models with the gene expression data with only the control arm (FULV) of C-07 resulted in good prognostic models, meaning that high- and low-risk groups achieved some additional separation when added to clinical covariates in the model.

A prognostic model built using both the FULV and FLOX arms of C-07. Genes for the model were identified by including genes in the prognostic model with a p value less than 0.00004, which corresponded to an FDR<0.1. Additionally, genes were filtered to ensure that 20% of the expression data for a particular gene had at least a 1.5-fold change in either direction from the gene's median value. Supervised principal component analysis (SuperPC) as a part of BRB ArrayTools was used to build a model using 3 principal components and 10-fold cross-validation. The model that included clinical covariates for treatment, T stage, and nodal status was built with 20 genes (Figure 1). The gene expression model was able to correctly classify patients into high- and low-risk groups and did add some additional separation to high- and low-risk patients, which were identified with only clinical variables included in the model.

Validation of the prognostic model built with samples from both arms of the C-07 discovery cohort. The prognostic model which was built with samples from both arms of the C-07 trial and using whole genome expression DASL data was tested in cases from C-08. Figure 2 demonstrates that the 20-gene prognostic model built with C-07 DASL data was validated in the C-08 DASL data. However, a comparison of the models built with only clinical covariates to models built with clinical covariates plus genes demonstrates that the gene expression did not improve the prognostic model (Figure 3).

C. Selection of Prognostic and Predictive Genes

Although the building of gene expression models with the DASL data was an important exercise for understanding how this data might be able to distinguish high- and low-risk patients, the ultimate goal of this work is to be able to devise a clinical test that can be used to determine

which patients can benefit from oxaliplatin. Because whole genome expression profiling is too complex and too expensive to be used as a clinical test, our strategy is to select genes from whole genome DASL expression profiling of C-07 and then to move to a different more clinically relevant platform for the development of a useful clinical test. Therefore, we have selected prognostic and oxaliplatin-predictive genes. These genes will be profiled on another platform which could be used as a clinical assay. Therefore, we have selected prognostic genes using the C-07 DASL data which were dependent and independent of clinical and pathological variables and treatment. We have also identified genes that are predictive of benefit from oxaliplatin when added to FULV.

Prognostic genes dependent and independent of clinical variables were identified by calculating the non-adjusted (crude) and adjusted HR and p-values for each of the 24,525 probes present on the Illumina Whole Genome DASL arrays. Among the top 20 genes (based on their ranking by p-value) 9 probes were identified in both the adjusted and non-adjusted analyses. Only the top 3 were significant at a FDR cut off of <0.01.

Predictive genes for oxaliplatin benefit were identified by calculation of interaction Cox proportional hazard models including treatment-by-gene expression interaction terms for each gene. Sixty-nine genes were identified with an interaction p-value below 0.01 which was used as a cut off to identify predictive genes. Predictive genes were also identified by 10-fold cross-validation and 39 genes were identified with an average p value below .01. A Kaplan Meier plot in which patients are stratified by the expression levels (using a median cut) of one of these predictive genes and by treatment is shown in Figure 4. Clearly, patients with a low level of expression of this gene appear to receive benefit from oxaliplatin while patients with a high level of expression do not.

It is unclear whether the identification of any of these genes will result in clinically useful clinical tests but because this information is potentially patentable, we will release the list of specific prognostic and predictive genes later, once appropriate patents have been filed.

Additional evaluation of prognostic and predictive genes. On the basis of previous results, we are aware that successful selection of genes across platforms requires additional data analysis beyond the examination of the prognostic or predictive values from whole genome expression data. Therefore, we are performing additional analyses for the selection of genes that will be used in a clinically relevant platform. We are currently selecting transcriptionally informative genes from DASL and Agilent arrays by comparison of expression measurements with DASL and Agilent arrays. The purpose of these analyses is to select genes which are concordant between different expression platforms as a mechanism of selecting meaningful and stable genes.

D. Additional Methodological Details

Gene expression profiling of C-08 with whole genome DASL arrays. Processing of samples for whole genome DASL arrays were done as recommended by Illumina. Any array with a signal to noise ratio (P95/P05) below 60 was removed from the data set. Illumina recommends that this ratio should be above 10.

Details of statistical analysis. The clinical covariates, age, gender, treatment, MSI status, T stage, and nodal status were tested for their effect on recurrence by analysis of maximum likelihood estimates for their association with survival. Among these covariates only T stage and nodal status were significantly associated with recurrence and were included as the clinical covariates.

Fig 1. 20 Gene Model

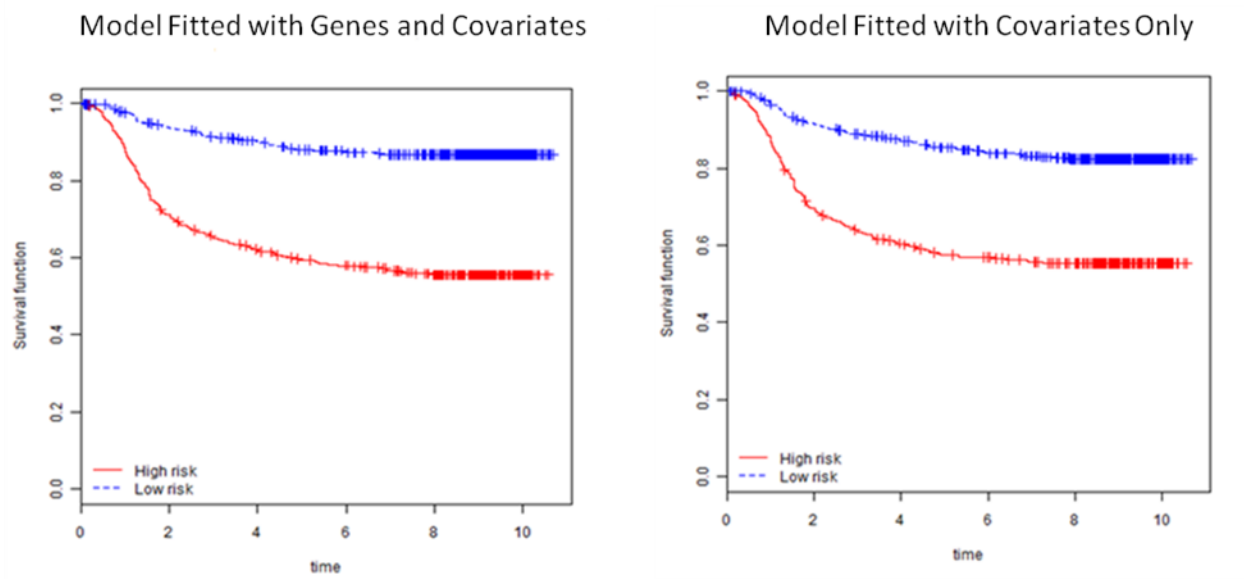


Fig. 2. 20 Gene Model is Validated in C08

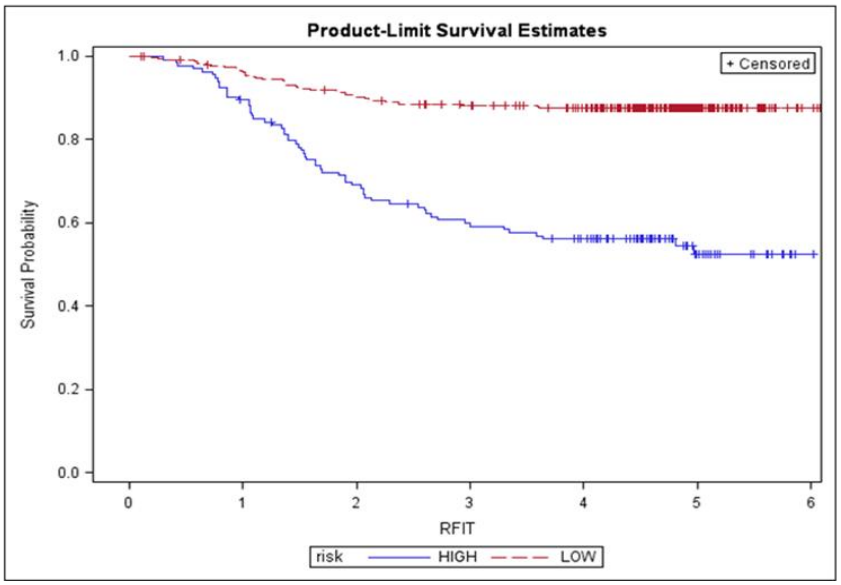
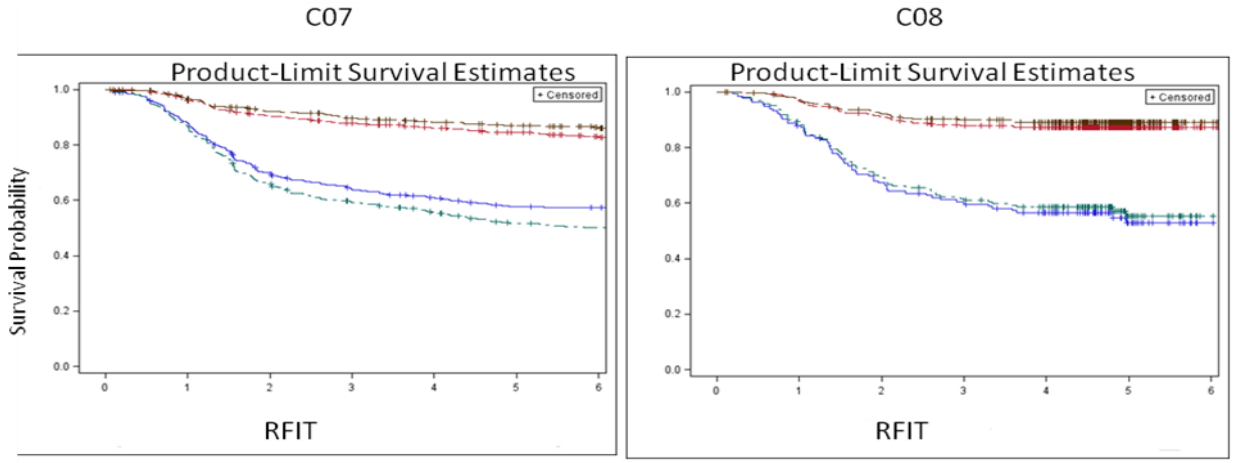


Fig 3. Kaplan Meier Comparisons of Clinical and Gene Models



C-index for COV only model=0.62
C-index for COV for COV + gene model=0.65

C-index for COV only model=0.63
C-index for COV for COV + gene model=0.65

Model= COV + genes, low risk
Model= COV, low risk
Model= COV, risk high
Model COV+ genes, high risk

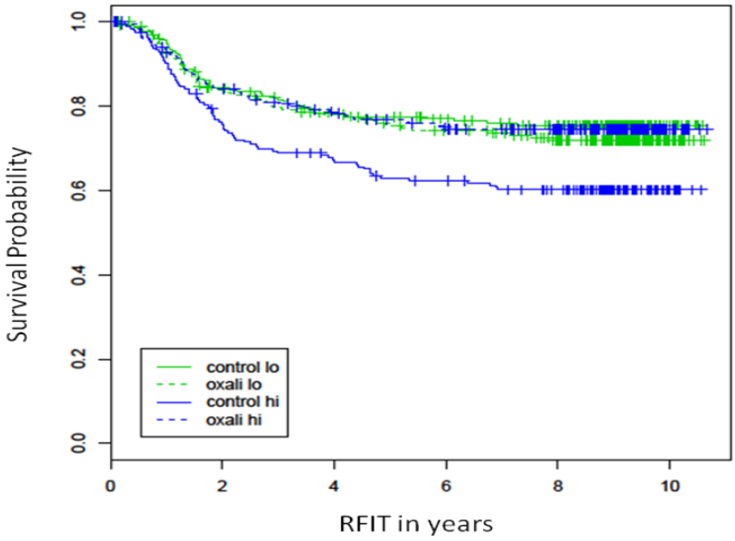



Fig 4. KM Plot based on Expression Levels of a Gene