National Surgical Adjuvant Breast and Bowel Project (NSABP) Foundation

Annual Progress Report: 2007 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 $1,306,064 in formula funds for the grant award period January 1, 2008 through December 31, 2011. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Mutation Profiling of Breast and Colon Cancer - Cancers are caused by mutations of the genes that control important functional components within the cells. These gene mutations, therefore, govern the behavior of cancer cells. Studies have shown that within a certain cancer type, such as breast, there are different sets of mutated genes. These differences (also called heterogeneity) of gene mutations result in different clinical behaviors of cancer cells, i.e., differential prognoses and responses to anti-cancer therapies. The goals of this project are to characterize gene mutations in breast and colon cancers and categorize them according to matched clinical behaviors so that the information can be used to optimize treatment for breast and colon cancers as well as to identify new targets for anti-cancer therapies.

Anticipated Duration of Project

1/1/2008 - 12/31/2011

Project Overview

It has been known for sometime now that human cancer is caused by the accumulation of mutations in oncogenes and tumor suppressor genes. A great deal of work has been done to identify a plethora of mutations in a variety of human cancers. However, until recently, the application of this information has had little impact on improving the treatment of cancer patients. The broad long-term goal of this project is to profile mutations in breast and colon cancers, which can be used to improve treatment through identifying mutations that may pinpoint new targets for new drugs.

There is mounting evidence that the heterogeneity that exists within breast and colon cancers may be due to different mutation profiles among different cancer sub-types (i.e., in the breast, luminal A and B, Her2, normal and basal). However, until recently there were various technical and pragmatic barriers to carrying out any systematic study of mutation profiles. The mass

spectrometric genotyping technology based on the Sequenom platform is suitable for the execution of such studies even using formalin-fixed, paraffin-embedded tissue specimens. The successful application of this approach was described by Thomas et al. (2007) during the past year. In their study, using this technology, 1000 tumor samples were tested for 238 known oncogene point mutations in 17 human oncogenes. The overall distribution of oncogene mutations found was consistent with previous literature reports, but many low frequency events involving both rare and common neoplasms also were detected. These included an FGFR1 mutation and a BRAF mutation in 2 breast tumors (in addition to prevalent PIK3CA mutations). These results endorse the notion that potentially 'drug-able' oncogene mutations may exist at low to moderate frequencies in many tumor types, including breast cancer, and that they are detectable using this approach.

In the project proposed, breast cancer tumors from neoadjuvant trial NSABP B-27 and colon carcinoma tumors from NSABP trial C-07 will be used for mutation screening. In our previous studies involving patients enrolled in NSABP neoadjuvant trial B-27, we have identified patients who had a poor prognostic gene signature and did not respond to chemotherapy, and, therefore, they are at very high risk of relapse and are resistant to chemotherapy. Cancer (CAN)-gene screening in such a cohort may yield rich information as to new treatment strategies beyond chemotherapy. Colon cancer tumors will be included in our project with the same goals.

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Expected Research Outcomes and Benefits

In the proposed project, we will identify mutations in key genes that are involved in the development of breast and colon cancer.

The proposed project will yield the following information:
1) The frequency of gene mutations in breast and colon cancer. This will be an important finding because it defines which genes may be the most important targets for the development of new therapies.

2) The prognostic significance of gene mutations in breast and colon cancer. This is of obvious importance to the development of strategies for selecting appropriate patients with regard to chemotherapy.

3) The role of gene mutations in predicting response to specific anti-cancer therapies.

4) The identification of gene mutations that can be targets for new drug development.

Potential benefits will include the following:
1) Better prognostication of patients diagnosed with breast and colon cancer
2) Better prediction of response to anti-cancer therapies

Summary of Research Completed

I. Additional Mutation Profiling of NSABP C-07

Completion of Mutation Profiling for C-07. In this report period, we completed all of the ColoCarta assays, which included assays for PIK3CA, NRAS, and KRAS on a cohort of C-07 patients (N=842) previously referred to as the validation cohort. Detailed methods for mutation profiling with ColoCarta and the Sequenom platform have been described in previous reports and in a recent manuscript by us (BMC Cancer. Fumagalli, et al, 10:101 http://www.biomedcentral.com/1471-2407/10/101).

II. Statistical Analysis of C-07 and C-08 Mutation Profiles

A variety of factors necessitated improving the analysis of the C-07 mutation data reported in our last progress report. On the basis of our initial analysis with a subset of the C-07 cases and other published reports, we decided to use our entire mutation data set as a "validation" of published reports for BRAF and KRAS mutations and as a comprehensive discovery set for PIK3CA, MET, and NRAS mutations.

In Stage II and III colon cancer, BRAF mutations were reported to be prognostic for overall survival (OS) but not for recurrence-free survival (RFS), and KRAS mutations were not prognostic for RFS or for OS. We also profiled other mutations, MET, NRAS, and PIK3CA, for which there are no definitive reports for their association with prognosis. PIK3CA mutations have been reported to be associated with poor cancer-specific survival in Stages I, II, and III resectable colon cancers but this association was not significant (p=.075). NRAS and MET mutations occur at a low frequency in colon cancer, 2.8 and 3.7%, respectively, and, therefore, it is difficult to establish them as useful prognostic markers. However, the current focus on using mutation data as possible biomarkers for determining the likelihood of patients to respond to targeted therapies make all of these mutations potentially important biomarkers for future clinical trials of targeted therapies.

Therefore, all of the appropriate cases* from C-07 for which we were able isolate DNA were combined and are currently being analyzed together with an additional appropriate 465 cases from C-08. These 465 cases were identified initially as a discovery cohort and are representative of the entire C-08 cohort. This combined cohort of C-07 and C-08 consists of more than 2000 cases. Thus, we anticipate that the results of analysis of this large data set will serve to definitively determine whether any of these mutations are useful as prognostic markers for Stage II and III colon cancers.

*Appropriate cases refers to cases from patients who have consented to use of their tissues for future research and who are considered eligible for the treatment protocol and have clinical follow-up information.

Statistical Analysis of C-07 and C-08 Mutations for Prognosis. Assay data for all mutations within a gene—identified by ColoCarta—were consolidated to determine the mutational status of each gene (BRAF, KRAS, NRAS, MET, PIK3CA). The number of assays for each gene varied from 2 to 7 and comprised all of the common mutations for each gene. Any mutation within a particular gene was scored as 0 to indicate no mutation and as 1 to indicate the presence of 1 or more mutations within a gene. (It is extremely rare that there is more than 1 mutation within a single gene.)

Table 1. shows the overall association for all mutations within a gene and for the specific mutation KRASG12V with recurrence (TTR), overall survival (OS) and survival after recurrence (SAR). Associations with a p value of less than 0.05 are considered to be significant and are highlighted. The KRASG12V mutation was included for analysis because it has been shown to be a marker for poor prognosis in a large meta-analysis. Of all the mutations examined, only the NRAS mutations were associated with TTR, but the low frequency of these mutations suggests that these mutations are unlikely to be of prognostic value in the clinical setting. Only BRAF mutations were associated with poor OS; this may be due to the association of BRAF mutations with poor SAR. KRAS, PIK3CA, and MET mutations were not prognostic for TTR, OS and SAR.

Details of Statistical Mutation Analysis. Cox proportional hazard models were used to calculate hazard ratios for each mutation. Analyses were controlled for treatment, and the combination of nodal status and T-Stage (N0 T3; N0 T4; N1 T1 or T2; N1 T3; N1 T4; N2 T1 or T2; N2 T3; and N2 T4). The endpoints for these analyses were TTR (even if preceded by a second primary cancer); OS; and SAR.

In summary we have made significant progress in this year and are close to completing the specific aims as proposed.

Table 1. Mutations and their Association with Recurrence, Overall Survival, and Survival After Recurrence

Recurrence (TTR)

Mutation	mutation rate(%)	Pr > ChiSq	Hazard Ratio	95% Lower Confidence Limit for Hazard Ratio	95% Upper Confidence Limit for Hazard Ratio
BRAFmutated	14.1959	0.8596	1.02	0.816	1.275
KRAS_G12V	8.4337	0.1563	1.219	0.927	1.603
KRASmutated	38.1067	0.2058	1.115	0.942	1.32
METmutated	3.7001	0.6383	1.106	0.728	1.68
NRASmutated	2.8851	0.0426	1.53	1.014	2.309
PIK3CAmutated	20.1817	0.2462	0.878	0.705	1.094

Overall Survival (OS)

Mutation	mutation rate(%)	Pr > ChiSq	Hazard Ratio	95% Lower Confidence Limit for Hazard Ratio	95% Upper Confidence Limit for Hazard Ratio
BRAFmutated	14.1959	0.0002	1.461	1.195	1.787
KRAS_G12V	8.4337	0.4882	1.105	0.833	1.466
KRASmutated	38.1067	0.3302	1.087	0.919	1.285
METmutated	3.7001	0.3989	1.193	0.792	1.797
NRASmutated	2.8851	0.3355	1.246	0.796	1.951
PIK3CAmutated	20.1817	0.3201	0.896	0.722	1.112

Survival after Recurrence (SAR)

Mutation	mutation rate(%)	Pr > ChiSq	Hazard Ratio	95% Lower Confidence Limit for Hazard Ratio	95% Upper Confidence Limit for Hazard Ratio
BRAFmutated	14.1959	<.0001	2.322	1.829	2.948
KRAS_G12V	8.4337	0.5486	0.907	0.66	1.247
KRASmutated	38.1067	0.2953	1.107	0.915	1.338
METmutated	3.7001	0.2746	1.308	0.808	2.117
NRASmutated	2.8851	0.1138	0.666	0.402	1.102
PIK3CAmutated	20.1817	0.0966	1.23	0.964	1.569