Blood Based Biomarkers Used as Predictors of Response to Therapy and Disease Activity in Ulcerative Colitis and Crohn’s Disease

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i. Summary of Project Aims:

Crohn’s Disease (CD) and Ulcerative Colitis (UC) are chronic relapsing inflammatory bowel diseases (IBD) of which the etiologies are not fully understood. Genetic and environmental factors are likely central to both diseases. In recent years, numerous discoveries in the genetics of CD and UC have dramatically increased our understanding into what initiates and then perpetuates IBD. Many of the advances in the biology of IBD are the result of newer available technologies. Gene expression (GE) profiling is commonly used to examine the differential expression of genes between disease states and normal.

Previous techniques included obtaining DNA from patient tissue samples that were hybridized to a gene chip that contained thousands of probes corresponding to individual genes. More recent advances in this field are the development of techniques to evaluate RNA from whole blood. Current serological inflammatory markers are not adequate to determine the severity of disease in IBD and therefore a GE assay using peripheral whole blood RNA would be a useful tool to predict disease activity and potentially response to targeted therapy. A GE analysis from peripheral whole blood is clinically advantageous over biopsy of tissue as it is minimally invasive and can be performed at any time. We previously published that GE profiles from whole blood can differentiate patients with active and inactive CD in one study and can differentiate between CD, UC, and non-inflammatory diarrheal disorders in another study.

In this proposed study, we anticipated the ability to outline discriminatory genes that will differentiate disease activity states in both UC and CD and potentially predict response to anti-TNF therapy. For patients with moderate to severe UC or CD, these tests may help predict one’s response to anti-TNF therapy, thereby allowing us to initiate patient-specific targeted therapy.

Aim 1: How would gene expression profiles derived from whole blood vary with the severity of inflammation in UC and CD, and can the gene expression profiles be used to predict disease activity?

Aim 2: Can gene expression profiles from whole blood be used to determine response to medical therapy?

In order to assess response to therapy, we have recruited subjects with moderate to severe disease activity based on a Mayo score for UC and a Harvey Bradshaw Index (HBI) for CD. We have enrolled subjects that were naive to anti-TNF agents and then started on anti-TNF therapy (infliximab, adalimumab, or certolizumab). Prior to commencement of anti-TNF therapy, patients underwent evaluation with calculation of Mayo score (without endoscopy subscore) for
UC or HBI for CD, as well as blood draw for baseline gene expression analysis. Response to therapy was determined by change in clinical score, with response to therapy in patients with UC defined as a decrease in modified Mayo score by ≥2 and response to therapy in patients with CD defined as a decrease in the HBI by ≥3.

The response to therapy was calculated on re-evaluation eight weeks after initiation of anti-TNF therapy, utilizing modified Mayo score for UC and HBI for CD. The interval of eight weeks was utilized given prior studies demonstrating a peak clinical response at 8 weeks (18, 22).

ii. Accomplishments towards meeting those aims:

We were funded in December, 2012 and as of February 18, 2015 the achievements for this project to date are categorized by aim of the study as follows.

Aim 1:
Identifying specific genes that are differentially expressed IBD flares may help stratify disease activity. The first aim of this study was to identify panels of genes to be able to distinguish disease activity in CD and UC. Patients were grouped into categories based on disease and severity determined by histological grading. Whole blood was collected by PAXgene Blood RNA collection tubes (PreAnalytiX) and gene expression analysis using mRNA was conducted. Logistic regression was performed on multiple combinations of common probe sets and data was evaluated in terms of discrimination by computing the area under the receiving operator characteristic curve (ROC-AUC).

Nine inactive CD, 8 mild CD, 10 moderate to severe CD, 9 inactive UC, 8 mild UC, 10 moderate to severe UC, and 120 controls were hybridized to Affymetrix U133 Plus 2 micro arrays. Panels of six individual genes discriminated stages of disease activity: CD with mild severity (ROC-AUC 0.89 [95% CI 0.84-0.95]); CD with moderate to severe severity (ROC-AUC 0.98 [95% CI 0.97-1.0]); UC with mild severity (ROC-AUC 0.92 [95% CI 0.87-0.96]); UC with moderate to severe severity (ROC-AUC 0.99 [95% CI 0.97-1.0]). Validation by real-time reverse transcription-polymerase chain reaction confirmed the Affymetrix microarray data.

From our work towards accomplishing aim 1, we were able to draw the following conclusions. The specific whole blood gene panels reliably distinguished CD and UC and determined the activity of disease, with high sensitivity and specificity in our cohorts of patients. This simple serological test has the potential to become a biomarker to determine the activity of disease.

Aim 2:
The original target enrollment for aim 2 was 40 subjects. For this aim, we are conducting gene expression analyses upon starting anti-TNF alpha therapy and two months after commencing therapy. Our initial stated goal was to compare gene expression profiles among responders to anti-TNF therapy to non-responders to anti-TNF therapy in patients with both CD and UC.

Among 25 patients with CD, we have collected paired samples from 18 patients who responded to anti-TNF therapy and 6 patients who were non-responders to anti-TNF therapy, with one
Among 12 patients with UC, we have collected paired samples from 4 patients who responded to anti-TNF therapy and 3 patients who were non-responders to anti-TNF therapy, with 5 patients’ evaluation pending at the time of the writing of this summary.

Thus far, the recruitment of patients with UC has lagged behind recruitments of patients with CD, as evidenced by the higher numbers of both responders and non-responders in the CD group. Based on the ability to collect more pairs of CD patients thus far, we have altered the ultimate goal of aim 2, such that we will now plan to focus our evaluation on responders and non-responders in CD alone. From the CD population, we plan to evaluate an initial test set of 10 pairs of responders comparing the gene expression analysis to that of 10 non-responders. From this initial test set of 10 pairs of responders/non-responders we hope to identify an accurate gene expression profile that can then be used as a biomarker to evaluate a separate training set of 10 responders and 10 non-responders with CD. Although we still plan to evaluate the gene expression profile in responders and non-responders with UC, this will require more funding and a separate proposal. Given the slow recruitment in the UC population, we felt that the ability to evaluate a test set in patients with CD and then develop a confirmatory profile to be used as a training set among patients with CD offered an overall stronger and more clinically meaningful result at this time.

We expect to complete our analysis of the test set of responders/non-responders with CD by April 2015, and then subsequently to complete the analysis of the training set of responders/non-responders with CD by September 1, 2015. Preliminary analysis of 5 responders and 2 non-responders with CD indicate a clustering profile with the ability to differentiate 4 out of 5 responders from non-responders. However, these results represent too small of an comparator set and thus are too premature to allow for full statistical analysis. Thus no concrete conclusions can be made based on this early gene expression analysis.

iii. A list of significant results:

Aim 1:
Previously we published results demonstrating that gene expression profiles from whole blood could differentiate between active an inactive CD and between CD, UC, and non-inflammatory diarrhea. In attempting to accomplish Aim 1, we were able to differentiate the severity of activity in CD and UC versus inactive CD and UC, and controls. We were able to identify combinations of discriminatory genes that were able to differentiate disease activity in both CD and UC. We demonstrated that gene panels were able to reliably distinguish CD and UC with high sensitivity and specificity. Our results were confirmed by real-time polymerase chain reaction.

A total of 152 subjects consented and qualified to participate in the study. After excluding 43 patients, 109 subjects were suitable for gene expression analysis. The 109 subjects comprised of 24 subjects with inactive CD, 19 with mild CD, 10 with moderate to severe CD, 22 with inactive UC, 24 with mild UC, and 10 with moderate to severe UC.
Based on the baseline characteristics, 10 samples from each group having the best match of age, sex, race, duration of disease, and extent of disease (Montreal classification) were selected. The following samples were successfully hybridized to Affymetrix U133 Plus 2 micro arrays: 9 inactive CD, 8 mild CD, 10 moderate to severe CD, 9 inactive UC, 8 mild UC, 10 moderate to severe UC. A few samples were excluded because of poor RNA quality or yield.

RT-PCR was performed on 20 samples with sufficient RNA remaining after the microarray experiment, using primers on a candidate panel of 22 genes selected from Affymetrix microarray experimental data. We compared the predictive performance based on ROC AUC analysis of the PCR data to the matching value based on Affymetrix data. The results were not statistically different, thereby validating the Affymetrix analysis.

The results from the “Null-set” analysis showed virtually no overlap between the distribution of the prediction accuracy for real data and predictions based on randomized disease status (p-value < 0.0001). This confirms that the sample size used in this study was sufficient. The prediction scores are presented in Figures 2 and 3. The sensitivity of the CD mild panel was 88% and the specificity ranged from 76-100% (Figure 2A). Notably the specificity against each of the UC groups was 100%. The CD moderate to severe panel separated samples according to severity by isolating the moderate to severe activity samples from all other samples (mild CD, inactive CD, all UC, and “other”), yielding a sensitivity of 100% and a specificity of 80-100% (100% specificity against CD mild and 89% against CD inactive) (Figure 2B). The sensitivity of the mild UC panel was 88% (Figure 3A). The specificity was 71-100%. The UC moderate to severe panel had a sensitivity of 90% and specificity of 78-100% (100% specificity against UC mild and 89% against UC inactive) (Figure 3B).

The six genes in the CD with mild severity panel were TAP2, ZFAS1, SIAH1, GMPR2, WAPAL, and ZNF45. These genes were able to discriminate mild CD from the other categories with a ROC AUC 0.89 [95% CI 0.84-0.95%], (Figure 4). The six genes in the CD with moderate to severe severity panel were KANSL1, PPP6C, LEPROTL1, MAP3K3, SRA1, and ZNF45 and the ROC AUC was 0.98 [95% CI 0.97-1.0], (Figure 4).

The six genes in the UC with mild severity panel were NLRP12, TAGAP, PDE7A, TNFRSF10C, ROPN1L, and SRA1. These genes were able to discriminate mild UC from the other categories with a ROC AUC of 0.92 [95% CI 0.87-0.96]. For moderate to severe UC, the genes were PGM1, FDFT1, HIST1H3H, CD24, C14orf119, and RTFDC1, and the ROC AUC was 0.99 [95% CI 0.97-1.0], (Figure 4).
Aim 2.
As noted previously, we expect to complete our analysis of the initial set of 10 responders and 10 non-responders with CD by April 2015. We will then subsequently complete the analysis of the training set of responders/non-responders with CD by September 1, 2015. Preliminary analysis of 5 responders and 2 non-responders with CD indicate a clustering profile with the ability to differentiate 4 out of 5 responders from non-responders. However, these results represent too small of an comparator set and thus are too premature to allow for full statistical analysis. Thus no concrete conclusions can be made based on this early gene expression analysis.

iv. A list of publications resulting from the grant:


v. A list of applications submitted to other funding agencies to continue work on this project, including the outcome of those proposals.

N/A
vi. Lay Summary of the Progress Report

Crohn’s Disease (CD) and Ulcerative Colitis (UC) are chronic relapsing inflammatory bowel diseases (IBD) of which the etiologies are not fully understood. Genetic and environmental factors are likely central to both diseases. Many of the advances in our understanding of the biology of IBD, as well as the potential implications for treatment are the result of newer available technologies. Gene expression (GE) profiling is commonly used to examine the differential expression of genes between disease states and normal.

In our initial study presented in this report, we performed mRNA gene expression analysis on peripheral whole blood in patients with CD and UC. Previously we published results demonstrating that gene expression profiles from whole blood could differentiate between active an inactive CD and between CD, UC, and non-inflammatory diarrhea. In this more recent study, we were able to differentiate the severity of activity in CD and UC versus inactive CD and UC, and controls. We were also able to identify combinations of discriminatory genes that were able to differentiate disease activity in both CD and UC. We demonstrated that gene panels were able to reliably distinguish CD and UC with high sensitivity and specificity. Our results were also confirmed by real-time polymerase chain reaction. We feel that the identified gene panels may have great potential as non-invasive biomarkers to determine the activity of disease in IBD patients.

We are using the same methodology in a subsequent study to identify gene panels that can determine response to biologic therapy. We have identified patients with either CD or UC that have not previously been treated with anti-tumor necrosis factor (TNF) medications such as infliximab, adalimumab, or certolizumab. Prior to the initiation of therapy with one of these medications we have attempted to measure the severity of their disease using standard scales and obtained blood samples to be used in gene expression analysis. Eight weeks after the initiation of therapy, we again measured the severity of disease and obtained a second sample of blood to be analyzed via gene expression analysis. With these measures we hope to identify specific patterns in gene expression biomarkers that may allow us to measure response to anti-TNF therapy.