Stool-Derived Eukaryotic RNA (seRNA) Biomarkers for Predicting Therapeutic Response and Monitoring Mucosal Healing for Patients With Inflammatory Bowel Disease

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Introduction

Inflammatory bowel disease (IBD) is a spectrum of difficult to manage diseases including ulcerative colitis (UC) and Crohn's disease (CD). IBD afflicts over 3 million individuals in the United States and has an annual economic burden of over $6.3B.[1,2,3] IBD typically requires a combination of multiple tests to make an initial diagnosis and can take many months to effectively treat.[4,5] Maintaining long-term remission is the goal of treatment to effectively avoid complications, surgery, malignancy, and iatrogenic side effects.[6–8] Currently, methods for diagnosis and assessing treatment efficacy primarily include invasive tests such as colonoscopy and sigmoidoscopy that require inconvenient and uncomfortable bowel preparation.[9]

Existing noninvasive diagnostics within IBD fall into three categories: blood-based protein biomarkers, stool-based protein biomarkers, or stool-based microbiome biomarkers. Serology markers include saccharomyces cerevisiae mannan antibodies, perinuclear anti-neutrophil cytoplasmic antibody, and IgA / IgG antibodies.[12,13] Fecal markers include calprotectin, lactoferrin, and lymphocyte markers.[14] The current intended use, sensitivity, and specificity of these tests are insufficient to assist physicians with predicting response to therapeutics and monitoring mucosal healing during treatment.[10,11]

This work leverages stool RNA biomarkers to explain disease activity, predict therapeutic response, and monitor mucosal healing. Ultimately, these signatures can be used by physicians to better diagnose and treat IBD.

Methods

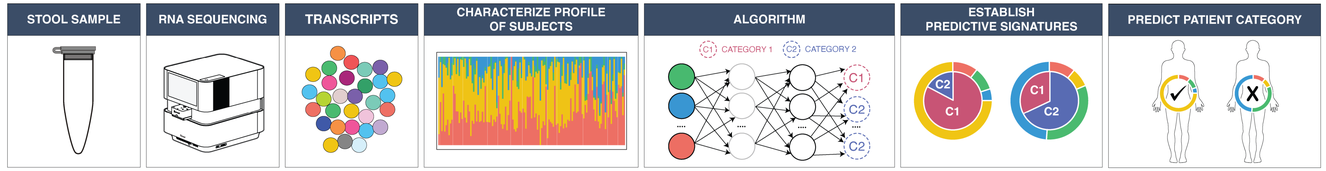
Sample collection – Individuals with refractory Crohn's Disease (CD) (n = 68) or Ulcerative Colitis (UC) (n = 12) were identified for enrollment. Stool samples were collected from individuals prior to treatment (Day 1) and at various timepoints after initiation of treatment (e.g., Day 14 and Day 28).

Therapy selection – For the individuals with CD, targeted therapies employed were based on physician recommendations. For the individuals with UC, patients were randomized to receive either a neutrophil modulator or placebo as part of a Phase 1 clinical trial.

Clinical correlates – For individuals with CD, Crohn's Disease Activity Index (CDAI) was used to determine disease activity. For individuals with UC, endoscopic procedures were used to define response.

seRNA isolation and sequencing – Stool samples collected from individuals at multiple timepoints were subjected to total RNA extraction and next-generation sequencing. Sequencing data was compared to clinical outcomes (CDAI or endoscopic response).

Figures



seRNA classifier models stratify IBD disease severity

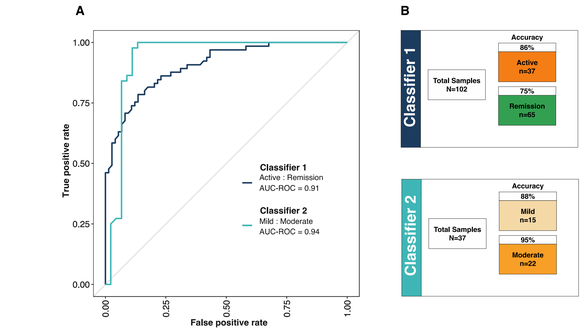


Figure 1. Disease activity classifier AUC-ROC curves
Two random forest binary classifiers were constructed to predict IBD disease activity. Labels were derived using the CDAI score. Features were selected using differentially expressed transcripts, PCA dimensionality reduction, gene set enrichment, and domain knowledge. (**A**) For all subjects, Classifier 1 predicted those with active disease (mild or moderate) relative to those in remission. For subjects with active disease, Classifier 2 predicted subjects as mild or moderate. (**B**) Classifier results are provided by disease activity label.

seRNA signatures reveal the nature of inflammation

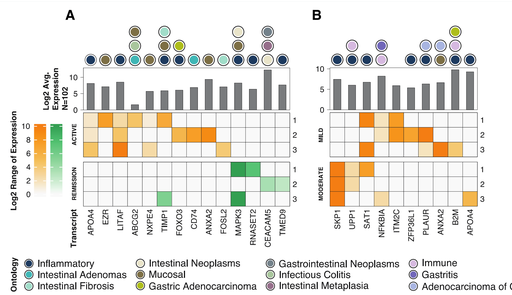


Figure 2. seRNA signatures and ontologies leveraged by the classifier models
Biomarker signatures for each disease activity class (active vs. remission and mild vs. moderate) are shown. Each row in the heatmap is considered a composite biomarker that is comprised of the highlighted transcripts. The top three composite biomarkers for each disease activity class are shown. This plot displays:
1. The log2 average range of expression of each transcript used in constructing the biomarker.
2. The log2 average expression across all samples in the study for each transcript.
3. The gene ontology term associated with each transcript used in each classifier (see **Figure 1**).
Note: Gene ontology terms only signify a general category and do not indicate directionality (increased or decreased) with regards to expression.

Deconvolution of cell type using seRNA biomarkers

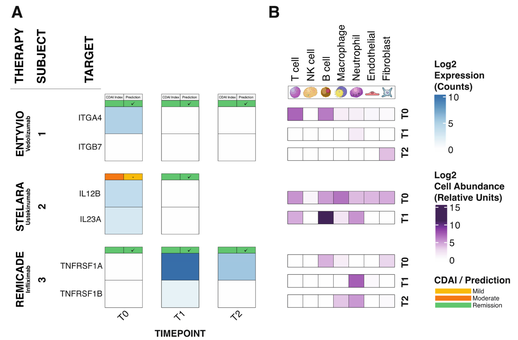


Figure 3. seRNA expression profiles and cell type deconvolution for on-treatment responders to Entyvio, Stelara, Remicade
(**A**) For each of the three subjects, therapy employed, expression of the therapy target (or receptor), and CDAI score with the associated model prediction (see **Figure 1**) are shown. Each column represents a different timepoint for each subject.

(**B**) For each of the three subjects, cell type deconvolution was performed to estimate immune cell abundance using marker genes from the entire expression profile of the sample at each time point. Cell abundance expression has been normalized within an individual sample.

Noninvasive assessment neutrophil-specific treatment

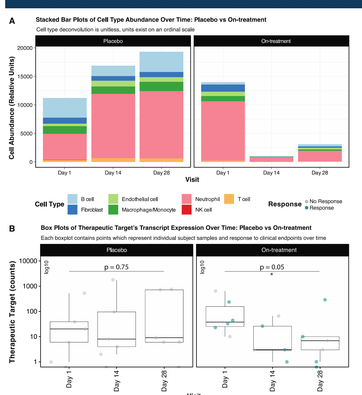


Figure 4. seRNA expression profiles deconvolute cell type for UC patients on placebo and UC patients on-treatment with a neutrophil modulator.
Transcriptome sequencing was performed on stool samples collected from 12 individuals before treatment (Day 1) and after treatment (Day 14 and Day 28). Six participants were treated with a neutrophil modulator and six participants were treated with placebo.

(**A**) Stacked barplots provide deconvolution estimates of immune cell abundance using marker genes from the entire expression profile of the sample at each time point.

(**B**) Boxplots provide expression of the neutrophil modulator target for all samples at three timepoints. Average expression at Day 1 and Day 28 was compared using a Mann Whitney U Test.

Results

Figure 1 – Using seRNA transcripts, two classifiers were generated to accurately predict disease activity for participants with CD (n = 102 stool samples from 68 unique patients). For all individuals, Classifier 1 predicted those with active disease from those in remission with an 86% and 75% accuracy, respectively. For individuals with active disease, Classifier 2 predicted those with mild disease from those with moderate disease with an 86% and 95% accuracy, respectively.

Figure 2 – Transcripts selected by Classifier 1 and 2 leveraged RNA signatures to predict disease activity. The signatures, and associated transcripts, correlated with ontologies that potentially indicated the mechanism of action of the inflammation.

Figure 3 – Three subjects were evaluated to demonstrate longitudinal transcriptome changes as a response to treatment. Subject 1 (on Entyvio), Subject 2 (on Stelara), and Subject 3 (on Remicade) showed decreased expression of the therapy target or increased expression in the therapy target receptor after treatment (T1 or T2) when compared to pre-treatment (T0). Deconvolution of cell types showed changes in immune signature during treatment.

Figure 4 – Relative to subjects on placebo (n = 6), subjects on the neutrophil modulator (n = 6) showed reduction of neutrophils after 14 and 28 days of treatment. Consequently, the expression of the therapy target was significantly decreased for subjects on treatment ($p = 0.05$) relative to those on placebo ($p = 0.75$). 67% of subjects on treatment showed endoscopic response.

Conclusions

Methods presented here demonstrate the ability to consistently detect a wide array of expression signals from isolated eukaryotic stool RNA (seRNA).

These methods preliminarily indicate that seRNA can be used to predict therapeutic response and monitor mucosal healing for patients with IBD.

Stool samples provide a noninvasive method to evaluate gastrointestinal health at frequent intervals during IBD treatment.

References

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