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Increased Expression of Interleukin-13 Receptor in Ileum Associated With Nonresponse to Adalimumab in Ileal Crohn's Disease

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Background: The terminal ileum poses a predilection for Crohn's disease (CD) but is less susceptible to undergo healing to treatment with biologics and small molecules. This study aimed to evaluate histologic features associated with endoscopic remission (ER).

Methods: This is a post hoc analysis of patients with moderately to severely active CD, defined as Crohn's disease activity index 220 to 450, and terminal ileal ulceration treated with antitumor necrosis factor (TNF)-α inhibitor adalimumab from the EXTEND trial. We studied whether baseline total Global Histologic Disease Activity Scores (GHAS), any individual histologic element, and specific immunohistochemical (IHC) markers of chronic inflammation from biopsy specimens were associated with postinduction (week 12) and maintenance (week 52) ER, defined as Simple Endoscopic Score for Crohn's Disease of 0. Multivariable logistic regression models adjusted for confounders were used to assess the relationship between histologic markers and 1-year outcomes.

Results: Seventy-one adult patients with CD affecting the ileum were included in this analysis. Both baseline ileal GHAS scores and individual histologic components were not found to be associated with ER at weeks 12 or 52. Increased expression of interleukin-13 receptor (IL-13R) on IHC stains was associated with reduced likelihood of achieving 1-year ER (adjusted odds ratio, 0.06; 95% CI, 0.01-0.92; $P = .044$). No other biomarker assessed was associated with 1-year ER.

Conclusions: Ileal histologic disease activity and IHC activation markers of chronic mucosal inflammation were not associated with 1-year ER. However, strong staining for IL-13 receptor in the ileum was associated with reduced odds of 1-year ER using adalimumab. Mucosal cellular disease profiles might pose an opportunity to guide treatment of CD.

Lay Summary

In this post hoc analysis, ileal histologic disease activity and IHC activation markers of chronic mucosal inflammation were not predictive of 1-year ER. However, strong staining for IL-13R in the ileum was associated with reduced odds of 1-year ER using adalimumab.

Key Words: Crohn's disease, inflammatory bowel disease, endoscopic remission, histologic remission, biomarkers, histology

Introduction

Crohn's disease (CD) is a chronic inflammatory disorder of the bowel characterized by transmural inflammation, ulceration, stricturing, and penetrating disease and can occur anywhere throughout the digestive tract. Most frequently, disease is located in the terminal ileum and/or colon. Treatment of CD has been revolutionized by biologic therapy, primarily by agents inhibiting tumor necrosis factor-alpha (TNF-α). In accordance with the latest guidelines, long-term therapy should be maintained with a target of clinical and endoscopic remission (ER).[1,2]

In CD, the potential role of treatment goals beyond ER remains unclear. The clinical relevance of histologic disease activity has been questioned repeatedly, with conflicting data on the correlation between histologic remission and ER, sometimes interpreted as sampling error.[2,3] More recently, with the acceptance of histologic end points in ulcerative colitis (UC), a rekindled interest in histologic disease activity in CD is observed. Histologic healing has been suggested to be predictive of clinical relapse-free survival in ileal CD.[4] Although numerous studies have evaluated histology as a predictor of relapse in UC, studies in CD are limited and have included only a small number of patients.[5]

Despite increasing availability of biologic therapies for patients with CD, many fail to respond to treatment. Forty

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Key Messages

- What is already known?

The terminal ileum poses a predilection for Crohn's disease (CD) but is less susceptible to undergo healing to treatment with biologics and small molecules.

- What is new here?

Both baseline ileal GHAS scores and individual histologic components were not found to be predictive of ER at weeks 12 or 52. Increased expression of IL-13-receptor on IHC stains was associated with reduced likelihood of achieving 1-year ER (adjusted odds ratio, 0.06; 95% CI, 0.01-0.92; $P = .044$).

- How can this study help patient care?

Mucosal cellular disease profiles might pose an opportunity to guide treatment of CD.

percent of patients experience primary nonresponse (lack of response to induction therapy), and up to 50% experience secondary loss of response (recurrence of symptoms during maintenance therapy).[6] Across intestinal disease locations, the ileum seems more difficult to heal, and rates of mucosal healing in the ileum are lower than in the colon.[7–10] Predictors of ileal mucosal healing are lacking. The role of histologic disease activity has not yet been explored.

The aim of this study was to assess whether histology or the expression of specific immunohistochemical (IHC) markers at baseline can predict ER in ileal CD.

Methods

Data from a clinical trial of moderate to severe CD was used for this study. Data from the EXTEND study (NCT00348283) was used by permission from Abbvie Inc. and obtained through VIVLI (Protocol #00006041).[11]

Ethics Approval

The Hamilton Integrated Research Ethics Board determined that a local ethics review was not necessary because previously collected and de-identified data were being used; therefore, no informed consent was needed.

Participants

The study design and inclusion criteria for EXTEND has previously been published.[11] Briefly, adult patients with CD who had a Crohn's disease activity score (CDAI) of 220 to 450 and mucosal ulceration on colonoscopy were eligible. Patients had inadequate response to conventional therapy such as immunomodulators or glucocorticoids or previous TNF-α antagonist use (excluding primary nonresponders). All patients were treated with adalimumab induction therapy for 4 weeks, then randomized to ongoing treatment 40 mg of adalimumab every other week or placebo. The entire study duration was 52 weeks. Endoscopy was performed at baseline, at week 12, and at week 52, all of which were centrally read. Histologic samples were obtained at each of these time points, as well. Endoscopic scoring was performed using the Simple Endoscopic Score for Crohn's Disease (SES-CD) in this study.[12] The EXTEND study enrolled 129 patients with baseline endoscopic assessments, but only those with endoscopic ileal involvement (SES-CD >2 in ileum and presence of ulcerations in the ileum) regardless of randomization arm were included in this analysis.

Patient Involvement

Participants were not involved in the study design or conduct.

Data Availability Statement

This publication (Vivli protocol #00006041) is based on research using data from Abbvie Inc. that has been made available through Vivli, Inc. Vivli has not contributed to or approved of nor is responsible for the contents of this publication. Data can be made available upon request from Vivli. All authors had access to the study data and reviewed and approved the final version of the manuscript.

Predictor Items From the Global Histologic Disease Activity Score and Outcomes of Interest

For the current analysis, the baseline individual components within the ileal Global Histologic Disease Activity Score (GHAS) were assessed for their association with ER, which was defined as SES-CD of 0 for the ileum. Endoscopic remission was chosen as the dependent variable because this is a treatment target for CD.[1]

The GHAS consists of 8 separate items: epithelial and architectural changes; inflammatory cell infiltrates (presence of mononuclear cells or polymorphonuclear cells in lamina propria and presence of neutrophils in the epithelium); erosions and ulcers; granulomas; and the extent of inflammation as measured by total number of biopsy specimens affected.[13] Details of scoring are provided in Supplementary Table 1. At each endoscopy, biopsies representative of the worst endoscopic inflammation in the ileum and colon were obtained.

A panel of specific monoclonal antibodies in conjunction with indirect IHC technique using immunoperoxidase was also performed on histologic specimens to detect for presence of certain inflammatory markers and cytokines. Omitting the primary antibody provided the negative control. The positive control was tissue that was known to be positive for these biomarkers. Expression of biomarkers was reported as the median percentage of positive-staining lamina propria mononuclear cells compared in 5 high-powered fields. Staining intensity of biomarkers was scored from 0 to 3 (0, none; 1, weak; 2, moderate; and 3, strong). The presence of these biomarkers at baseline was also evaluated for their association with 1-year ER.

All biopsies were processed and scored by a central pathology reader (Professor Karel Geboes). The IHC data used for this post hoc analysis were largely made possible by the efforts of Professor Geboes. Because the investigation of biomarkers was conducted as part of exploratory analyses, biomarkers were selected per the recommendations of the central reader. Although this analysis was intended to include a greater variety of staining for biomarkers for exploration, due to technical limitations of the lab this was not performed. However, all biomarker analyses done as part of this exploratory analysis are presented in the current manuscript. All data used for the current analysis was obtained from the centrally read histologic scores. Therefore, further details regarding the methodology of IHC staining are unavailable. The IHC images are not presented in the current study, as they are not available.

Statistical Analysis

For each analysis, descriptive statistics were provided and unadjusted odds ratios (ORs) for achieving the outcomes of interest were calculated. Continuous variables were presented as means and standard deviations (SD) or as medians and interquartile ranges (IQRs). Dichotomous variables were presented as proportions or percentages. Multivariate logistic regression models were also used to evaluate the relationship between baseline histologic characteristics and achievement of the outcomes of interest by adjusting for known confounders for ER such as treatment allocation and other variables found to have a *P* value of <0.15 on univariate analyses. Results are presented as ORs and adjusted ORs (aORs) with 95% confidence intervals (CIs). Statistical significance was chosen to be at $P < .05$. Data were analyzed using Stata SE version 15.0.

Results

Demographics

Baseline characteristics from the 127 potentially eligible participants are provided in Supplementary Table 2. From these participants, there were 117 who had ileal histologic information available at baseline, including 113 with ileal and colonic GHAS scores available; 4 had only ileal GHAS scores. The remaining 10 had only colonic GHAS scores available. Out of these 117, 71 patients had active endoscopic ileal involvement (SES-CD >2 in ileum and presence of ulcerations in the ileum) and were included in this analysis. Baseline characteristics for the 71 participants are summarized in Table 1. There were colonic GHAS scores available in 30 participants for the ascending colon, 25 in the transverse colon, 34 in the descending colon, and 34 in the rectum. Due to small sample size for each of these segments, colonic analyses were not performed due to lack of power and lack of information regarding the exact location of the colonic biopsies.

Disease location was isolated to the ileum for 52 (73.2%) of the participants, and 19 had ileocolonic disease. The majority of included participants had medium (0.5-2 cm, 39 of 71 participants, 54.9%) or large (>2 cm, 11 of 71, 15.5%) ulcers present in the ileum, per SES-CD criteria. The baseline SES-CD score of the ileum was 6.5 (SD 2.2). This improved to 3.7 (SD 3.0) by week 12 and was 3.9 (SD 3.3) at week 52. Twelve of 71 (16.9%) participants achieved ER of the ileum at week 52. At baseline, the ileal mean GHAS score was 5.7 (SD 3.2) and improved to 3.2 (SD 2.8) by week 12 (Table 2). At week 52, the ileal mean GHAS score was 2.7 (SD 3.3), and 26 of 71 (36.6%) participants had achieved histologic remission in the ileum (GHAS score of 0).

Impact of Baseline Histologic Ileal Components on Likelihood of Achieving Endoscopic Remission

Table 3 shows results of univariate analyses between baseline variables of interest and 1-year ER. The variables prior anti-TNF-α exposure, baseline ileal SES-CD score, baseline ileal ulcer size, and disease duration had *P* values <0.15 and were selected for inclusion in multivariable logistic regression models. Table 4 provides results of the logistic regression models examining outcomes of patients with endoscopic ileal activity at baseline. When adjusted for treatment allocation, prior anti-TNF-α exposure, baseline ileal SES-CD score, baseline ileal ulcer size, and disease duration, no individual histologic component of the GHAS at baseline had

Table 1. Characteristics of the study population.

Variable	All participants (*n* = 71)	Adalimumab induction only/placebo (*n* = 31)	Adalimumab (*n* = 40)
Age, mean (SD)	36.6 (11.1)	36.6 (11.5)	36.6 (11.0)
Male, *n* (%)	29 (40.9)	13 (41.9)	16 (40.0)
Body mass index, mean (SD)	24.7 (4.9)	24.5 (4.8)	24.9 (5.0)
Caucasian, *n* (%)	70 (98.6)	31 (100.0)	39 (97.5)
Prior anti-TNF-α exposure, *n* (%)	33 (46.5)	15 (48.4)	18 (45.0)
Concomitant immunomodulator use, *n* (%)	26 (36.6)	12 (38.7)	14 (35.0)
Concomitant corticosteroid use, *n* (%)	11 (15.5)	9 (29.0)	2 (5.0)
Disease duration, mean (SD)	10.9 (9.2)	10.5 (9.8)	11.3 (8.9)
Baseline ileal ulcer size as per SES-CD score, *n* (%)			
Small	21 (29.6)	11 (35.5)	10 (25.0)
Medium	39 (54.9)	15 (48.4)	24 (60.0)
Large	11 (15.5)	5 (16.1)	6 (15.0)
Disease location, *n* (%)			
Isolated ileal	52 (73.2)	25 (80.7)	27 (67.5)
Ileocolonic	19 (26.8)	6 (19.4)	13 (32.5)
Baseline hemoglobin (g/L), mean (SD)	132.6 (15.3)	134.4 (15.4)	131.2 (15.3)
Baseline C-reactive protein (g/L), mean (SD)	18.7 (22.4)	18.7 (26.0)	18.8 (19.4)
Baseline CDAI score, mean (SD)	316.4 (62.3)	307.4 (55.3)	323.4 (67.1)
Baseline SES-CD score, mean (SD)	15.8 (9.2)	15.8 (9.3)	15.8 (9.3)
Baseline SES-CD score in ileum, mean (SD)	6.5 (2.2)	6.1 (2.3)	6.8 (2.1)
Baseline ileum GHAS score, mean (SD)	5.7 (3.2)	5.9 (3.5)	5.6 (3.0)
Baseline ileum GHAS score of 0, *n* (%)	10 (14.1)	4 (12.9)	6 (15.0)

Table 2. Endoscopic and histologic outcomes attained by study population at week 12 and one year.

	All participants (n = 71)	Adalimumab induction only/placebo (*n* = 31)	Adalimumab (*n* = 40)
Ileum GHAS score at week 12, mean (SD)	3.2 (2.8)	3.8 (3.0)	2.7 (2.6)
SES-CD score in ileum at week 12, mean (SD)	3.7 (3.0)	5.1 (3.0)	2.4 (2.4)
SES-CD score in ileum at one year, mean (SD)	3.9 (3.3)	4.3 (4.0)	3.5 (2.5)
Ileum GHAS score at one year, mean (SD)	2.7 (3.3)	2.8 (3.2)	2.6 (3.4)
Endoscopic remission of ileum at week 12, *n* (%)	9 (12.7)	2 (6.5)	7 (17.5)
Endoscopic remission of ileum at one year, *n* (%)	12 (16.9)	7 (22.6)	5 (12.5)
Histologic remission of ileum at week 12, *n* (%)[a]	18 (25.4)	7 (22.6)	11 (27.4)
Histologic remission of ileum at one year, *n* (%)[a]	26 (36.6)	11 (35.5)	15 (37.5)

[a]Defined as IGHAS score of 0.

Table 3. Univariate analyses for baseline variables of interest and 1-year ER.

Predictor	Univariate	
	OR (95% CI)	*P*
Disease duration	0.93 (0.85-1.02)	0.113
Disease location (ileocolonic vs isolated ileal)	N/A	N/A
Arm	0.58 (0.16-2.16)	0.420
Concomitant immunomodulator use	0.75 (0.19-2.91)	0.678
Concomitant corticosteroid use	0.52 (0.06-3.76)	0.559
Age	1.00 (0.95-1.05)	0.955
Sex (Female vs Male)	0.84 (0.23-3.13)	0.795
Smoking (Current vs not/never smoker)	0.93 (0.24-3.64)	0.915
Race (Caucasian vs non)	N/A	N/A
Baseline albumin	1.05 (0.90-1.23)	0.516
Baseline CRP	1.00 (0.97-1.03)	0.738
Baseline ileal SES-CD	0.68 (0.49-0.95)	0.025
Baseline medium or large ileal ulcer	0.35 (0.09-1.40)	0.137
Baseline CDAI	1.00 (0.99-1.01)	0.997
BMI	0.99 (0.85-1.15)	0.881
Fistula	0.87 (0.24-3.22)	0.838
Low hemoglobin (<115 g/L)	0.28 (0.03-2.29)	0.236
Prior anti-TNF-α exposure	0.15 (0.03-0.76)	0.023

an association with likelihood of 1-year ER. Specifically, the presence of extensive epithelial damage (P = .259), severe architectural changes (P = .803), moderate/severe mononuclear cell infiltrate (P = .801) and polymorphonuclear cells (0.932) in the lamina propria, epithelial neutrophils (P = .564), erosions or ulceration (P = .929), or extensive number of affected biopsies (P = .934) involved did not have any association with 1-year ER.

Impact of Baseline Histologic Ileal GHAS Scores on Likelihood of Achieving Endoscopic Improvements

Table 5 provides results of the logistic regression models examining whether elevated ileal GHAS scores impact likelihood of achieving ER. When analyzed as a continuous variable and adjusted for treatment allocation, prior anti-TNF-α exposure, baseline ileal SES-CD score, baseline ileal ulcer size, and disease duration, elevation in the ileal GHAS score had no association with likelihood of achieving ER at week 12. Similarly, no association was observed between elevated baseline ileal GHAS scores and 1-year ER.

Baseline Immunohistochemistry Characteristics and Likelihood of Achieving Endoscopic Remission

All inflammatory biomarkers and cytokines that were evaluated in the EXTEND trial were assessed for their association with likelihood to achieve ER, as shown in Table 6. Among the 62 nonremitters at week 12, 41 of 62 (66.1%) had strong baseline interleukin-13 receptor (IL-13R) staining; 17 of 62 (27.4%) had moderate baseline IL-13R staining; and 4 of 62 (6.5%) had weak baseline IL-13R staining. Similarly, among the 59 nonremitters at 1 year, 43 of 59 (72.9%) had strong baseline IL-13R staining; 10 of 59 (16.9%) had moderate staining; and 6 of 59 (10.2%) had weak staining. Patients with strong staining for IL-13R in the ileum had reduced odds for achievement of 1-year ER compared with those with mild or no IL-13R present, once adjusted for other covariates (aOR, 0.06; 95% CI, 0.01-0.92; P = .044). No other significant associations were observed with the other biomarkers and cytokines assessed with 1-year ER.

Discussion

In this post hoc analysis of the EXTEND trial,[11] no association was seen between baseline ileal GHAS scores and likelihood of ER at week 12 or 52. Although individual histologic components as assessed by the GHAS did not appear to be predictive for ER, we observed the presence of high levels of IL-13R on IHC stains of the ileum was associated with less likelihood of achieving ER at 1-year. Understanding factors that impact the ability for the ileum to heal is important, as numerous studies have demonstrated lower rates of ileal healing compared with the colon when using biologic therapies.[8,9]

The factors influencing poor ileal healing are not well understood, but differences in genetic expression, microbiome composition, and immune tolerance may play a role. In a large multicenter study, Cleynen et al[14] demonstrated that predictive models based on genetic risk scores could accurately

Table 4. Impact of baseline histologic components of ileum on achieving 1-year endoscopic remission in the ileum (segment SES-CD of 0; *n* = 71).

Predictor	OR (95% CI)	Unadjusted *P*	Adjusted OR[a] (95% CI)	Adjusted *P*
Epithelial damage		0.516		0.259
Focal	1.89 (0.52-6.82)		0.36 (0.06-2.01)	
Extensive	0.94 (0.14-6.19)		N/A	
Epithelial damage (extensive vs not)	0.61 (0.12-3.14)	0.555	N/A	N/A
Architectural changes		0.943		0.568
Moderate (<50%)	1.20 (0.29-5.07)		0.36 (0.05-2.51)	
Severe (>50%)	0.95 (0.12-7.28)		0.34 (0.02-6.51)	
Architectural changes (severe vs not)	0.82 (0.15-4.37)	0.818	0.72 (0.06-9.21)	0.803
Mononuclear cells in lamina propria (moderate/severe increase vs not)	1.15 (0.39-3.45)	0.800	0.81 (0.16-4.18)	0.801
Polymorphonuclear cells in lamina propria (moderate/severe increase vs not)	0.87 (0.28-2.69)	0.807	1.07 (0.22-5.29)	0.932
Neutrophils in epithelium (cryptits/crypt abscess vs not)	1.33 (0.20-8.71)	0.764	1.94 (0.20-18.47)	0.564
Erosion or ulceration	0.67 (0.16-2.70)	0.570	1.09 (0.18-6.69)	0.929
Granuloma	N/A	N/A	N/A	N/A
Number of affected biopsies		0.966		0.934
<33%	0.88 (0.17-4.54)		0.24 (0.03-2.03)	
33-66%	0.71 (0.14-3.50)		0.41 (0.04-3.87)	
>66%	0.67 (0.08-5.30)		0.16 (0.01-3.19)	
Number of affected biopsies > 33%	0.77 (0.26-2.24)	0.627	0.72 (0.14-3.67)	0.692
Number of affected biopsies > 66%	0.82 (0.15-4.37)	0.818	0.42 (0.03-5.35)	0.505

[a]Adjusted for arm, prior anti-TNF-α exposure, baseline ileal SES-CD score, baseline ileal ulcer size, and disease duration.

Table 5. Impact of ileal GHAS scores at baseline on achieving outcomes among those with isolated ileal or ileocolonic disease (*n* = 71).

Outcome	OR (95% CI)	Unadjusted *P*	Adjusted OR[a] (95% CI)	Adjusted *P*
Endoscopic remission (SES-CD of 0) at 1 year	0.86 (0.71-1.04)	0.128	0.85 (0.67-1.07)	0.164
Endoscopic remission (SES-CD of 0) at week 12	0.97 (0.73-1.28)	0.826	0.67 (0.43-1.05)	0.082

[a]Adjusted for arm, prior anti-TNF-α exposure, baseline ileal SES-CD score, baseline ileal ulcer size, and disease duration.

predict subphenotype across multiple genetic variants, thereby showing that ileal CD had distinct gene expression vs UC, with colonic CD as an intermediate. This suggests a continuum of disorders, identifying ileal CD as a distinct entity. Tyler et al[15] studied microbiome variability across distinct intestinal locations, noting significant changes in concentration of intestinal microbes between ileal mucosa vs sigmoid, afferent, and pouch mucosa. Analysis of paired colonic and ileal samples by Mann et al[16] demonstrated that ileal dendritic cells produced more pro-inflammatory cytokines (TNF-α, IL-1β) vs their colonic counterparts, as well as different expression of regulatory T-cells, demonstrating unique immunity tolerance and imprinting in the ileum vs other intestinal segments.

We observed a discrepancy in baseline GHAS scores and endoscopic disease activity, whereby 14.1% of all participants had GHAS scores of 0, despite inclusion criteria requiring SES-CD >1 and ulceration of the ileum. This discrepancy calls into question the correlation between endoscopic and histologic findings and suggests that there may be a degree of sampling error with respect to number and location of biopsies during ileocolonoscopy, whereby biopsies of normal tissue were obtained in actively diseased segments. Protocols for biopsy sampling in clinical trials are somewhat heterogeneous; although guidance exists regarding a standardized consensus approach in obtaining biopsy samples from UC patients, there remains uncertainty about sampling in CD.[17]

The IL-13R complex is composed of 2 subunits, IL-13RA1 and IL-13RA2. Verstock et al[18] demonstrated that baseline elevations in IL-13RA2 predicted anti-TNF-α nonresponse in patients treated with either infliximab or adalimumab—but not vedolizumab. Furthermore, elevated IL-13RA2 levels correlated with elevated mucosal TNF-α levels, which were more often seen in nonhealers. Interestingly, baseline serum TNF-α were lower in nonhealers. The effect of IL-13RA2 on epithelial cells is well established, but in a study of knockout mice and mucosal biopsies of active IBD patients, Verstock et al[19] demonstrated that expression of IL-13RA2 on epithelial cells negatively influenced goblet cell recovery, goblet cell function, and epithelial restoration after injury. Activation of IL-13RA2 leads to the production of transforming growth factor (TGF-ß), a potent fibrogenetic factor in CD that may lead to aggressive disease.[20]

This study had several strengths. Study outcomes were measured using well-defined clinical, endoscopic, and histologic disease activity scores, namely the CDAI, SES-CD, and

Table 6. Impact of baseline immunohistochemistry analysis of ileum biopsies on achieving endoscopic remission in the ileum (segment SES-CD of 0).

Predictor	OR (95% CI)	Unadjusted *P*	Adjusted OR[a] (95% CI)	Adjusted *P*
IL-13R		0.087		0.143
Weak staining	0.78 (0.22-2.77)		0.29 (0.02-3.51)	
Moderate staining	0.12 (0.01-1.12)		1.36 (0.12-15.38)	
Strong staining	0.11 (0.01-1.02)		0.05 (0.01-0.95)	
IL-13R (strong staining vs not)	0.17 (0.02-1.43)	0.103	0.06 (0.01-0.92)	0.044
IL-13R (moderate or strong staining vs not)	0.13 (0.03-0.63)	0.011	0.46 (0.09-2.47)	0.367
HLA-DR		0.255		0.715
Weak staining	0.48 (0.12-1.81)		1.55 (0.20-12.17)	
Moderate staining	0.33 (0.07-1.54)		0.49 (0.05-4.99)	
Strong staining	0.15 (0.01-1.39)		N/A	
HLA-DR (strong staining vs not)	0.25 (0.03-2.12)	0.203	N/A	N/A
HLA-DR (moderate or strong staining vs not)	0.35 (0.10-1.24)	0.104	0.23 (0.03-1.95)	0.177
HLA-DR (percentage of cells staining)	0.85 (0.75-0.97)	0.012	0.89 (0.78-1.03)	0.120
HLA-DR (≥20% staining vs less)	0.27 (0.07-1.06)	0.061	0.49 (0.08-3.09)	0.451
HLA-DR (≥15% staining vs less)	0.34 (0.11-1.06)	0.064	0.44 (0.09-2.20)	0.320
MPO (percentage of cells staining, continuous)	0.93 (0.86-0.99)	0.046	0.92 (0.83-1.03)	0.162
MPO (≥10% staining vs less)	0.40 (0.13-1.26)	0.118	0.44 (0.07-2.61)	0.363
MPO (≥20% staining vs less)	0.11 (0.01-0.94)	0.043	0.06 (0.01-2.11)	0.123
Tenascin		0.247		0.269
Weak staining	0.33 (0.09-1.21)		0.24 (0.02-3.02)	
Moderate staining	0.70 (0.15-3.29)		N/A	
Strong staining	N/A		N/A	
Tenascin (moderate/strong vs not)	0.84 (0.20-3.55)	0.818	N/A	N/A
TNF-α(percentage of cells staining, continuous)	0.87 (0.76-1.00)	0.051	0.98 (0.84-1.14)	0.799
TNF-α (≥5% staining vs less)	0.81 (0.26-2.49)	0.715	0.86 (0.16-4.59)	0.855
TNF-α (≥10% staining vs less)	0.32 (0.08-1.27)	0.106	0.87 (0.16-4.74)	0.868

[a]Adjusted for arm, prior anti-TNF-α exposure, baseline ileal SES-CD score, baseline ileal ulcer size, and disease duration.

GHAS. Patients' endoscopic disease activity at week 12 and week 52 and all histologic assessments were centrally read to reduce bias. Endoscopic and histologic assessment was done at prespecified intervals, providing measures of disease activity at baseline, postinduction, and again at 1-year, allowing for likely adequate time to assess endoscopic outcomes. Furthermore, the inclusion of several patients with ileal disease specifically allowed us to answer questions about a distinct population seen in clinical practice that has been historically difficult to study and treat. We observed consistent associations and adjusted for numerous confounders of endoscopic healing, including treatment received and disease duration.

Findings of this study may not be generalizable, as all patients were treated with adalimumab; therefore, further confirmation of these findings using cohorts treated with alternative biologics, small molecule inhibitors, or anti-inflammatory medications are needed. Another limitation is that study participants were predominantly Caucasian, possibly reducing the applicability to more diverse populations. Further, patients in the EXTEND study were randomized to placebo or adalimumab at week 4 and were included in the current analysis, which evaluated outcomes at week 12 and 1 year. Further analyses stratified by arm would be ideal, but given the small samples size, this was not feasible. Other histological markers not included in the GHAS or IHC markers not analyzed within this study may also be predictive of treatment response but have not been considered in this analysis due to technical limitations. Furthermore, we present the results of several different comparisons, and there remains a small probability that some significant results are due to chance alone (multiple comparisons). Details that may inform the reproducibility of this study, such as the source and clones of antibodies used for IHC, are unavailable. Further, the only significant association in our study was relatively weak. Our study was meant to be hypothesis-generating, and correction for multiplicity was not performed. Thus, statistically significant findings in our study should not be considered confirmatory, but rather as having a potential association with the outcome. Further studies that are adequately powered are warranted to confirm our findings. Additionally, differentiation between intensity of staining (eg, strong vs moderate) was subjective, as all biopsies were read by a single reader. Additionally, our study does not differentiate between the subunits of the IL-13R complex; therefore, we cannot confirm whether anti-TNF-α nonresponse is mediated by the expression of the IL-13RA1 or IL-13RA2 subunit—or both. However, as IL-13RA2 is membrane-bound yet lacks a cytoplasmic domain, this suggests it may not be a signal mediator. Verstockt et al have previously reported the expression of IL-13RA2 as a predictor of anti-TNF-α nonresponse.[18] Further studies are needed to better understand the mechanism of anti-TNF-α nonresponse, particularly the dichotomy between

IL-13RA1 and IL-13RA2. Inasmuch as this was a post hoc analysis with a relatively small sample size, findings from this study should be considered hypothesis-generating at this stage and should be validated in larger cohorts of patients with ileal CD. In addition, we did not use unsupervised learning models, which can be effective in identifying potential biomarkers of interest. Techniques such as clustering and dimensionality reduction have been used in omics research to reveal molecular patterns in IBD pathogenesis using existing patient-level data to predict IBD outcomes.[21] Future studies should use a more rigorous and systematic approach to biomarker selection and consider unsupervised machine-learning approaches. It remains unknown whether some of these biomarkers are implicated in the pathogenesis of CD and whether targeting some of these biomarkers can be used as a treatment strategy.

In conclusion, our study suggests that histologic assessment at baseline does not appear to be associated with likelihood of ileal ER in patients with CD. However, we observed that strong staining of IL-13R in the ileum was associated with a reduced odds of achieving 1-year ER. Early identification of anti-TNF-α nonresponders may allow clinicians to identify if an alternative treatment strategy may be preferable and possibly lead to improved abilities to achieve treatment targets and improve patient outcomes. Further research is needed to identify and confirm histologic, molecular, and genetic factors that predict individualized patient response to specific treatments in CD.

Supplementary Data

Supplementary data is available at *Inflammatory Bowel Diseases* online.

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Author Contribution

E.C.L.W.—acquisition and compilation of data; statistical analysis; drafting of the manuscript;

A.Y.—study design; drafting of the manuscript

J.P.—study design; drafting of the manuscript

P.S.D.—study concept and design; statistical analysis; data interpretation; drafting of the manuscript;

J.F.C.—study design; drafting of the manuscript

J.K.M.—study design; drafting of the manuscript

W.R.—study concept and design; acquisition and compilation of data; data interpretation; drafting of the manuscript

N.N.—study concept and design; acquisition and compilation of data; statistical analysis; data interpretation; drafting of the manuscript

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Data Availability

Data can be made available upon request to third parties.

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