

The Effect of Etrasimod on Fecal Calprotectin and High-sensitivity C-reactive Protein: Results From the ELEVATE UC Clinical Program

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Background: Biomarkers offer potential alternatives to endoscopies in monitoring ulcerative colitis (UC) progression and therapeutic response. This post hoc analysis of the ELEVATE UC clinical program assessed potential predictive values of fecal calprotectin (fCAL) and high-sensitivity C-reactive protein (hsCRP) as biomarkers and associated responses to etrasimod, an oral, once-daily, selective sphingosine 1-phosphate (S1P)_{1,4,5} receptor modulator for the treatment of moderately to severely active UC, in 2 phase 3 clinical trials.

Methods: In ELEVATE UC 52 and ELEVATE UC 12, patients were randomized 2:1 to 2 mg of etrasimod once daily or placebo for 52 or 12 weeks, respectively. Fecal calprotectin/hsCRP differences between responders and nonresponders for efficacy end points (clinical remission, clinical response, endoscopic improvement-histologic remission [EIHR]) were assessed by Wilcoxon *P*-values. Sensitivity and specificity were presented as receiver operating characteristics (ROC) curves with area under the curve (AUC).

Results: In ELEVATE UC 52 and ELEVATE UC 12, 289 and 238 patients received etrasimod and 144 and 116 received placebo, respectively. Baseline fCAL/hsCRP concentrations were generally balanced. Both trials had lower week-12 median fCAL levels in week-12 responders vs nonresponders receiving etrasimod for clinical remission, clinical response, and EIHR (all *P* < .001), with similar trends for hsCRP levels (all *P* < .01). For etrasimod, AUCs for fCAL/hsCRP and EIHR were 0.85/0.74 (week 12; ELEVATE UC 52), 0.83/0.69 (week 52; ELEVATE UC 52), and 0.80/0.65 (week 12; ELEVATE UC 12).

Conclusions: Fecal calprotectin/hsCRP levels decreased with etrasimod treatment; ROC analyses indicated a prognostic correlation between fCAL changes during induction and short-/long-term treatment response.

Lay Summary

We show associations between fecal calprotectin (fCAL) and high-sensitivity C-reactive protein (hsCRP) levels with efficacy outcomes among patients receiving 2 mg of etrasimod once daily, and that fCAL levels may be an early indicator of the achievement of long-term efficacy end point achievement.

Key Words: ulcerative colitis, biomarker, etrasimod, sphingosine 1-phosphate, phase 3 clinical trial.

Key Messages

What is already known?

Etrasimod is a once-daily, oral sphingosine 1-phosphate (S1P)_{1,4,5} receptor modulator for the treatment of moderately to severely active ulcerative colitis (UC).

What is new here?

We show associations between fecal calprotectin (fCAL) and high-sensitivity C-reactive protein (hsCRP) levels with efficacy end points among patients treated with etrasimod and that fCAL levels may be an early indicator of long-term efficacy end point achievement.

How can this study help patient care?

The use of fCAL as a biomarker for efficacy outcomes in patients with UC treated with etrasimod could reduce the need for invasive endoscopy procedures.

Introduction

Ulcerative colitis (UC) is a chronic inflammatory disease caused by continuous mucosal colonic inflammation and characterized by intermittent symptom recurrence that may include bowel urgency, increased frequency of bowel movements, abdominal pain, and rectal bleeding.^{1,2} Treatment for UC aims to achieve symptomatic control and improve the appearance of the mucosa on endoscopy.³ Endoscopy with biopsies is the only way to establish a diagnosis of UC and, given that endoscopic healing is associated with improved remission rates and reduced risk of colectomy, is key in assessing disease severity.¹ However, repeated endoscopic assessments in routine clinical practice are invasive, expensive, and may be impractical, leaving a need to explore noninvasive alternatives for disease monitoring.⁴

The identification of reliable, noninvasive biomarkers for UC disease activity can provide a surrogate to invasive endoscopy procedures.⁵ Fecal calprotectin (fCAL) is a complex consisting of calcium-binding proteins used as a biomarker of intestinal inflammation and is expressed by activated neutrophils (and, to a lesser extent, by macrophages and monocytes); fecal levels correlate with the number of neutrophils in the gut. fCAL has thus demonstrated utility as a monitoring biomarker in patients with UC.⁶ Meanwhile, high sensitivity C-reactive protein (hsCRP) is an acute phase protein expressed by hepatocytes in response to inflammatory cytokines. Recent American Gastroenterological Association (AGA) guidelines recommend monitoring fCAL as a reliable biomarker to determine treatment response and endoscopy assessment, depending on the individual patient's medical history.⁴ Additionally, recent updates to treatment target statements from the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE-II) initiative recommend normalization of fCAL (to 100-250 µg/g) and hsCRP (to values under the upper limit of normal [2.87 mg/L]⁷) as intermediate treatment targets in UC.³ Both fCAL and hsCRP have been shown to reflect clinical and histologic findings as observed in the phase 2 trials of patients with UC treated with ozanimod⁸ and tofacitinib.⁵ fCAL concentrations may be a predictor of clinical relapse in patients with UC,⁹ and,

although nonspecific, fCAL is specific to the gastrointestinal tract.¹⁰ Conversely, hsCRP is a widely used surrogate biomarker in UC but may only be useful for cases of acute severe UC² and can be influenced by extraneous noninflammatory factors such as age, sex, and body mass index.^{11,12}

Etrasimod is an oral, once-daily, selective sphingosine 1-phosphate (S1P)_{1,4,5} receptor modulator for the treatment of moderately to severely active UC. The S1P₁ receptor modulation regulates the trafficking of specific lymphocyte subsets out of the lymph nodes, leaving fewer peripheral immune cells available to traffic to sites of inflammation.¹³⁻¹⁵ Sphingosine 1-phosphate₄ has been implicated in dendritic cell trafficking, and S1P₅ in both the localization of natural killer cells and the egress of patrolling monocytes from bone marrow; however, their functions are not well understood.^{13,16} In the ELEVATE UC clinical program, the efficacy and safety of etrasimod in patients with moderately to severely active UC were demonstrated in a phase 3 induction and maintenance trial with a treat-through design (ELEVATE UC 52; NCT03945188) and a phase 3 induction trial (ELEVATE UC 12; NCT03996369).¹³ The clinical program found that greater proportions of patients receiving 2 mg of etrasimod once daily (QD) achieved the primary efficacy end point of clinical remission vs placebo, in addition to meeting all key secondary end points, in ELEVATE UC 52 at both weeks 12 and 52, and at week 12 in ELEVATE UC 12.¹³

This post hoc analysis assessed the potential predictive value of fCAL and hsCRP and their associations with responses to etrasimod among patients in the ELEVATE UC clinical program.

Methods

Patients and Trial Design

The trial designs and full inclusion and exclusion criteria for ELEVATE UC 52 and ELEVATE UC 12 were as previously described.¹³ Briefly, in 2 multicenter, randomized, double-blind, placebo-controlled studies, patients aged 16 to 80 years with moderately to severely active UC were randomized 2:1 to receive either 2 mg of etrasimod QD or placebo for 52 weeks or 12 weeks for ELEVATE UC 52 and ELEVATE UC 12, respectively. ELEVATE UC 52 featured a treat-through design comprising a 12-week induction period, followed by a 40-week maintenance period. ELEVATE UC 12 comprised an independent 12-week induction period. Patients were permitted to continue therapeutic doses of nonbiologic therapy for UC (oral 5-aminosalicylates, oral corticosteroids, or medicinal probiotics), provided the dose was stable prior to randomization and during treatment periods. In ELEVATE UC 52, patients receiving oral corticosteroid therapy at baseline were required to maintain their stable dose during the 12-week induction period; investigators were directed to taper corticosteroids after the week 12 assessment.

fCAL, hsCRP and Clinical Efficacy Measurement and End Points

For both the ELEVATE UC 52 and ELEVATE UC 12 trials, baseline fCAL was measured during the screening period and at weeks 2, 4, 8, and 12. For ELEVATE UC 52, fCAL was also measured at weeks 24 and 52. The hsCRP was measured during the screening period, on day 1 (baseline), and at weeks 2, 4, 8, and 12, and at 2-week follow-up. For ELEVATE UC

52, hsCRP was additionally measured at weeks 16, 20, 24, 32, 40, 48, and 52, and at 2-week follow-up. Sample handling is described in the [Supplementary Methods](#).

The primary efficacy end points of ELEVATE UC 52 and ELEVATE UC 12 were the proportion of patients who achieved clinical remission at weeks 12 (both studies) and 52 (ELEVATE UC 52 only).¹³ Efficacy end points assessed in this post hoc analysis were clinical remission, clinical response, and endoscopic improvement-histologic remission (EIHR). For completeness, endoscopic improvement and histologic remission were also analyzed separately. Clinical remission was defined as stool frequency (SF) of the following levels: subscore = 0, or = 1 with a ≥ 1 -point decrease from baseline; rectal bleeding (RB) subscore = 0; and endoscopic subscore (ES) ≤ 1 (excluding friability). Clinical response was defined as a ≥ 2 -point and $\geq 30\%$ decrease from baseline in Modified Mayo score (MMS) and a ≥ 1 -point decrease from baseline in RB subscore or an absolute RB subscore ≤ 1 . Endoscopic improvement-histologic remission was defined as an ES of ≤ 1 with histologic remission measured by Geboes Index score (< 2.0). Endoscopic improvement and histologic remission were separately defined as an ES of ≤ 1 and as Geboes Index score < 2.0 , respectively. Patients were classified as responders or nonresponders based on the criteria for each efficacy end point.

Statistical Analysis

Data from patients with a baseline MMS of 4 to 9 in the full analysis set (FAS) populations of ELEVATE UC 52 and ELEVATE UC 12 were analyzed. The FAS included all randomized patients who received ≥ 1 dose of trial treatment. Median fCAL and hsCRP levels at protocol-specified visits were analyzed by visit and by treatment group, comparing responders vs nonresponders for clinical efficacy end points separately for the 2 trials.

For responder vs nonresponder analyses, 2-sided *P* values were based on the Wilcoxon rank-sum test comparing the distributions (medians) of fCAL and hsCRP concentrations at a given visit between patients who responded to treatment, as assessed by the achievement of clinical end points compared with those who did not respond in a treatment arm at the same visit.

The sensitivity and specificity of predicting a response in a clinical end point at a visit were calculated based on fCAL and hsCRP concentration and presented as receiver operating characteristic (ROC) curves with area under the curve (AUC). Youden Index was estimated as sensitivity + specificity - 1. A higher Youden Index value indicates a better balance of sensitivity and specificity.¹⁷ Sensitivity and specificity were also calculated at different prespecified cut-offs for fCAL and hsCRP concentrations based on the estimated ROC curves. For each clinical efficacy end point at a visit and for each treatment group separately, a logistic regression model was fitted to evaluate the association between efficacy end point and biomarker value at the same visit as the only covariate. The AUC for each ROC curve was calculated from this model.

Based on Youden Indexes for different efficacy end points, another logistic regression model was fitted to clinical efficacy end points at a visit on dichotomized fCAL ≤ 250 $\mu\text{g/g}$ (yes/no) as the only covariate, providing odds ratios (ORs) and 95% confidence intervals (CIs), in addition to descriptive statistics.

For all analyses described previously, missing fCAL values at weeks 12 and 52 were imputed using the LOCF method; missing baseline fCAL values were not imputed. Missing binary clinical end point data were handled by setting to nonresponse. Data from ELEVATE UC 52 and ELEVATE UC 12 were analyzed separately.

Results

Patient Demographics and Baseline Characteristics

In ELEVATE UC 52 and ELEVATE UC 12, 289 and 238 patients received etrasimod, and 144 and 116 received placebo, respectively. Patient demographics and baseline characteristics were generally similar between treatment groups in both ELEVATE UC 52 and ELEVATE UC 12 ([Table 1](#)). The median (range) age of patients enrolled in both trials ranged from 35.5 years (17-78 years; placebo; ELEVATE UC 52) to 40 years (18-78 years; etrasimod; ELEVATE UC 52). Male sex accounted for between 52.6% (etrasimod; ELEVATE UC 52) and 62.9% (placebo; ELEVATE UC 12) of patients ([Table 1](#)). Median (range) baseline fCAL concentrations were 1135.7 $\mu\text{g/g}$ (5.4-46,189.8 $\mu\text{g/g}$; placebo; ELEVATE UC 52), 1084.1 $\mu\text{g/g}$ (3.8-33,464.1 $\mu\text{g/g}$; etrasimod; ELEVATE UC 52), 834.6 $\mu\text{g/g}$ (4.2-29,656.0 $\mu\text{g/g}$; placebo; ELEVATE UC 12), and 953.5 $\mu\text{g/g}$ (7.9-41,794.2 $\mu\text{g/g}$; etrasimod; ELEVATE UC 12). Median (range) baseline hsCRP were 3.2 mg/L (0.3-108.4 mg/L; placebo; ELEVATE UC 52), 4.3 mg/L (0.2-102.4 mg/L; etrasimod; ELEVATE UC 52), 2.7 mg/L (0.2-96.7 mg/L; placebo; ELEVATE UC 12), and 3.5 mg/L (0.2-84.6 mg/L; etrasimod; ELEVATE UC 12).

Median Absolute fCAL and hsCRP Values Over Time

In ELEVATE UC 52, significant reductions in median fCAL concentrations were observed as early as week 4 among patients in the FAS receiving etrasimod compared with those receiving placebo ([Supplementary Figure 1A](#)); significant differences were maintained through week 12. Although there were numerical differences between patients receiving etrasimod compared with placebo at week 52, the differences between treatment arms were not significant. An overall reduction from baseline in median fCAL was also observed in patients receiving placebo ([Supplementary Figure 1A](#)).

In ELEVATE UC 12, median fCAL decreased over time for patients receiving either etrasimod or placebo. No significant differences were observed over the trial duration between treatment arms, although median fCAL levels were generally lower in patients receiving etrasimod compared with placebo ([Supplementary Figure 1A](#)).

Median hsCRP concentrations trended downward over time for patients receiving etrasimod in both ELEVATE UC 52 and ELEVATE UC 12. Median hsCRP concentrations also trended downward over time for patients receiving placebo in ELEVATE UC 52, but no change was observed among those receiving placebo in ELEVATE UC 12 ([Supplementary Figure 1B](#)).

fCAL and hsCRP Levels in Responders vs Nonresponders to Etrasimod and Placebo Treatments

When patients receiving etrasimod were stratified by treatment response, median levels at the time-of-response

Table 1. Patient demographics and baseline characteristics (FAS, MMS 4-9).

	ELEVATE UC 52		ELEVATE UC 12	
	Placebo (N = 144)	2 mg of etrasimod QD (N = 289)	Placebo (N = 116)	2 mg of etrasimod QD (N = 238)
Age, years; median (range)	35.5 (17-78)	40 (18-78)	38.0 (17-72)	37.5 (16-73)
Male, <i>n</i> (%)	88 (61.1)	152 (52.6)	73 (62.9)	135 (56.7)
Race, White; <i>n</i> (%)	129 (89.6)	256 (88.6)	88 (75.9)	176 (73.9)
BMI, kg/m ² ; median (range)	25.2 (16.2-49.7)	24.5 (13.0-51.1)	24.3 (15.4-39.1)	23.5 (15.8-40.4)
Duration of UC, years; median (range)	4.5 (0.2-30.8)	4.7 (0.3-37.9)	4.8 (0-35)	4.7 (0-31)
Extent of disease; <i>n</i> (%)				
Left-sided colitis/ proctosigmoiditis	90 (62.5)	172 (59.5)	63 (54.3)	146 (61.3)
Pancolitis	47 (32.6)	93 (32.2)	41 (35.3)	77 (32.4)
Proctitis	6 (4.2)	22 (7.6)	12 (10.3)	15 (6.3)
MMS, median (range)	7.0 (4.0-9.0)	7.0 (4.0-9.0)	7.0 (4.0-9.0)	7.0 (4.0-9.0)
Naïve to biologic/JAKi therapy, <i>n</i> (%)				
No	45 (31.3)	84 (29.1)	43 (37.1)	89 (37.4)
Yes	99 (68.8)	205 (70.9)	73 (62.9)	149 (62.6)
Prior immunomodulator use, ^a <i>n</i> (%)				
No	95 (66.0)	181 (62.6)	67 (57.8)	149 (62.6)
Yes	49 (34.0)	108 (37.4)	49 (42.2)	89 (37.4)
Corticosteroids use (weeks) over prior 12 months median (range)	8.0 (0-56)	8.0 (0-57)	8.0 (0-54)	8.0 (0-52)
Baseline corticosteroid use, <i>n</i> (%)				
No	102 (70.8)	196 (67.8)	82 (70.7)	173 (72.7)
Yes	42 (29.2)	93 (32.2)	34 (29.3)	65 (27.3)
Baseline MMS group, <i>n</i> (%)				
4-6	57 (39.6)	113 (39.1)	53 (45.7)	109 (45.8)
7-9	87 (60.4)	176 (60.9)	63 (54.3)	129 (54.2)
Baseline ES, <i>n</i> (%)				
2	56 (38.9)	123 (43.6)	56 (48.3)	109 (45.8)
3	88 (61.1)	163 (56.4)	60 (51.7)	129 (54.2)
fCAL, ^b µg/g; median (range)	1135.7 (5.4-46,189.8)	1084.1 (3.8-33,464.1)	834.6 (4.2-29,656.0)	953.5 (7.9-41,794.2)
hsCRP, mg/L; median (range)	3.2 (0.3-108.4)	4.3 (0.2-102.4)	2.7 (0.2-96.7)	3.5 (0.2-84.6)

^aImmunomodulators include azathioprine and/or mercaptopurine only.

^bBaseline groups for fCAL values: ELEVATE UC 52: placebo, *n* = 143; etrasimod, *n* = 286; ELEVATE UC 12: placebo, *n* = 114; etrasimod, *n* = 238.

Full demographics and baseline characteristics were presented previously.¹³

Baseline was the last measurement taken prior to the first dose of trial treatment.

Abbreviations: BMI, body mass index; ES, endoscopic subscore; FAS, full analysis set; fCAL, fecal calprotectin; hsCRP, high-sensitivity C-reactive protein; JAKi, Janus kinase inhibitor; MMS, Modified Mayo score; N, number of patients in cohort; *n*, number of patients with characteristic; QD, once daily; UC, ulcerative colitis.

assessment of fCAL were significantly lower in responders compared with nonresponders in both ELEVATE UC 52 and ELEVATE UC 12, indicating a correlation between fCAL concentration and achievement of end points (Figure 1). This was observed across clinical remission, EIHR, and clinical response end points at weeks 12 and 52 (all *P* < .01; Figure 1), and separately for endoscopic improvement and histologic remission (all *P* < .01; Supplementary Table 1). This correlation was also seen in patients receiving placebo; median fCAL levels were significantly lower in patients treated with placebo and achieving all efficacy end points compared with those who did not (Figure 1; Supplementary Table 1).

Likewise, median hsCRP values were significantly lower in responders compared with nonresponders among patients

receiving etrasimod in both ELEVATE UC 52 and ELEVATE UC 12 across clinical remission, EIHR, and clinical response end points (all *P* < .01; Figure 2). Consistent with fCAL, this correlation was also observed among patients receiving placebo. However, there was no significant difference between hsCRP levels in EIHR responders and nonresponders among patients receiving placebo in ELEVATE UC 12 at week 12 (Figure 2A).

Relationship Between Efficacy End Points and fCAL and hsCRP in Etrasimod and Placebo Treatments

The prognostic correlation between fCAL and hsCRP in patients treated with etrasimod and clinical remission and EIHR end points is presented in Figure 3 and clinical response end point in Supplementary Figure 2.

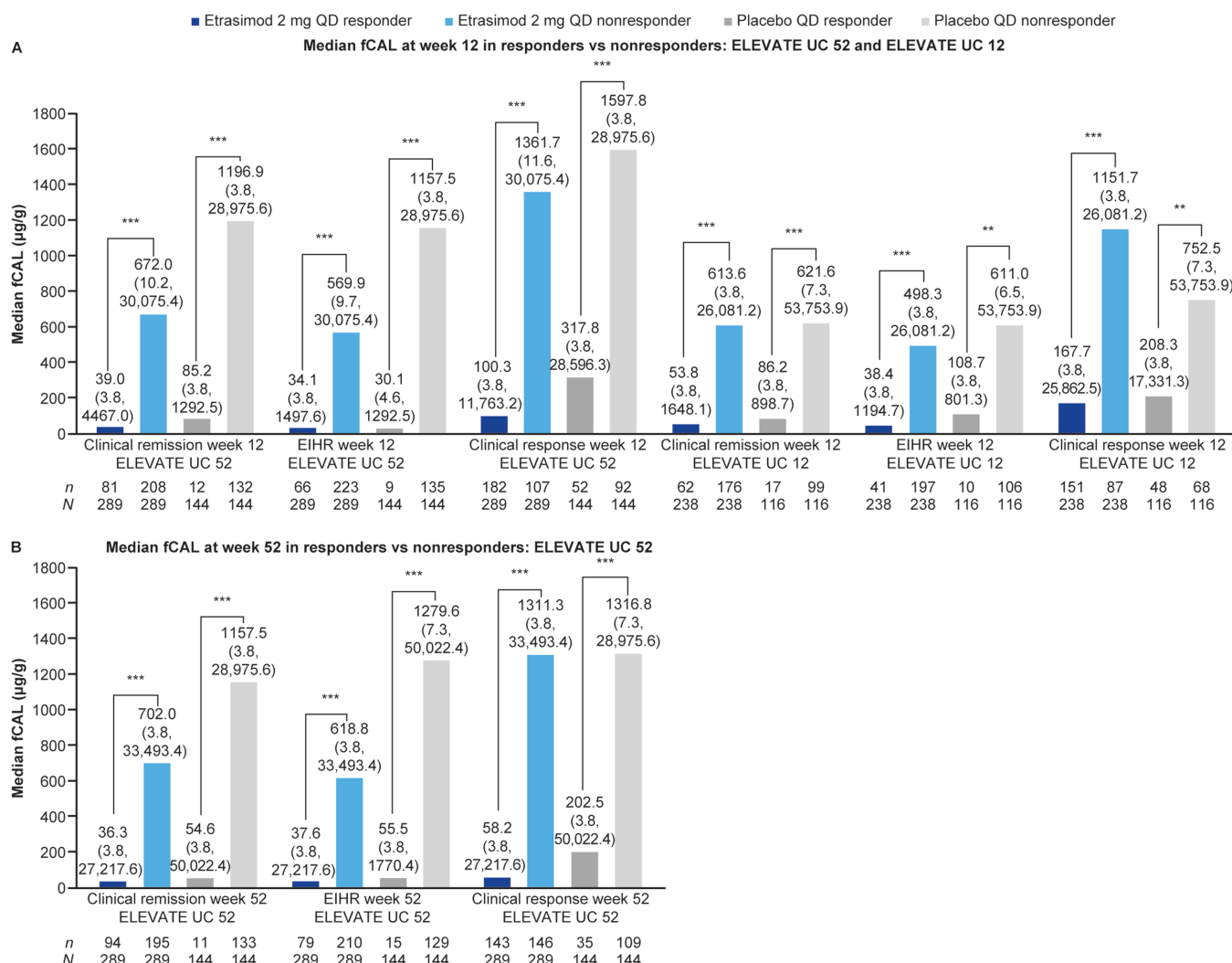


Figure 1. Median (range) fCAL (µg/g) by responders and nonresponders receiving etrasimod and placebo in (A) ELEVATE UC 52 at week 12 and week 52 and (B) ELEVATE UC 12 at week 12 (FAS, baseline MMS 4-9). ** $P < .01$; *** $P < .001$. P values were based on the Wilcoxon rank-sum test comparing the distributions (medians) of fCAL between responders and nonresponders within each treatment arm for each end point. Abbreviations: EIHR, endoscopic improvement-histologic remission; FAS, full analysis set; fCAL, fecal calprotectin; MMS, Modified Mayo score; n , number of responders/nonresponders for the end point; N , number of patients in cohort; QD, once daily; UC, ulcerative colitis.

In ELEVATE UC 52, the AUC for the correlation between clinical remission and fCAL was 0.8210 at week 12 and 0.8379 at week 52 (Figure 3A and 3B; Supplementary Table 2). The AUC for the correlation between EIHR and fCAL was 0.8452 at week 12 and 0.8265 at week 52 (Figure 3A and 3B; Supplementary Table 2). Similarly, the AUC for the correlation between clinical remission and fCAL in ELEVATE UC 12 was 0.8185 at week 12 (Figure 3C; Supplementary Table 2) and 0.8049 for the correlation between EIHR and fCAL (Figure 3C; Supplementary Table 2).

The AUC for the correlation between clinical remission and hsCRP in ELEVATE UC 52 was 0.7542 and 0.7469 at week 12 and week 52, respectively (Figure 3D and 3E; Supplementary Table 2); the correlation between EIHR and hsCRP was 0.7379 and 0.6945 at week 12 and week 52, respectively (Figure 3D and 3E; Supplementary Table 2). In ELEVATE UC 12, the AUC for the correlation between each of these end points and hsCRP at week 12 was 0.6838 and 0.6520, respectively (Figure 3F; Supplementary Table 2).

Similar relationships were observed with these biomarkers and the clinical response end point (Supplementary Figure 2 and Supplementary Table 2). Consistent with the clinical remission and EIHR end points, AUC values for fCAL and clinical response were generally greater than the corresponding AUC values observed with hsCRP (Supplementary Figure 2 and Supplementary Table 2).

The prognostic correlations between fCAL and hsCRP and the efficacy end points in patients receiving placebo were generally consistent with the corresponding relationships observed in patients receiving etrasimod, with the exception of fCAL and clinical remission at week 52 in ELEVATE UC 52 (Supplementary Table 2).

For clinical remission at week 12, fCAL concentrations of 350 µg/g and 300 µg/g provided the highest Youden Indexes of 0.512 and 0.552 among patients receiving etrasimod in ELEVATE UC 52 and ELEVATE UC 12, respectively. For clinical remission at week 52, an fCAL concentration of 250 µg/g provided the highest Youden Index of 0.502 among patients

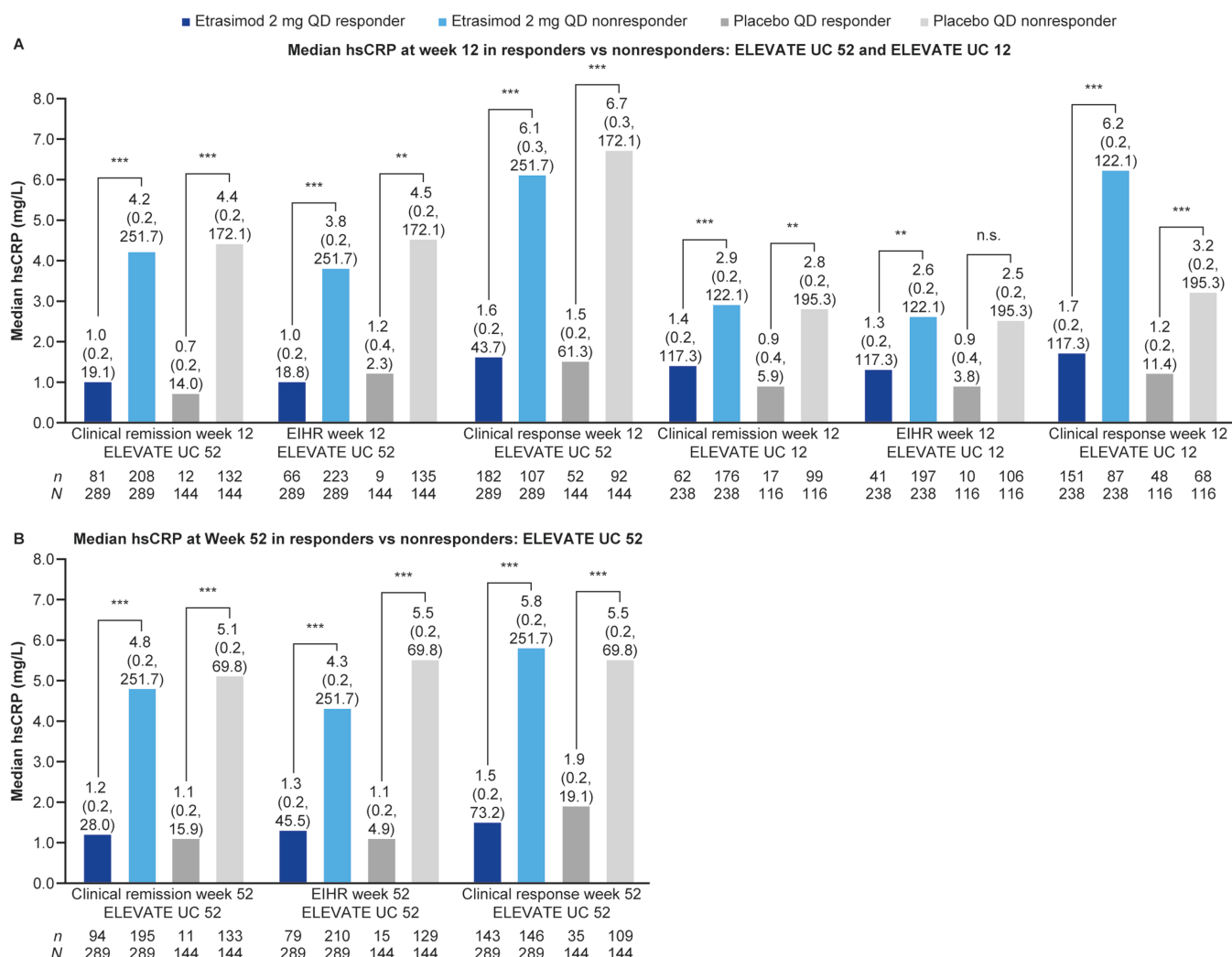


Figure 2. Median (range) hsCRP (mg/L) by responders and nonresponders receiving etrasimod or placebo in (A) ELEVATE UC 52 at week 12 and week 52 and (B) ELEVATE UC 12 at week 12 (FAS, baseline MMS 4-9). ** $P < .01$; *** $P < .001$. P values were based on the Wilcoxon rank-sum test comparing the distributions (medians) of hsCRP concentrations between the response and nonresponse groups within each treatment arm for each end point. Abbreviations: EIHR, endoscopic improvement-histologic remission; FAS, full analysis set; hsCRP, high-sensitivity C-reactive protein; MMS, Modified Mayo score; n , number of responders/nonresponders for the end point; N , number of patients in cohort; n.s., nonsignificant; QD, once daily; UC, ulcerative colitis.

receiving etrasimod in ELEVATE UC 52 (Supplementary Table 3).

For EIHR at week 12, fCAL concentrations of 100 $\mu\text{g/g}$ and 300 $\mu\text{g/g}$ provided the highest Youden Indexes of 0.540 and 0.482 among patients receiving etrasimod in ELEVATE UC 52 and ELEVATE UC 12, respectively. For EIHR at week 52, an fCAL concentration of 200 $\mu\text{g/g}$ provided the highest Youden Index of 0.542 among patients receiving etrasimod in ELEVATE UC 52 (Supplementary Table 3).

For clinical response at week 12, fCAL concentrations of 250 $\mu\text{g/g}$ and 350 $\mu\text{g/g}$ provided the highest Youden Indexes of 0.411 and 0.340 among patients receiving etrasimod in ELEVATE UC 52 and ELEVATE UC 12, respectively. For clinical response at week 52, an fCAL concentration of 300 $\mu\text{g/g}$ provided the highest Youden Index of 0.488 among patients receiving etrasimod in ELEVATE UC 52 (Supplementary Table 3).

Among patients receiving etrasimod, fCAL concentrations of 250 $\mu\text{g/g}$ provided generally higher Youden Indexes for

the different efficacy end points (Supplementary Table 3). A threshold of 250 $\mu\text{g/g}$ fCAL was therefore selected for patient stratification by fCAL concentration. The summations and Youden Indexes for varying concentrations of fCAL are shown in Supplementary Tables 3 and 4 and in Supplementary Table 5 for hsCRP.

Proportions of Patients Meeting Efficacy End Points Stratified by fCAL Concentrations

Using the threshold of 250 $\mu\text{g/g}$ fCAL, patients receiving etrasimod were stratified by fCAL concentrations ≤ 250 $\mu\text{g/g}$ and > 250 $\mu\text{g/g}$ at each visit. A greater proportion of patients achieving efficacy end points at weeks 12 and 52 had ≤ 250 $\mu\text{g/g}$ fCAL than those who had > 250 $\mu\text{g/g}$ fCAL across both ELEVATE UC 52 (Figure 4A) and ELEVATE UC 12 (week 12 only; Figure 4B). The OR (95% CI) values for patients achieving EIHR with fCAL concentrations ≤ 250 $\mu\text{g/g}$ compared with > 250 $\mu\text{g/g}$ were 13.48 (6.12, 29.66; week 12) and 15.97 (7.30, 34.97; week 52) for ELEVATE UC 52

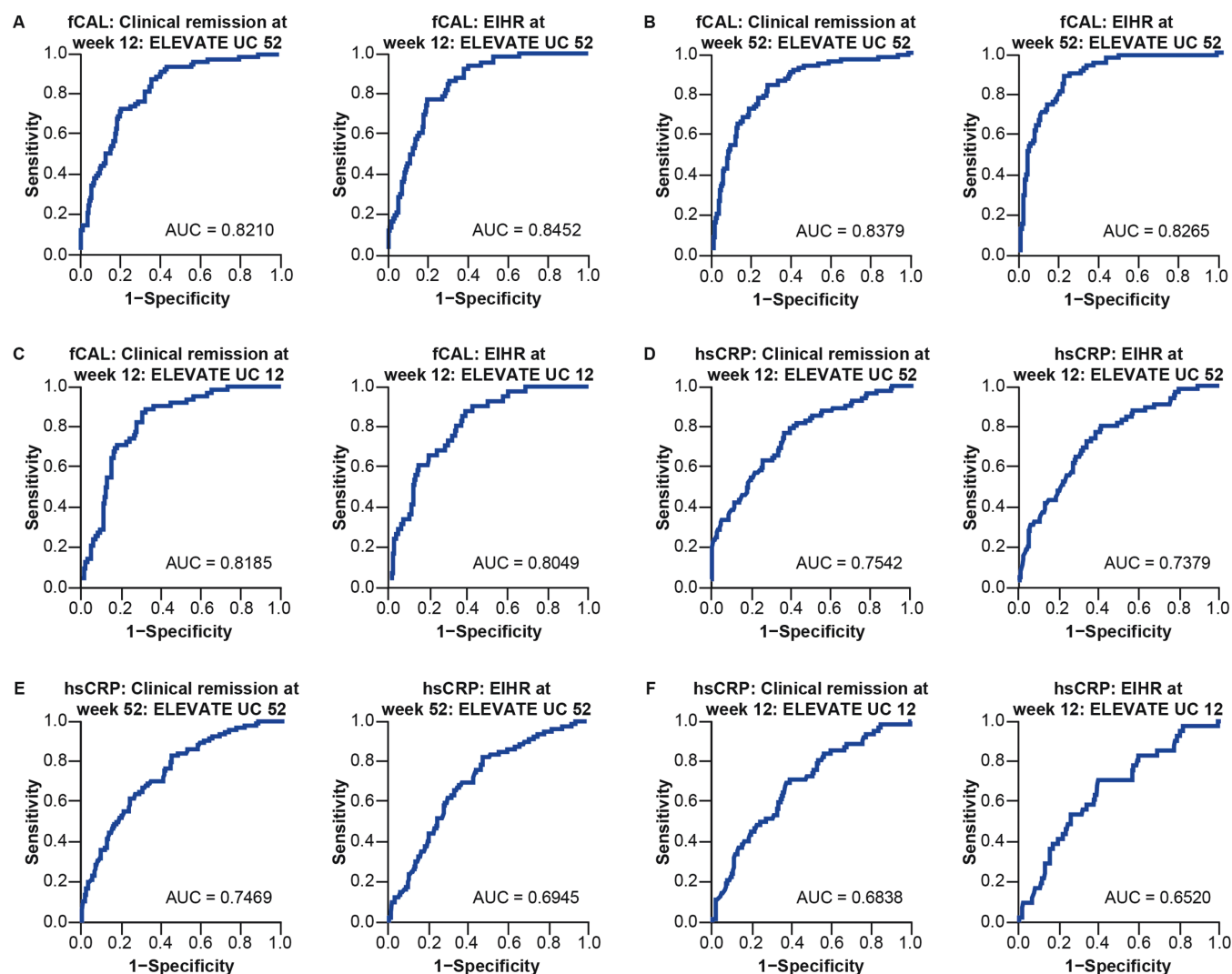


Figure 3. ROC (AUC) of (A-C) fCAL and (D-F) hsCRP with clinical remission and EIHR in patients treated with etrasimod in ELEVATE UC 52 and ELEVATE UC 12. fCAL and hsCRP values presented are representative of the same timepoint as clinical remission and EIHR efficacy end points presented. Abbreviations: EIHR, endoscopic improvement-histologic remission; fCAL, fecal calprotectin; hsCRP, high-sensitivity C-reactive protein; ROC (AUC), area under the receiver operating characteristic curve; UC, ulcerative colitis.

(Figure 4A), and 8.43 (3.56, 20.00; week 12) for ELEVATE UC 12 (Figure 4B).

Findings for the placebo group were generally similar, in that a greater proportion of patients who had ≤ 250 $\mu\text{g/g}$ fCAL achieved efficacy end points in both studies compared with those who had fCAL concentrations >250 $\mu\text{g/g}$ (Supplementary Figure 3).

Median fCAL Concentrations at Baseline, Week 4, and Week 12 Among Patients Achieving Efficacy End Points at Week 12 and Week 52

Median fCAL concentrations among patients who received etrasimod and were subsequent week-12 responders vs nonresponders in ELEVATE UC 52 and ELEVATE UC 12 are shown in Figure 5, at baseline (Figure 5A, B, respectively), and week 4 (Figure 5C, D, respectively). Week 4 and week 12 median fCAL concentrations for patients who were subsequent week-52 responders vs nonresponders are also presented (Figure 5E, F, respectively). In ELEVATE UC 12, there were significant differences in median baseline fCAL

concentrations in patients who received etrasimod and were subsequent responders at week 12 for clinical remission and clinical response end points compared with nonresponders at week 12 ($P < .05$; Figure 5B).

However, this was not observed in patients who were subsequent responders at week 12 for EIHR compared with nonresponders (Figure 5B). Among patients who received placebo in ELEVATE UC 52, baseline median fCAL concentrations in patients who were subsequent week-12 responders were numerically, but not significantly, lower for all efficacy end points except clinical response, where the difference was significant (Supplementary Figure 4A). In ELEVATE UC 12, differences in baseline median fCAL concentrations were not significantly different between patients who were subsequent responders and nonresponders for all end points (Supplementary Figure 4B).

At week 4, patients receiving etrasimod who were subsequent responders for efficacy end points at week 12 had significantly lower median fCAL concentrations compared with nonresponders at week 12 in both ELEVATE UC 52

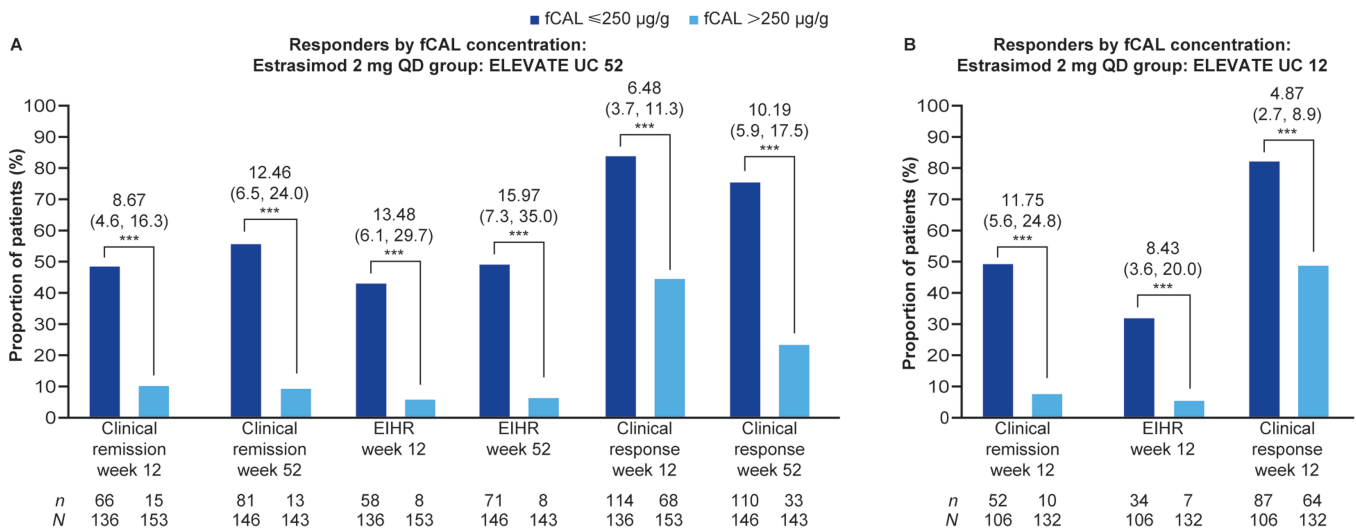


Figure 4. Proportion of patients treated with etrasimod achieving efficacy end points, stratified by fCAL concentrations ≤ 250 $\mu\text{g/g}$ vs > 250 $\mu\text{g/g}$ in (A) ELEVATE UC 52 and (B) ELEVATE UC 12 (OR [95% CI]; FAS, baseline MMS 4-9). *** $P < .001$. OR (95% CI) compares the odds of clinical end point response among patients receiving etrasimod with fCAL ≤ 250 $\mu\text{g/g}$ concentrations vs fCAL > 250 $\mu\text{g/g}$ concentrations at weeks 12 and 52. OR (95% CI) values and P values were obtained from a logistic regression model. Missing fCAL values at weeks 12 and 52 were imputed using the LOCF method; a missing baseline fCAL value was not imputed. Abbreviations: CI, confidence interval; EIHR, endoscopic improvement-histologic remission; FAS, full analysis set; fCAL, fecal calprotectin; LOCF, last observation carried forward; MMS, Modified Mayo score; n , number of responders/nonresponders for the end point; N , number of patients in cohort; OR, odds ratio; QD, once daily; UC, ulcerative colitis.

(all $P < .001$; Figure 5C) and ELEVATE UC 12 (all $P < .001$; Figure 5D). This was also observed at week 4 and week 12 for subsequent responders at week 52 in ELEVATE UC 52 (all $P < .001$; Figure 5E and 5F). Among patients receiving placebo, median fCAL concentrations were significantly lower for responders vs nonresponders for the end points of clinical remission and EIHR (both $P < .05$), but not for clinical response in ELEVATE UC 52 (Supplementary Figure 4C), and for clinical remission ($P < .001$), but not EIHR or clinical response, in ELEVATE UC 12 (Supplementary Figure 4D). At week 12 for subsequent responders receiving placebo at week 52 in ELEVATE UC 52, median fCAL concentrations were significantly lower among responders compared with nonresponders for all end points (clinical remission and EIHR, $P < .05$; clinical response, $P < .01$) (Supplementary Figure 4F).

Discussion

This post hoc analysis assessed the relationship between fCAL and hsCRP as biomarkers of UC, with clinical efficacy end points in patients with moderately to severely active UC enrolled in the ELEVATE UC clinical program. Overall, these results indicate that fCAL and hsCRP levels numerically decreased over time with etrasimod treatment. The ROC analyses indicated a predictive relationship between biomarkers and treatment response. Based on ROC relationships with efficacy end points, fCAL—but not hsCRP—was a reliable predictor of clinical efficacy. These findings thus contribute to the understanding of the use of biomarkers in UC, their potential utility in predicting treatment outcomes, and reducing the overall cost and impact of UC care and disease monitoring, particularly among patients treated with etrasimod. By contributing to the understanding of the relationship between biomarkers, such as fCAL and hsCRP, and efficacy end points, patients with UC may be able

to benefit from increased use of noninvasive monitoring in place of invasive endoscopies, which in turn has the potential to improve their health-related quality of life.

Clinical remission was used as the primary end point, consistent with US Food and Drug Administration (FDA) guidance for industry for UC clinical trial design and drug development.^{18,19} Meanwhile, EIHR was used as one of the key secondary end points, given that its stringent definition encompasses both endoscopic and histologic outcomes. Differences in fCAL concentrations between responders vs nonresponders were apparent as early as week 4, and they became more pronounced by week 12. When patients were stratified by response to treatment based on the achievement of clinical end points, there was a prominent relationship between biomarkers and efficacy end points of clinical remission, clinical response, and EIHR for both fCAL and hsCRP. Given that the relationship between fCAL and hsCRP and efficacy end points in responders was observed in patients receiving placebo and those receiving etrasimod, it is likely this relationship is a reflection of disease improvement, independent of treatment.

A greater proportion of patients achieved certain end points among those enrolled in ELEVATE UC 52 compared with ELEVATE UC 12; this may have been due to the attrition of nonresponders in the longer-term ELEVATE UC 52 trial. Due to the treat-through study design, patients remaining in the trial beyond week 12 likely also experienced an improvement while receiving placebo, given that they remained in the trial rather than enrolling in the open-label extension due to lack of disease improvement or disease worsening compared with baseline. This may explain the reduction in absolute fCAL at later timepoints in the placebo group in the responder analysis.

The ROC analyses for fCAL suggested that the fCAL cut-off concentration of 250 $\mu\text{g/g}$ generally resulted in the highest summation of specificity and sensitivity. Indeed, a greater

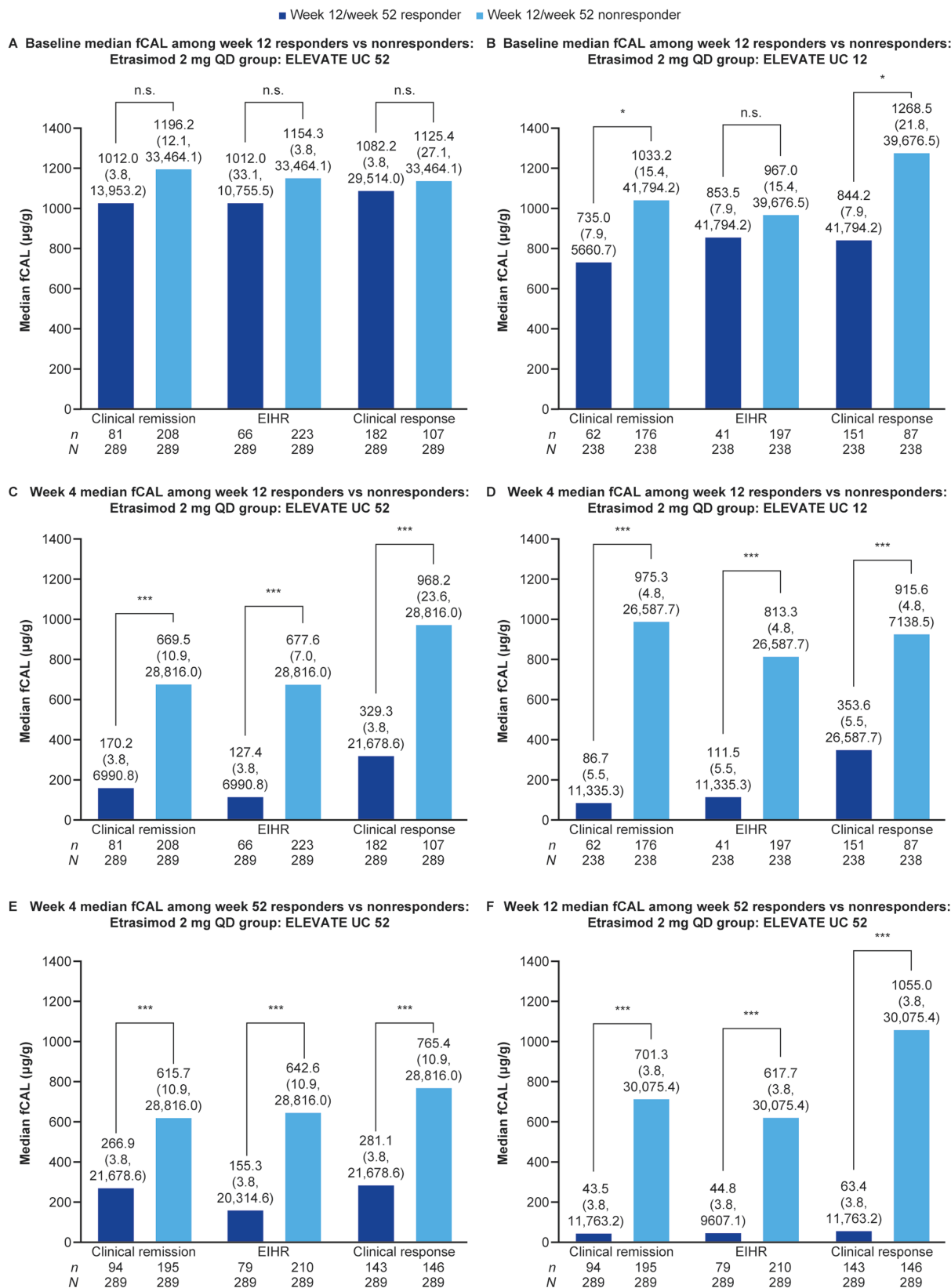


Figure 5. Responder analysis of median fCAL concentration at (A-B) baseline and (C-D) week 4 by responders vs nonresponders at week 12 in ELEVATE UC 52 and ELEVATE UC 12, (E) week 4 by responders and nonresponders at week 52 in ELEVATE UC 52, and (F) week 12 by responders vs nonresponders at week 52 in ELEVATE UC 52 who received etrasimod (median [range]; FAS, MMS 4-9). * $P < .05$; *** $P < .001$. P values were based on Wilcoxon rank-sum test comparing the distributions (medians) of fCAL concentrations between responder and nonresponder groups within treatment arms for each end point. Missing fCAL values at weeks 4 and 12 were imputed using the LOCF method; missing baseline fCAL value was not imputed. Abbreviations: EIHR, endoscopic improvement-histologic remission; FAS, full analysis set; fCAL, fecal calprotectin; MMS, Modified Mayo score; n , number of responders/nonresponders for the end point; N , number of patients in cohort; n.s., nonsignificant; QD, once daily; UC, ulcerative colitis.

proportion of patients who had fCAL concentrations ≤ 250 $\mu\text{g/g}$ achieved the efficacy end points compared with those with fCAL concentrations > 250 $\mu\text{g/g}$. Nonresponders for both week 12 and week 52 had significantly higher fCAL concentrations at early timepoints compared with nonresponders. These data indicate that low fCAL concentrations ≤ 250 $\mu\text{g/g}$ correlate with a greater likelihood of a response in clinical end points and support the consideration of fCAL testing in lieu of an endoscopic assessment. Additionally, low fCAL at week 4 or week 12 may be associated with achieving endoscopic end points at week 12 or week 52. The use of a 250 $\mu\text{g/g}$ threshold is consistent with STRIDE-II guidelines, which state normalization of fCAL to 100 to 250 $\mu\text{g/g}$ as an intermediate treatment target for UC.³ This is above the long-established threshold of 50 $\mu\text{g/g}$ fCAL as a diagnostic criterion for differentiating between inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS).^{20,21} Understanding how fCAL concentrations above the diagnostic threshold correlate to treatment outcomes, using a 250 $\mu\text{g/g}$ threshold, provides important context for the use of fCAL in disease monitoring among patients treated with etrasimod.

Meanwhile, ROC analyses for hsCRP suggested that the relationship between hsCRP and efficacy end points did not carry the same predictive value as that seen with fCAL, given that AUC values were generally lower for the relationships between hsCRP and efficacy end points than with fCAL.

Collectively, these results indicate that fCAL is a reliable biomarker of clinical efficacy for etrasimod, and that early changes in this biomarker may help to predict the subsequent achievement or maintenance of efficacy. In contrast, based on ROC relationships with efficacy end points, hsCRP was not observed to be a useful predictor of clinical efficacy in our analysis. Our results are consistent with both STRIDE-II and ECCO guidelines, which emphasize the utility of fCAL as a surrogate biomarker; ECCO guidelines cite a strong correlation between endoscopic inflammation and fCAL levels in UC, suggesting fCAL levels as an early target in IBD using a 4-phase approach to monitoring.^{3,22} Meanwhile, STRIDE-II guidelines note that hsCRP level normalization after treatment initiation should be considered a minimal obligatory short- to medium-term target but is insufficient for the longer term.³ As a general marker of inflammation, hsCRP has been noted to have poor sensitivity, with a negative hsCRP test not necessarily excluding the presence of a disease flare.²² Furthermore, hsCRP is not IBD-specific, and its elevation cannot differentiate IBD from infectious, or other causes of, colitis.²² Our results therefore inform the biochemical process involved with UC, while providing context for the use of fCAL in disease monitoring among patients receiving etrasimod.

These trials provide interesting data for patients treated with placebo. While clinical symptoms may improve in a clinical trial, this trial provides information to inform power calculations for studies of UC, especially for treat-through designs, with respect to the low but nonnegligible proportion of patients treated with placebo with biochemical improvements in fCAL and hsCRP.

These findings are consistent with those from a phase 2 trial of patients with UC treated with ozanimod, in which both fCAL and hsCRP concentrations decreased quickly after treatment induction and remained low throughout the ≥ 4 year open-label extension study.⁸ In addition to fCAL and hsCRP levels reflecting clinical and histologic findings

among patients receiving ozanimod,²³ the prominent difference in fCAL concentrations among responders compared with nonresponders has been noted in patients with UC receiving tofacitinib.⁵ fCAL as a biomarker has been validated in the broader UC patient population; a 6-month prospective study of patients with UC reported that, although there was a weak correlation between fCAL and symptoms, there was a strong correlation between fCAL and endoscopic activity or histopathology.²⁴ This may be particularly relevant among certain patients with either low or high fCAL concentrations. In patients with UC, monitoring was found to be a successful surrogate for endoscopy among those with fCAL concentrations of either ≤ 50 $\mu\text{g/g}$ or ≥ 250 $\mu\text{g/g}$, with a $< 5\%$ false negative or false positive rate, respectively.²⁵ Given that fCAL can be used to differentiate between IBD and IBS,^{20,21} the use of fCAL pre-endoscopic screening could substantially reduce the number of invasive measurements required in IBD diagnosis.²⁶ In turn, it has been estimated that introducing routine fCAL monitoring could approximately halve the cost of IBD care, while aiding changes in clinical management in a similar proportion to historical colonoscopy-only monitoring.²⁷

Limitations of this study included the post hoc nature of this analysis; additionally, these data were collected under a phase 3 controlled clinical trial setting that may not be fully representative of a general UC population. Further analyses using clinical registry or disease-specific observational data collection will help to further assess the generalizability of the data.

Conclusions

fCAL and hsCRP values corresponded with the end points of clinical remission, clinical response, and EIHR in both responders and nonresponders. fCAL and hsCRP levels were shown to decrease in patients treated with 2 mg of etrasimod QD. The use of ROC curves to compare fCAL and hsCRP to treatment response indicated a predictive correlation between biomarkers and treatment response; this relationship was more pronounced at week 52 in all end points. Collectively, these results suggest that fCAL and, to a lesser extent, hsCRP are reliable biomarkers of clinical efficacy end points and that early changes in these biomarkers may help to predict the subsequent achievement or maintenance of efficacy.

Supplementary Data

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

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Author contributions

V.J. and B.S. contributed to the conception or design of the study. D.T.R., M.T.A., C.W.L., M.F., J.C.W., and C.C. contributed to the conception or design of the study, and analysis of data. B.V. contributed to the conception or design of the study, patient recruitment, and analysis of data. A.H.Ç. contributed to patient recruitment and the acquisition of data. D.H. and G.G. contributed to the conception or design of the study, patient recruitment, and acquisition of data.

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Conflicts of Interest

V.J. has received consulting/advisory board fees from AbbVie, Alimentiv, Arena Pharmaceuticals, Asahi Kasei Pharma, Asieris Pharmaceuticals, AstraZeneca, Avoro Capital, Bristol Myers Squibb, Celltrion, Eli Lilly, Endpoint Health, Entera, Ferring, Flagship Pioneering, Fresenius Kabi, Galapagos, Gilde Healthcare, GlaxoSmithKline, Genentech, Gilead, Innomar, JAMP, Janssen, Merck, Metacrine, Mylan, Pandion, Pendopharm, Pfizer Inc, Protagonist, Prometheus Biosciences, Reistone Biopharma, Roche, Roivant, Sandoz, SCOPE, Second Genome, Sorriso Pharmaceuticals, Takeda, TD Securities, Teva, Topivert, Ventyx Biosciences, and Vividion Therapeutics; and has received speaker's fees from AbbVie, Ferring, Bristol Myers Squibb, Galapagos, Janssen, Pfizer Inc, Shire, Takeda, and Fresenius Kabi.

D.T.R. has received consultancy/advisory board fees from AbbVie, AltruBio, Aslan Pharmaceuticals, Athos Therapeutics, Bellatrix Pharmaceuticals, Boehringer Ingelheim, Bristol Myers Squibb, Celgene, Chronicles, ClostraBio, Connect Biopharma, EcoR1, Genentech/Roche, Gilead Sciences, Iterative Health, Janssen, Kaleido Biosciences, Lilly, Pfizer Inc, Prometheus Biosciences, Reistone Biopharma, Seres Therapeutics, Syneos Health, Takeda, Target RWE, and Trellus Health; has received grant support from Takeda, Helmsley Charitable Trust, and GI Research Foundation; has participated in the Board of Trustees for Crohn's & Colitis Foundation, and Cornerstones Health; and has stock options with Alike Health, AltruBio, Datas Health, and Iterative Health.

B.V. has received research support from AbbVie, Biora Therapeutics, Landos Biopharma, Pfizer Inc, Sosei Heptares, and Takeda; has received speaker's fees from AbbVie, Biogen, Bristol Myers Squibb, Celltrion, Chiesi, Falk, Ferring, Galapagos, Janssen, Lilly, MSD, Pfizer Inc, R-Biopharm, Sandoz, Takeda, Tillotts Pharma, Truvion Healthcare, and Viatrix; and has received consultancy fees from AbbVie, Alimentiv, Applied Strategic, Atheneum, BenevolentAI, Biora Therapeutics, Bristol Myers Squibb, Galapagos, Guidepoint, Landos Biopharma, Lilly, Mylan, Inotrem, Ipsos, Janssen, Pfizer Inc, Progenity, Sandoz, Santa Ana Bio, Sosei Heptares, Takeda, Tillotts Pharma, and Viatrix.

A.H.Ç. declares no conflicts of interest.

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Data Availability

Upon request, and subject to review, Pfizer will provide the data that support the findings of this study. Subject to certain criteria, conditions, and exceptions, Pfizer may also provide access to the related individual de-identified participant data. See <https://www.pfizer.com/science/clinical-trials/trial-data-and-results> for more information.

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