Assessment of Disease-Related Therapeutic Protein Drug-Drug Interaction for Etrolizumab in Patients With Moderately to Severely Active Ulcerative Colitis

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Abstract

The efficacy and safety of etrolizumab, a humanized IgG1 mAb, were evaluated in patients with ulcerative colitis (UC) in a phase 2 study (EUCALYPTUS). The current study assessed the risk of therapeutic protein drug-drug interaction (TP-DDI) of etrolizumab on CYP3A activity in patients with UC. Literature review was performed to compare serum proinflammatory cytokine levels and pharmacokinetic (PK) parameters of CYP3A substrate drugs between patients with inflammatory bowel disease (IBD) and healthy subjects. Treatment effect of etrolizumab on CYP3A activity was evaluated by measuring colonic CYP3A4 mRNA expression and serum C-reactive protein (CRP) in EUCALYPTUS patients. Literature data suggested similar levels between IBD patients and healthy subjects for serum proinflammatory cytokines and PK parameters of CYP3A substrate drugs. Additionally, treatment with etrolizumab did not change colonic CYP3A4 mRNA expression or serum CRP levels in UC patients. In conclusion, our results indicate a low TP-DDI risk for etrolizumab in UC patients, particularly on medications metabolized by CYP3A.

Keywords

etrolizumab, therapeutic protein drug-drug interaction, ulcerative colitis

The extensive clinical use of therapeutic proteins (TPs) in various diseases has drawn increased attention to assessing the potential risk of therapeutic protein drug-drug interactions (TP-DDI).[1,2] Disease-drug interaction was found under some inflammatory situations where significantly elevated proinflammatory cytokines can down-regulate the expression and activity of specific drug-metabolizing cytochrome P450 (CYP) enzymes, leading to the decreased clearance of coadministered small-molecule drugs that are substrates of the affected CYP enzymes.[3,4] TPs that modulate proinflammatory cytokines can result in a "normalization" of CYP activities and a subsequent increase in the clearance of concomitant small-molecule drugs. This is referred to as disease-related TP-DDI.[4–7] Proinflammatory cytokines such as interleukin (IL)-6, IL-1β, tumor necrosis factor α (TNF-α), and interferon α (IFNα), have been reported to be capable of down-regulating CYP enzyme expression.[8–11] At present, in vitro and preclinical systems have shown limited values in predicting a clinically relevant effect of cytokine-mediated TP-DDI, and clinical evidence is preferred for informing the evolving risk assessment for TP-DDI.[11,12] To facilitate the determination of the necessity for a dedicated clinical DDI study, a 4-step approach was proposed by the IQ Consortium/FDA TP-DDI workshop (San Diego, 2012), including in-

vestigations of (1) the disease effect on cytokine levels and CYP expression, (2) TP mechanism and its impact on cytokine-mediated DDI, (3) DDI liability of the concurrently used small-molecule drugs, and (4) the clinical approaches in determining TP-DDI risk for cytokines or cytokine modulators on CYP enzymes.[13]

Inflammatory bowel disease (IBD) consists of 2 major forms: ulcerative colitis (UC) and Crohn disease (CD). Both are chronic inflammatory diseases of the gastrointestinal (GI) tract caused by immune dysregulation in genetically susceptible individuals in response to commensal microbiota and other environmental

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triggers.[14,15] IBD often associates with dysregulation of a complex cytokine network.[16,17] CD can affect the whole GI tract, whereas UC is limited to the large intestine.[14,15] Theoretically, a clinically significant overexpression of proinflammatory cytokines in the circulation or along the intestinal mucosa may suppress CYP activity in IBD patients; and the normalization of cytokine network expression due to TP treatment may reverse the suppression of CYP enzymes.

Etrolizumab, a humanized IgG1 monoclonal antibody (mAb) that selectively targets the $\beta 7$ subunit of the a4$\beta 7$ and aE$\beta 7$ integrins with high affinity, blocks the trafficking and retention of inflammatory leukocytes to the gut.[18] This gut-selective treatment approach avoids broad-spectrum immunosuppression and possibly reduces disease severity in patients with UC or CD.[19–21] Etrolizumab has been evaluated in patients with moderate to severe UC in 2 clinical studies (1 phase 1 study [ABS4262g] and 1 phase 2 study [ABS4986g, EUCALYPTUS]).[21,22] In EUCALYPTUS, clinically meaningful induction of disease remission was observed in etrolizumab-treated patients without significant safety concerns.[21] Phase 3 studies of etrolizumab are currently ongoing in patients with moderate to severe UC or CD. Based on its mechanism of action, etrolizumab does not (1) act as a cytokine or cytokine modulator, (2) induce the proliferation of human lymphocytes or the release of proinflammatory cytokines and chemokines from human peripheral blood mononuclear cells, or (3) induce either antibody-dependent cell-mediated cytotoxicity or complement-dependent cytotoxicity in vitro (unpublished data). Therefore, etrolizumab is not expected to affect CYP enzyme activities directly via an effect on circulating proinflammatory cytokines. However, it is possible that improvement of the inflammatory condition in the gut by etrolizumab treatment may potentially modify the gut cytokine network regulation, which may in turn impact the CYP enzyme expression via a secondary disease-drug-drug interaction.

To assess the potential TP-DDI risk for etrolizumab in patients with UC, this study followed the stepwise assessment approach recommended by the IQ Consortium/FDA TP-DDI workshop by (1) reviewing the disease effect of IBD on CYP activity using literature data, (2) evaluating serum levels of pro- and anti-inflammatory cytokines, drug exposure and PK parameters of CYP3A substrates in IBD patients and healthy controls, and (3) determining the potential treatment effect of etrolizumab on serum C-reactive protein (CRP) and colonic CYP3A4 mRNA expression levels in samples collected pre- and postetrolizumab or placebo treatment from patients with moderate to severe UC in the EUCALYPTUS.

Methods

Literature Review

Circulating Cytokine Levels in IBD Patients vs Healthy Subjects. Baseline serum levels of proinflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8) and the anti-inflammatory cytokine IL-10 in patients with UC or CD and healthy control subjects were collated from recent publications (1995 to 2012) that reported quantitative measurements.[23–26]

PK Parameters of CYP3A Substrate Drugs in IBD Patients vs Healthy Subjects or Subjects Free of Active IBD. Information on drug exposure and PK parameters of CYP3A substrate drugs (prednisolone,[27] budesonide,[28] as intravenous [IV] administration, and cyclosporine,[29,30] as oral [PO] administration) that are commonly used for IBD patients were reviewed and compared between IBD patients and healthy subjects (or subjects free of active IBD).

Clinical Assessment of Potential TP-DDI Effect of Etrolizumab in Patients With Moderately to Severely Active UC From a Phase 2 Study (EUCALYPTUS)

Clinical Study and Patients. EUCALYPTUS (NCT01336465) was a phase 2 global, randomized, double-blind, placebo-controlled multicenter study to evaluate the efficacy and safety of 2 etrolizumab dose regimens compared with placebo in patients with moderate to severe UC who failed to respond to conventional therapy. Patients were recruited from 40 referral centers in 11 countries (Australia, Belgium, Canada, Czech Republic, Germany, Hungary, Israel, New Zealand, Spain, the United Kingdom, and the United States). Institutional review boards at each study site approved the protocol, and all patients provided written informed consent. The study was undertaken and reported in accordance with the study protocol.[21]

Details of the study design and outcome are reported elsewhere.[21] Briefly, eligible patients were 18–75 years of age, with a diagnosis of UC and a Mayo Clinic Score (MCS) of 5 points or higher. A total of 124 patients (57% male and 43% female) were randomized in a 1:1:1 ratio to subcutaneous injections of etrolizumab 100 mg (weeks 0, 4, and 8 and with placebo at week 2), etrolizumab 300 mg + LD (300 mg on weeks 2, 4, and 8 plus a 420-mg loading dose [LD] at week 0), or matching placebo for 4 doses. The primary efficacy endpoint was clinical remission at week 10, defined as the proportion of patients with a MCS of 2 points or less with no individual subscore greater than 1 point. Clinical remission at week 6 was evaluated as a secondary endpoint.[21]

Determination of CYP3A4 mRNA Levels in Colonic Biopsy Samples. Each patient enrolled in the EUCALYPTUS had colonic biopsies collected by flexible

sigmoidoscopy or full colonoscopy from the most inflamed area of the colon at baseline, week 6, and week 10 post-etrolizumab-treatment. The same depth of flexible sigmoidoscope was used for each collection in each patient to ensure consistency in location of the biopsy samples across all 3 sampling time points. RNA from biopsies was isolated using the RNeasy Mini Kit (Qiagen), and the RNA integrity (RIN) was assessed using an Agilent 2100 Bioanalyzer. Samples with RIN < 5 were excluded from analysis. Real-time polymerase chain reaction (PCR) was used to determine CYP3A4 mRNA levels in colonic biopsies (ViiA 7 instrument, Life Technologies). The total number of patients with biopsy samples that yielded usable RNA (RIN $\geq$ 5) was 30 for the 100-mg group, 34 for the 300-mg + LD group, and 38 for the placebo group.

Relative changes in mRNA levels were assessed by the comparative cycle time ($\Delta\Delta$ cycle threshold [Ct]) method.[31] The mRNA expression of CYP3A4 was normalized to GAPDH mRNA levels, which is the difference between the Ct values of CYP3A4 and Ct values of the housekeeping gene GAPDH (ΔCt = Ct[GAPDH] – Ct[CYP3A4]). These ΔCt values were summarized by means and standard deviations (SD) in each treatment arm. $\Delta\Delta$Ct values were baseline corrected ΔCt values and were calculated as the difference between ΔCt at week 6 or week 10 and ΔCt at baseline, and fold change (vs baseline) was derived as $2^{\Delta\Delta Ct}$. Mean differences in fold change in CYP3A4 mRNA expression level between each etrolizumab arm and placebo at weeks 6 and 10 were estimated via a baseline-adjusted analysis of covariance (ANCOVA) model, along with the associated 95% confidence interval (CI).

Determination of Serum CRP Levels. A highly sensitive enzyme-linked immunosorbent assay (ELISA) method (Roche Immunoturbidimetric) was used to determine the serum CRP levels from patients at baseline (prior to drug treatment), and at week 6 and week 10 post-treatment of etrolizumab. Changes in serum CRP levels from baseline were plotted at week 6 and week 10 in patients who had elevated baseline CRP (>0.4 mg/dL).

Results

Literature Review on the Potential Effect of IBD on CYP3A Activity

Baseline Cytokine Levels in IBD Patients. Baseline levels of the pro- and anti-inflammatory serum cytokines in patients with UC or CD as compared to those in healthy subjects (or UC patients in remission) were collected from the literature and are summarized in Table 1.[23–26] Overall, healthy subjects had negligible levels of these cytokines (<10 pg/mL). However, levels of serum cytokines were elevated in patients with UC or CD in a statistically significant manner but remained in a low range when compared to average serum IL-6

level in patients with rheumatoid arthritis (RA) (50–500 pg/mL). UC or CD patients had average serum levels ranging from 1-17 pg/mL for TNF-α, 1–3 pg/mL for IL-1β, 8–36 pg/mL for IL-6, 32–64 pg/mL for IL-8, and 2–4 pg/mL for IL-10. In 1 paper, the highest levels in individual IBD patients were <10 pg/mL for TNF-α, IL-1β, and IL-10, <13 pg/mL for IL-6, and <150 pg/mL for IL-8.[25]

Effect of IBD on the PK of CYP3A Substrate Drugs. Corticosteroids such as prednisone, prednisolone, and budesonide or immunosuppressant drugs such as cyclosporine are conventionally used IBD medications and are metabolized via CYP3A (Table 2). Prednisolone is partially metabolized by CYP3A (fraction metabolized [f_m] by CYP3A $\approx$ 0.26), whereas CYP3A is the major enzyme involved in metabolism of budesonide ($f_m \approx$ 0.76) and cyclosporine ($f_m \approx$ 0.79).[32] Literature data examining the effect of IBD on the PK of these medications concluded that there was comparable exposure of these drugs in IBD patients and healthy subjects.[33]

As illustrated in Figures 1A and 1B, following intravenous (IV) administration of prednisolone or budesonide, drug exposure (observed maximum plasma concentration [C_{max}] and area under the curve [AUC] or dose-normalized AUC for budesonide) and PK parameters (total clearance [CL] and elimination half-life [$t_{1/2}$]) were similar in patients and healthy subjects.[27,28] Similar trends were reported following oral administration of cyclosporine[29,30] (Figure 1C). Furthermore, disease severity, as indicated by the Crohn Disease Activity Index (CDAI) score (CDAI < 150 vs CDAI $\geq$ 150), did not significantly alter the PK of cyclosporine (Figure 1D).[29]

Clinical Assessment on Potential TP-DDI Effect of Etrolizumab in UC Patients From a Phase 2 Study (EUCALYPTUS)

Effect of Etrolizumab Treatment on Colonic CYP3A4 mRNA Expression. In EUCALYPTUS, a significant improvement in clinical remission rates was observed following etrolizumab treatment at week 10 for both 100-mg and 300-mg + LD groups in patients with UC. No significant treatment effect was noted for clinical remission at week 6.[21] The variation and ranges for relative CYP3A4 mRNA expression in individual patients (Figure 2A) as well as the mean profiles of the time course (Figures 2B and 2C) at baseline (screening) or posttreatments were similar between the etrolizumab arms and the placebo arm. By week 10, there was no substantial difference in the fold changes from baseline in CYP3A4 mRNA expression levels between the placebo and the treatment arms (Table 3). At week 6, the ratio of fold change in CYP3A4 mRNA levels at 100 mg vs placebo (baseline adjusted) was 2.5 (95%CI: 0.9, 6.5),

Table 1. Literature Reported Baseline Levels (pg/mL, Mean $\pm$ SD) of Serum Proinflammatory Cytokines (TNF-α, IL-1β, IL-6, IL-8) and Anti-Inflammatory Cytokine IL-10 in Patients With IBD (UC or CD) and Healthy Subjects

UC or CD (n)	Patients With UC or CD					Healthy Subjects/UC Patients in Remission						References/Assay
	TNF-α	IL-1β	IL-6	IL-8	IL-10	n	TNF-α	IL-1β	IL-6	IL-8	IL-10	
UC[a] (20)	1.18 ± 0.73 (0.1–2.5)	1.35 ± 1.21 (0.4–4.6)	8.63 ± 2.14 (4.8–12.8)	31.8 ± 12.9 (14.6–61.3)	4.40 ± 1.55 (2.0–6.7)	15	0.61 ± 0.28 (0.1–1.0)	0.12 ± 0.10 (0–0.2)	1.59 ± 0.90 (0.6–3.2)	9.77 ± 7.65 (0–22.1)	1.35 ± 0.96 (0–2.8)	25/Highly sensitive ELISA (Quantikine)
CD[a] (12)	3.12 ± 2.42 (1.5–7.8)	0.94 ± 0.84 (0.3–2.8)	8.24 ± 1.75 (5.8–10.8)	53.7 ± 41.5 (28–150)	2.16 ± 1.46 (0–3.9)							
UC (50)	0.96 ± 0.06	3.06 ± 0.27	8.03 ± 0.7	n/a	n/a	n/a[b]	0.62 ± 0.04[b]	1.47 ± 0.12[b]	5.13 ± 0.4[b]	n/a	n/a	23/Highly sensitive ELISA (Quantikine)
UC (19)	16.5 ± 36.4	n/a	12.1 ± 13.2	63.5 ± 115	n/a	10	5.0 ± 0	n/a	0.4 ± 0.1	3.1 ± 0.8	n/a	26/IL-6: CLEIA IL-8,TNF-a:ELISA
UC (15)	n/a	n/a	10 ± 4	n/a	n/a	25	n/a	n/a	7.3 ± 1.2	n/a	n/a	24/Immunoradiometric
CD (28)	n/a	n/a	36 ± 8	n/a	n/a		n/a	n/a		n/a	n/a	

CD, Crohn's disease; CLEIA, chemiluminescent enzyme immunoassay; ELISA, enzyme linked immunosorbent assay; IL, interleukin; IBD, inflammatory bowel disease; TNF, tumor necrosis factor.; UC: ulcerative colitis.

[a]Range in parentheses.

[b]From UC patients in remission, n value was not specified in the original literature.

n/a: not reported.

Table 2. Summary of Involvement of CYP Metabolic Pathways for a List of Concomitant Medications Commonly Used to Treat Ulcerative Colitis

Medication Class	Medication Name	Metabolic Pathway and Enzyme Involved
Corticosteroids	Prednisone	Partially through CYP3A
	Methylprednisolone	CYP3A
	Prednisolone	CYP3A
	Budesonide	CYP3A
Immunosuppressants	Azathioprine	Non-CYP pathway
	Cyclosporine	CYP3A substrate with narrow therapeutic range
	Tacrolimus (prograf)	CYP3A substrate with narrow therapeutic range
Antimetabolites	Methotrexate	Non-CYP pathway
	6-mercaptopurine	Non-CYP pathway
5-aminosalicylic acid	Mesalazine (mesalamine)	Non-CYP pathway
	Balsalazide disodium	Non-CYP pathway
	Sulfasalazine	Non-CYP pathway
Antidiarrheals	Lomotil (diphenoxylate + atropine)	Non-CYP pathway
	Loperamide hydrochloride	CYP2C8, CYP3A
Antibiotics	Penicillin	Non-CYP pathway
	Ciprofloxacin	Non-CYP pathway
	Fluoroquinolones	Non-CYP pathway
	Flagyl (metronidazole)	Non-CYP pathway
	Rifaximin	Non-CYP pathway
	Cephalosporins	Non-CYP pathway
	Cefuroxime axetil	Non-CYP pathway

CYP, cytochrome P450.

Sources: http://www.druginteractioninfo.org (a metabolism and transport drug interaction database built by the University of Washington; used to search for metabolic pathway and CYP enzymes involved for the concomitant medications in the table); http://www.pdr3d.com (used to search for metabolic pathway and drug-drug interaction information from package inserts [labels] for the concomitant medications listed in the table); http://en.wikipedia.org/wiki (used to search for pharmacokinetic information for medications whose pharmacokinetic and metabolism information was not identified from the other two Web sites).

which was a non–statistically significant trend for an increase. This increase was not sustained at week 10 when a significant clinical remission (vs placebo) was observed. Furthermore, such a difference at week 6 was not observed in the higher-dose group (Table 3). In addition, disease severity as measured by baseline Mayo Clinic Score had no correlation with the changes in CYP3A4 mRNA expression following treatment with etrolizumab ($r^2 < 0.02$) (Figure 3). Therefore, compared with placebo, there was no consistent trend suggesting a change in colonic CYP3A4 mRNA expression following etrolizumab treatment in patients with moderate to severe UC.

Treatment Effect of Etrolizumab on Serum CRP Levels. At week 10 (Figure 4), serum CRP levels were comparable to those with placebo in both etrolizumab treatment arms for patients who had an elevated baseline CRP (>0.4 mg/dL). No statistically significant treatment effect of etrolizumab was observed on changes in serum CRP levels.

Discussion

The initiation and aggregation of IBD inflammatory processes can be associated with the dysregulation of a complex cytokine network mostly manifested as an overexpression of proinflammatory cytokines or inactivation of anti-inflammatory cytokines.[16,17] Quantitative measurements of the expression level changes in IBD patients relative to healthy controls have only been reported for limited cytokines as shown in Table 1. Cross-study variability in the reported levels of proinflammatory cytokines was noticed and was found primarily driven by differences between the highly sensitive and well-qualified Quantikine ELISA assays and other less-well-qualified assays. As shown in Table 1, the less-well-qualified assays tend to produce higher cytokine levels and higher within-assay variability. However, both patients and healthy subjects were measured with each assay format[23–26]; therefore, cross-population comparison can be made reasonably based on the results produced by the same assay method. Although circulating levels of these cytokines were significantly increased in patients with UC or CD compared to the healthy controls, the mean levels of these cytokines remained low in IBD patients (1–65 pg/mL) when compared to IL-6 level in RA patients (50–500 pg/mL). Among these cytokines, IL-8 was found to have the highest serum concentration in IBD patients but is still <150 pg/mL. Moreover, the role of IL-8 on CYP activity is not clear, and to date there are no data reporting the potential effect of IL-8 on the alteration of CYP activities. Although elevation of TNF-α, IL-6, and IL-1β has been shown to down-regulate the expression of multiple CYP enzymes,[8–11] the relatively low baseline levels of these cytokines in IBD patients do not appear to be sufficient to suppress CYP activities in vivo.[34] For instance, IL-6 is a potent proinflammatory mediator in modulating CYP activity.[10,12] As shown in Table 1, the mean serum level of IL-6 in IBD patients is approximately 10 pg/mL in a majority of studies. This is in contrast to the significantly increased mean IL-6 levels measured in RA patients (50−500 pg/mL) or postsurgery patients (100–500 pg/mL), in whom IL-6 concentration−dependent suppression of CYP3A activity was observed.[34] A physiologically based pharmacokinetic (PBPK) approach allowed a quantitative relationship between elevated serum IL-6 levels and changes in hepatic CYP3A activity to be established. Such an exposure and response relationship appears to be mainly driven by the changes in IL-6 levels and is
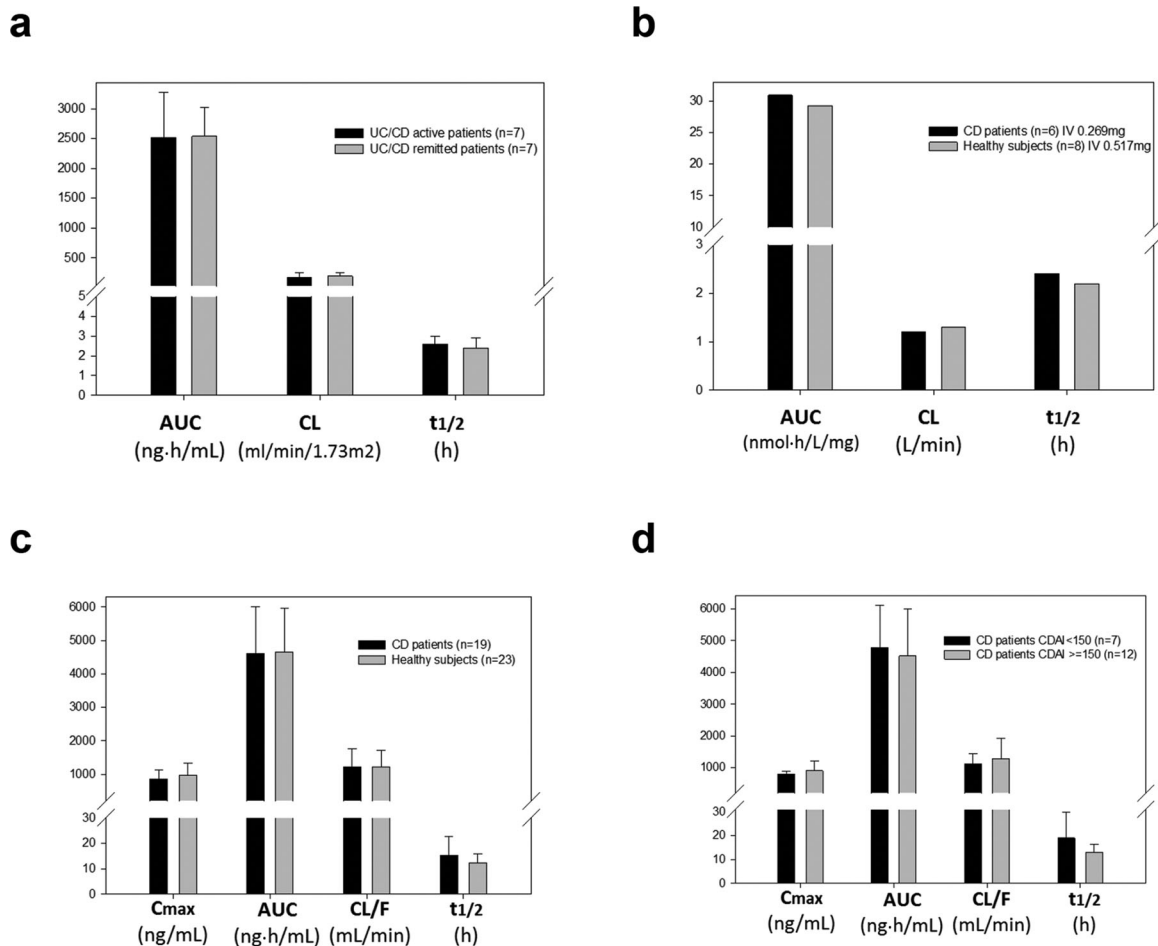
Figure 1. Literature data comparing drug exposure and pharmacokinetic parameters of CYP3A substrates in patients with inflammatory bowel disease vs healthy subjects. (a) Prednisolone (f_m by CYP3A ~0.26) following prednisolone IV 32 mg in UC/CD active vs patients in remittance.[30] (b) Budesonide (f_m by CYP3A ~0.85) following budesonide IV 0.269 mg in CD patients vs IV 0.517 mg in HS.[29] (c) Cyclosporine (f_m by CYP3A ~0.68) following cyclosporine PO 300 mg in CD patients vs HS.[31,32] (d) Cyclosporine (f_m by CYP3A ~0.68) following cyclosporine PO 300 mg in CD patients with CDAI < 150 vs CD patients with CDAI ≥150.[31] CD, Crohn disease; CDAI, Crohn Disease Activity Index; f_m, fraction metabolized; HS, healthy subjects; IV, intravenous; PO, per oral dose; UC, ulcerative colitis.

independent of disease type because similar relationships were established independently in multiple case studies of different diseases.[34] According to predictions from this PBPK model, the impact of IL-6 on CYP3A activity was negligible when the IL-6 concentration was less than 10 pg/mL, although the CYP3A activity was significantly suppressed when the IL-6 concentration was higher than 50 pg/mL.[34] This PBPK model also predicted that an increase in IL-6 levels to 100 pg/mL would result in an elevation of simvastatin AUC in virtual RA patients (59%), comparable to the observed clinical data (58%),[34,35] and that 500 pg/mL of IL-6 is needed to produce a similar increase in cyclosporine AUC in virtual bone marrow transplant patients (45%) to that of observed levels (39%).[34,36] Given that a sufficiently high serum IL-6 level (>50 pg/mL) is needed to induce a clinical DDI effect, the serum level of IL-6 in IBD patients (~10 pg/mL in most cases except

~40 pg/mL in 1 case) appears to be insufficient to suppress CYP enzyme activity to a clinically meaningful extent and elicit disease DDI effect on CYP substrates. The potential risk of a disease DDI effect due to overexpression of TNF-α and IL-1β is likely even lower, as increases in serum levels of these cytokines in IBD patients are minimal.[10] Although data from a thorough assessment of potential impact on CYP enzyme activity were not available for every family of cytokines involved in IBD, critical information could be derived based on the circulating levels of the cytokines listed in Table 1, as they represent the currently identified key cytokines capable of down-regulating CYP enzyme activities at sufficiently high levels. Given the reported low serum levels of these cytokines in IBD patients, it is unlikely that CYP activity would be modulated by the dysregulation of these circulating cytokines in patients with IBD.
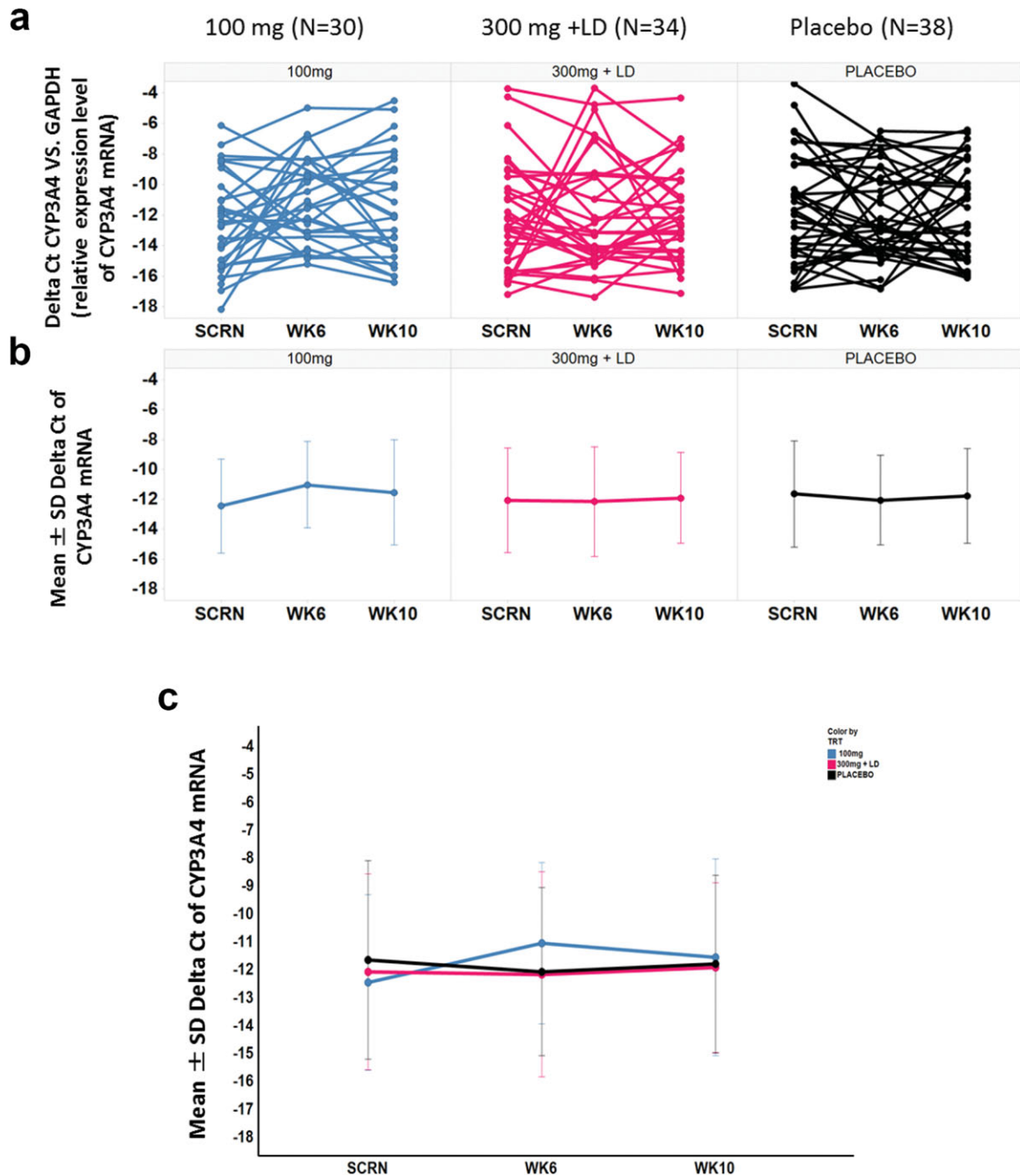
Figure 2. Relative expression levels of CYP3A4 mRNA from colonic biopsies before and after etrolizumab treatment (a) in individual patients and (b, c) as mean ± SD from 3 study arms. ΔCt, Ct(GAPDH) − Ct(CYP3A); LD, loading dose; SCRN, screening (at baseline); WK6, week 6; WK10, week 10. Blue dots and curve represent 100 mg group; red dots and curve represent 300 mg + LD group; black dots and curve represent placebo group. Actual drug amounts for the nominal 100-mg and 300-mg doses are 105 mg (1 injection of drug) and 315 mg (3 injections of drug), respectively (150 mg/mL drug concentration, 0.7 mL volume per injection).

Consistent with this low likelihood for CYP enzyme suppression in IBD patients as a result of minimal increase in serum proinflammatory cytokine levels, the literature data also demonstrated that the inflammatory status of IBD does not significantly alter the PK of evaluated CYP3A substrate drugs. Following IV administration, drug exposures of prednisolone and budesonide in UC or CD patients were similar to those found in healthy controls and achieved the target therapeutic serum levels,[27,28] indicating that IBD itself does not affect the disposition of drugs that are partially or extensively metabolized by hepatic CYP3A. Furthermore, following oral administration, cyclosporine drug exposure in patients with CD did not differ from that

found in healthy subjects and had no relationship with the disease severity.[29,30] These data suggest that there is no significant disease effect of CD on intestinal CYP3A activity, as approximately 50% of oral cyclosporine is metabolized by intestinal CYP3A.[37] In addition, it is reported that marked increase in circulating level of IL-6 (500 pg/mL) was able to induce a 1.7-fold increase in cyclosporine exposure.[34,36] Because the disease status of UC and CD has a small impact on serum IL-6 levels,[23,25] and PK of cyclosporine was not altered in patients with CD,[29,30] one can infer that IBD does not result in an elevation of proinflammatory cytokines to clinically sufficient levels that can alter the PK of CYP3A substrate drugs. It is recognized that these literature data comparisons have some limitations such as a relatively small subject size in the data sets for UC patients and potential confounding factors in using corticosteroids to evaluate the pure inflammatory disease effect on CYP activity due to the ability of corticosteroids to suppress inflammation. However, 2 lines of the above-mentioned evidence from the literature showed a minimum IBD disease effect either on the changes in the serum cytokine levels or on PK and exposure of CYP3A substrate drugs,[23,25,27–30] suggesting a low risk of IBD disease effect on CYP activity.

To our knowledge, this is the first report in which expression levels of CYP3A4 mRNA from colonic biopsy tissue were determined to assess the potential TP-DDI effect on CYP3A expression at the site of disease pathology. Our results showed that treatment with etrolizumab did not result in a consistent change in colonic CYP3A4 mRNA expression compared to placebo patients. Further, etrolizumab did not show a significant treatment effect on serum CRP levels at

Table 3. Fold Change of CYP3A4 mRNA; Relative Expression Level From Baseline in Etrolizumab-Treated Patients Versus Placebo-Treated Patients (Baseline-Adjusted Geometric Mean Ratio [95%CI])
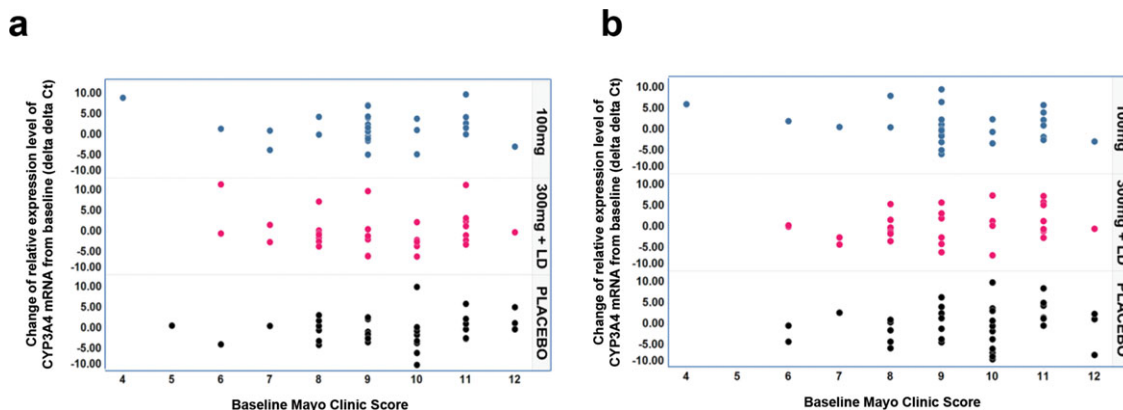
	Week 6	Week 10
Etrolizumab 100 mg versus placebo (fold)	2.5 (0.93, 6.5)	1.3 (0.35, 3.0)
Etrolizumab 300 mg +LD versus placebo (fold)	1.0 (0.35, 3.0)	1.0 (0.35, 3.0)

CI, confidence interval; CYP, cytochrome P450; LD, loading dose.

the target therapeutic dose level. Serum CRP has been proposed as a sensitive biomarker for inflammation-mediated CYP3A changes because production of CRP by hepatocytes is mediated via the same transcription factors that regulate the dynamics of IL-6. In addition, changes in CRP levels were found to correlate with CYP3A activity from in vivo studies.[12,35,38,39] These results are in agreement with the outcome of our literature-based assessment and confirm that a TP-DDI effect on CYP3A expression is unlikely with etrolizumab treatment.

Although the colon is not commonly found to be a major drug metabolism tissue, examination of the treatment effect of etrolizumab on colonic CYP3A4 mRNA levels from patients with UC was thought to be useful for assessing the potential TP-DDI in this study, based on the following considerations:

1. UC is a disease of the large intestine, and inflammation is mainly localized in the colon.[15] Hence, systemic inflammatory reactions of UC may not fully reflect the local inflammation status of the colon. Even though quantitative data comparing the cytokine levels between the systemic circulation and the colon are not available, significantly

Figure 3. Correlation of baseline MCS with changes from baseline in colonic CYP3A4 mRNA relative expression level (a) at week 6 and (b) at week 10 in 3 study arms. Baseline MCS ranges 0–12 in UC patients. Disease severity increases with increased MCS score. Eligible patients with moderate to severe UC were enrolled in the phase 2 ABS4986 study with a baseline MCS of 6–12 in the United States and a MCS of 5–12 outside the United States. A patient with an MCS of 4 was inadvertently enrolled in the ABS4986 study and was included in CYP3A4 mRNA analysis. MCS, Mayo Clinic Score; UC, ulcerative colitis. Actual drug amounts for the nominal 100-mg and 300-mg doses are 105 mg (1 injection of drug) and 315 mg (3 injections of drug), respectively (150 mg/mL drug concentration, 0.7 mL volume per injection).

increased expression of mRNA for TNF-α, IL-6, and IL-1β was found in the biopsy samples of large intestinal mucosa in patients with active UC compared to healthy subjects or patients in remission.[23,40] It is possible that, with higher levels of colonic proinflammatory cytokines, the effects of cytokine levels on CYP enzyme activity may be more pronounced in colon tissue than in hepatic tissue.

2. Systemic monitoring of drug exposure may not be sensitive enough to detect changes in colonic CYP enzyme activity because drug absorption and metabolism would likely be completed in the upper small intestine for most orally administered drugs.[41,42] Direct examination of changes in colonic CYP3A mRNA allows more definite determination of the effect on enzyme activity due to UC disease-drug interaction.

3. Although the total content of CYP enzymes in the intestine is much lower than that in the liver, CYP genes such as CYP3A4, 3A5, and 2E1 were found expressed at all locations throughout the GI tract.[43] Human studies have demonstrated that the enteric CYP3A can contribute significantly, and in some cases equally to hepatic CYP3A, to the first-pass metabolic effect on CYP3A substrates.[41,43] Among the tested areas along the human GI tract (jejunum, ileum, proximal colon, and distal colon), CYP3A content and activity are highest in the jejunum and lowest in the colon.[41,44] Still, CYP3A-mediated drug metabolism is detectable in human colonic tissues, based on ex vivo studies.[45]

4. Assessing the levels of CYP enzyme mRNA is the most sensitive approach to detecting changes in enzyme expression. Despite the large intersubject variability in CYP3A content, there is significant correlation between CYP3A4 mRNA and protein levels within each of the various human intestinal regions, including the jejunum, ileum, proximal colon, and distal colon.[41] CYP3A4 gene expression was also found to correlate well with CYP3A4 enzymatic activity in 466 human liver samples.[46] In addition, CYP3A4 mRNA measurement can distinguish clinically meaningful inducers from noninducers when an appropriate fold change cutoff value is defined from in vitro human hepatocytes.[47]

It is also recognized that CYP3A4 mRNA data typically exhibit considerable (>11-fold) intersubject and between-sample variability in both liver and intestine samples, probably due to the inherent variation in gene expression and the high sensitivity of the quantitative PCR assay.[44,46] Despite the inherent high variability, our data provide convincing evidence of no meaningful changes in colonic CYP3A4 mRNA levels by etrolizumab treatment.

This TP-DDI assessment for etrolizumab focused specifically on CYP3A, as CYP3A is the most common drug-metabolizing enzyme and is responsible for the elimination of approximately 50% of marketed drugs that undergo oxidative metabolism.[48] In addition, as summarized in Table 2, metabolism of the most frequently used concomitant medications for IBD patients does not involve CYP enzymes, with the exception of corticosteroids and some immunosuppressants (use limited to more severe IBD patients[49]),both of which are CYP3A substrates. Furthermore, among the CYP enzymes (CYPs 1A2, 2B6, 2C9, 2C19, 2D6, and 3A4) affected by multiple proinflammatory cytokines such as IL-6, IL-1β, and TNF-α, CYP3A was found to be most sensitive to the cytokine-mediated suppression, based on in vitro data from hepatocyte cultures.[8,9,50] The in vitro findings are consistent with tocilizumab clinical DDI data where treatment with tocilizumab led to a decrease in exposure of CYP3A probe substrate (simvastatin) by 57% but to a lesser extent in exposure of CYP2C19 probe substrate (omeprazole) by 28%.[35] Therefore, it may be reasonable to deduce that if the change in proinflammatory cytokine levels is not sufficient to alter CYP3A enzyme activity, other CYPs such as CYP2C19 are also unlikely to be impacted. Hence, focusing the TP-DDI risk assessment of etrolizumab on CYP3A is clinically meaningful.

To clearly rule out a possible TP-DDI risk, the ideal approach would be to conduct a dedicated clinical study by comparing the PK of specific CYP probe substrate(s) in patients with UC pre- and posttreatment with TPs. To date, clinical DDI studies have
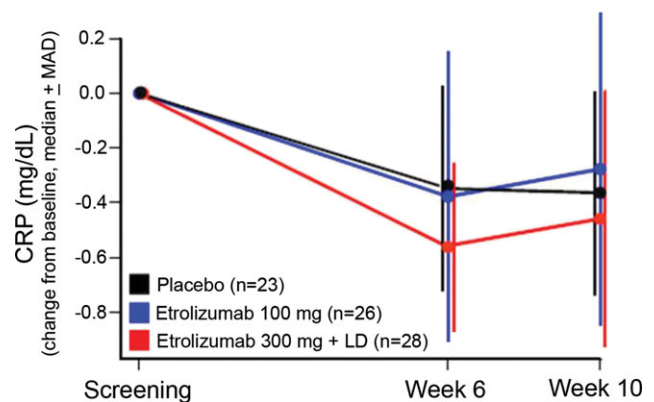
Figure 4. Change in serum CRP (median $\pm$ MAD) from baseline in treated and placebo arms in patients with elevated baseline CRP > 0.4 mg/dL. CRP, C-reactive protein; MAD, median absolute deviation. This figure was presented by Vermeire et al.[21] Actual drug amounts for the nominal 100-mg and 300-mg doses are 105 mg (1 injection of drug) and 315 mg (3 injections of drug), respectively (150 mg/mL drug concentration, 0.7 mL volume per injection).

been conducted with a limited number of TPs that are cytokines or cytokine modulators. Compared to the potent CYP inhibitors (eg, ketoconazole) or CYP inducers (eg, rifampin), the magnitude of clinical DDI effects of these cytokine modulator TPs is negligible or relatively modest. Such modest DDI effects have not warranted any dose adjustment of coadministered CYP substrates.[11,12] A clinical study investigated the disease-related TP-DDI potential for tocilizumab in patients with RA who have mean plasma IL-6 levels 10-fold higher than healthy subjects.[35] Consistent with the strong IL-6 effect of lowering CYP, treatment with tocilizumab, an anti–IL-6 receptor mAb, reversed the IL-6–induced suppression of CYP activity and led to a decrease in exposure of simvastatin (CYP3A probe substrate) and omeprazole (CYP2C19 probe substrate) by 57% and 28%, respectively.[35] Similarly, an 18%-45% exposure reduction for CYP substrates (3A4, 2C9, and 2C19) was also observed following sirukuzumab (anti– IL-6 mAb) treatment in RA patients.[51] In contrast, the lack of effect on PK of sensitive probe substrate was recently demonstrated in a clinical study on CYP3A (midazolam) with the treatment of denosumab, an inhibitor of cytokine RANKL, in postmenopausal patients with osteoporosis.[52] And treatment with da-clizumab (anti–α-chain of IL-2 receptor) did not alter the PK of several drugs that were substrates to different CYP isoforms (CYPs 1A2, 2C9, 3A, 2C19, and 2E1) in multiple sclerosis patients,[53] although serum IL-2 levels increased up to 2-fold post treatment, and the elevated IL-2 was found to alter the activities of multiple CYP isoforms from in vitro data.[11] These currently available in vivo data suggest that (1) the disease-related TP-DDI may exist but were mostly observed on those TPs with anti–IL-6 mechanisms, and such an observed TP-DDI effect is modest without requiring dose modification; (2) the TP-DDI risk assessed in vitro alone may not translate to clinical settings, especially in those diseases in which cytokine elevations are very low.[54]

Conducting a disease-related clinical DDI study in IBD patients could be challenging, and interpretation of results could be confounded by multiple complex factors. These include patient heterogeneity (eg, anti–TNF-α naive vs anti–TNF-α inadequate responders), concomitant use of anti-inflammatory medications, and potential drug malabsorption due to intestinal mucosal lesion. The 4-step TP-DDI risk assessment recommended by the IQ Consortium/FDA TP-DDI Workshop (San Diego, 2012) is a useful tool in justifying a need for conducting a dedicated DDI study. Our assessments of the potential TP-DDI risk of etrolizumab on CYP enzymes followed this 4-step approach and provided a convincing body of evidence indicating a low risk of clinical TP-DDI effect for etrolizumab. This holistic approach, which combines literature review and experimental assessments, may have broader applications to other therapeutic proteins in evaluating the TP-DDI risks on the relevant drug-metabolizing CYP enzymes.

Conclusions

Based on (1) literature findings of insignificant increase in baseline levels of serum proinflammatory cytokines in IBD patients and similar PK properties of CYP3A substrates in IBD versus healthy subjects, and (2) lack of etrolizumab treatment effect on the colonic tissue CYP3A4 mRNA expression and serum CRP levels in UC patients, we conclude that the disease-related TP-DDI risk for etrolizumab is low, particularly on medications metabolized by CYP3A.

Author Contributions

X.W. led the research design, reviewed the literature data, and wrote the manuscript; J.K. guided research design and data interpretation; L.D. performed qPCR assay on colonic CYP3A4 mRNA samples and helped with data interpreta-tion; R.M. conducted statistical analysis on the qPCR data and helped with data interpretation; C.L. prepared patients' samples for the qPCR assay; M.T.T. guided research design and wrote the manuscript. All authors reviewed and approved the manuscript.

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