Manuscript Doi: 10.1093/ecco-jcc/jjaa239

Tissue exposure does not explain non-response in ulcerative colitis patients with adequate serum vedolizumab concentrations

Nathalie Van den Berghe[1], Bram Verstockt[2,3], Ann Gils[1], João Sabino[2,3], Marc Ferrante[2,3], Séverine Vermeire[2,3], Paul Declerck[1], Debby Thomas[1]

[1] Laboratory for Therapeutic and Diagnostic Antibodies, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven, Belgium.

[2] Department of Gastroenterology and Hepatology, University Hospitals Leuven, KU Leuven, Leuven, Belgium.

[3] Translational Research in Gastrointestinal Disorders, Department of Chronic Diseases, Metabolism and Ageing, KU Leuven, Leuven, Belgium.

Address correspondence to:

Dr. Debby Thomas

Department of Pharmaceutical and Pharmacological Sciences, KU Leuven

Campus Gasthuisberg O&N2, PB 820; Herestraat 49, B-3000 Leuven, Belgium

Phone: +32 16 37 74 93

Email: debby.thomas@kuleuven.be

Abstract

Background and aims: Some patients with ulcerative colitis (UC) do not respond to vedolizumab treatment despite adequate drug exposure in serum. This study aimed to investigate vedolizumab in tissue and questioned whether insufficient tissue exposure could explain non-response in UC patients with adequate serum vedolizumab concentrations.

Methods: A paired serum sample and colonic mucosal biopsy was collected from 40 UC patients (20 endoscopic responders, 20 non-responders) at week 14 of vedolizumab treatment. Vedolizumab, soluble (s)-mucosal addressin cell adhesion molecule-1 (MAdCAM-1), s-vascular cell adhesion molecule-1 (VCAM-1) and s-intercellular adhesion molecule-1 (ICAM-1) were measured in serum and/or tissue. Endoscopic response was defined as Mayo endoscopic sub-score ≤1.

Results: A significant positive correlation was observed between vedolizumab serum and colonic tissue concentrations (ρ = 0.84, $p<0.0001$), regardless of the macroscopic inflammatory state of the tissue. Vedolizumab tissue concentrations were lower in non-responders than in responders (0.07 vs 0.11 µg/mg, p = 0.04). In the subgroup of patients with adequate vedolizumab serum concentrations (>14.6 µg/mL), tissue vedolizumab was not significantly different between responders and non-responders (0.15 vs 0.13 µg/mg; p = 0.92). Serum sMAdCAM-1, but not serum sICAM-1 or sVCAM-1 concentrations, were significantly higher in responders than non-responders with adequate vedolizumab serum concentrations (1.04 vs 0.83 ng/mL, p =0.03).

Conclusions: Vedolizumab concentrations in colonic mucosal tissue of UC patients reflect the concentration in serum regardless of the macroscopic inflammatory state of the tissue. Our data shows that insufficient tissue exposure does not explain non-response in UC patients with adequate serum vedolizumab concentrations.

Keywords: vedolizumab, colonic mucosal tissue, therapeutic drug monitoring

Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) characterized by continuous inflammation of the colonic mucosa. The intestinal inflammation has been attributed to the infiltration of leukocytes from the blood circulation into the gut mucosa, where these immune cells are activated and release pro-inflammatory cytokines.[1] Inhibiting leukocyte trafficking with vedolizumab is one of the therapeutic options for the treatment of IBD.

Vedolizumab is a humanized monoclonal antibody directed against the $\alpha_4\beta_7$ integrin on circulating leukocytes. Binding to $\alpha_4\beta_7$ results in prevention of the interaction of leukocytes with mucosal addressin cell adhesion molecule-1 (MAdCAM-1) on the intestinal endothelium, and consequently inhibits leukocytes trafficking to the gut.[2] The randomized controlled GEMINI trials showed vedolizumab to be a safe and effective drug in both moderate-to-severe UC and Crohn's disease (CD).[3-5]

Despite the proven therapeutic efficacy of vedolizumab, some patients experience primary non-response, while others initially respond to treatment but lose response over time.[6] Several explanations for this non-response or loss of response have been put forward. Serum drug concentrations might be too low due to a high inflammatory burden or the presence of anti-drug antibodies, leading to underexposure to the drug.[7,8] Alternatively, merely inhibiting lymphocyte trafficking through $\alpha_4\beta_7$ might not be sufficient to achieve response, and the disease might be driven by an alternative pathway, a so-called mechanistic failure.

Several studies on the concentration-response relationship for vedolizumab in UC patients revealed that non-response is associated with lower vedolizumab serum concentrations.[9-12] There is however limited knowledge on the correlation between vedolizumab concentrations in serum and tissue, and how this is associated with therapeutic response. Interestingly, in the ATLAS study focusing on the anti-tumor necrosis factor (anti-TNF) biologicals adalimumab and infliximab, serum anti-TNF concentrations correlated with anti-TNF concentrations in uninflamed but not inflamed tissue. Moreover, patients with active disease in that study had relatively low concentrations of tissue anti-TNF, despite elevated concentrations of serum anti-TNF.[13] It is therefore of interest to evaluate whether a similar observation could be made in vedolizumab-treated UC patients. Since several studies have challenged the 'inhibition of T-cell migration concept' as the sole mechanism of action, vedolizumab might have an additional anti-inflammatory function in tissue.[14-17]

This study aimed to investigate vedolizumab in tissue and questioned whether insufficient tissue exposure could explain non-response in UC patients with adequate serum vedolizumab concentrations.

Materials and Methods

Study design and patients

This study was conducted at University Hospitals Leuven (Leuven, Belgium) in accordance with the ethical principles of the Declaration of Helsinki. All patients provided written informed consent to participate in the Institutional Review Board-approved IBD Biobank (B322201213950/S53684) where serum, mucosal biopsies, and clinical characteristics are collected on predefined time points. Consecutive UC patients treated with vedolizumab for

which a paired serum sample and colonic mucosal biopsy was available at week 14 of treatment were considered for this study. Vedolizumab was administered intravenously at a dose of 300 mg at weeks 0, 2, 6, and 14 and every 8 weeks thereafter. All patients had active disease at baseline with a Mayo endoscopic sub-score ≥2. Patients who showed endoscopic disease activity limited to the rectum were excluded.

Sample collection

Serum samples and colonic mucosal biopsies were collected at week 14 and stored at -20 °C and -80 °C, respectively. Mucosal biopsies were stored in RNA-later. In non-responders, defined as a Mayo endoscopic sub-score of ≥2, inflamed colonic biopsies were taken near an ulceration or erosion in the most affected rectosigmoid area. In responders, defined as a Mayo endoscopic sub-score ≤1, a biopsy was taken in a macroscopically uninflamed rectosigmoid area. Endoscopic remission was defined as Mayo endoscopic sub-score equal to 0.

Four tissue samples were excluded because the sample was not collected at trough, i.e. right before the infusion. The final analysis included 17 macroscopically inflamed (Mayo endoscopic sub-score 2-3) and 19 uninflamed (Mayo endoscopic sub-score 0-1) colonic mucosal tissue samples, all collected from individual UC patients. For the analysis of serum samples, all 40 patients were considered.

Lysis of biopsies

After removing the biopsy from the RNA-later solution, the tissue sample was lysed by addition of 10 µl lysis buffer (50 mM Tris, 0.1% Triton X-100, 100 mM NaCl and cOmplete Mini Protease Inhibitor Cocktail (Roche)) per mg tissue and vortexed for 10-15 sec every 5

min during a 1 hour incubation period on ice. Thereafter, the sample was centrifuged for 5 min at maximum speed at 4°C and the supernatant was transferred to a clean labelled test tube. Tissue extracts were kept at -80°C until further analysis.

Measurements

The total protein content was measured in the tissue extracts using the Pierce™ BCA Protein Assay Kit (Thermo Scientific) and diluted to 3 mg/mL before application on the below-mentioned assays.

Vedolizumab concentrations

Vedolizumab concentrations in serum and tissue extracts were measured with an in-house developed enzyme-linked immunosorbent assay (ELISA) using MA-VDZ6E6 for capture and biotinylated MA-VDZ6F3 for detection.[9] Tissue extracts were diluted 1/50-1/400 to allow the quantification of vedolizumab concentrations down to 0.04 µg/mL. For tissue extracts, results are expressed as µg/mg total protein content. Spiking biopsies of vedolizumab-naïve IBD patients without or with vedolizumab (10 µg/mL), subsequent lysis, and analysis of the tissue extracts on the MA-VDZ6E6/MA-VDZ6F3 ELISA showed that vedolizumab could accurately be measured in tissue extracts (**Supplementary Table 1**). Tissue extracts without vedolizumab did not give a background signal.

MAdCAM-1, VCAM-1 and ICAM-1 concentrations

Soluble MAdCAM-1, vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) concentrations were determined using the Human MAdCAM-1 DuoSet ELISA (R&D Systems, DY6056-05), Human VCAM-1/CD106 DuoSet ELISA (R&D Systems,

DY809) and Human ICAM-1/CD54 DuoSet ELISA (R&D Systems, DY720) according to the manufacturer's instructions, respectively.

Statistical analyses

Percentages were used for discrete variables and median with interquartile range (IQR) for continuous variables. Spearman's rank correlation coefficient (ρ) was used to investigate the relationship between two continuous variables. Unpaired data were analysed using the Mann–Whitney U-test for continuous variables. To determine a rank-based trend, the non-parametric Jonckheere-Terpstra test for trend was used. Receiver-operator characteristic (ROC) curve analysis was performed to identify a vedolizumab serum concentration cut-off for response, and concentrations higher than this cut-off were considered adequate. A cut-off was chosen based on the performance of the Youden J statistic. All statistical analyses were performed with GraphPad Prism 8.4.0 (GraphPad Software, San Diego, CA, U.S.A.). The threshold for significance was set at 5%.

Results

Patient characteristics

A total of 40 consecutive UC patients (50% responders, 50% non-responders) who initiated vedolizumab therapy and of whom a paired serum sample and colonic mucosal biopsy at week 14 of treatment were available were included in this study. Baseline demographic and clinical characteristics are summarized in **Table 1**. Responders and non-responders had a median disease duration of 8.9 years and 7.3 years, respectively. Of all patients, 24 (60%) were previously treated with at least one anti-TNF agent. At baseline, 58% of all patients received concomitant corticosteroid therapy (70% topical, 30% systemic).

Correlation of vedolizumab serum and tissue concentrations

The median vedolizumab concentration in serum and tissue was 14.93 µg/mL (IQR 9.82–21.78 µg/mL) and 0.08 µg/mg (IQR 0.06–0.14 µg/mg), respectively. Two patients had undetectable vedolizumab concentrations in tissue, both had active endoscopic disease. A significant positive correlation was observed between vedolizumab concentrations in colonic mucosal tissue and matched serum ($\rho = 0.84$, $p < 0.0001$, **Figure 1**). This correlation was retained upon stratification based on the macroscopic inflammatory state of the tissue ($\rho = 0.84$ for inflamed and $\rho = 0.82$ for uninflamed; both $p < 0.0001$, **Supplementary Figure 1**).

A vedolizumab tissue dose-response relationship

Tissue vedolizumab concentrations were lower in non-responders than in responders (0.07 vs 0.11 µg/mg, respectively; $p = 0.04$, **Figure 2A**). A similar observation could be made when taking endoscopic remission, defined as Mayo endoscopic sub-score equal to 0, as the desired outcome. Vedolizumab concentrations in tissue of patients in remission were significantly higher compared to tissue of patients not achieving this outcome (0.12 vs. 0.07 µg/mg, respectively; $p = 0.02$, **Figure 2B**). Moreover, a trend was observed towards higher tissue vedolizumab concentrations in patients with lower Mayo endoscopic sub-scores and consequently a better response ($p<0.01$ for trend, **Supplementary Figure 2**).

Tissue exposure of vedolizumab in patients with adequate serum vedolizumab concentrations

Vedolizumab tissue concentrations were compared between non-responders and responders in whom vedolizumab serum concentrations were deemed adequate. Serum vedolizumab concentrations >14.6 µg/mL were considered adequate, as determined by ROC curve analysis (**Supplementary Figure 3**). This cut off value corresponds to what is reported in literature.[9] In this patient subgroup, responders did not have higher tissue vedolizumab concentrations compared to non-responders (0.15 vs 0.13 µg.mg; p = 0.92, **Figure 3**). Accordingly, insufficient tissue exposure does not explain non-response in UC patients with adequate serum vedolizumab concentrations.

Serum sMAdCAM-1 concentrations in patients with adequate serum vedolizumab concentrations

As insufficient tissue exposure cannot explain non-response in UC patients with adequate serum vedolizumab concentrations, a mechanistic explanation was sought. It was hypothesized that in these non-responders, the MAdCAM-1/$\alpha_4\beta_7$ axis might not be the main pathway for lymphocytes to enter the gut mucosa. MAdCAM-1 expression has been correlated with the infiltration of β7-integrin positive lymphocytes.[18,19] Consequently, higher MAdCAM-1 expression on the intestinal endothelium increases the probability of lymphocytes to enter the mucosa through this cell adhesion molecule. Therefore, soluble MAdCAM-1 (sMAdCAM-1) concentrations in serum, which is a reflection of transmembrane MAdCAM-1[20], were compared between responders and non-responders with adequate vedolizumab serum concentrations.

The sMAdCAM-1 concentration in serum was significantly higher in responders with adequate vedolizumab serum concentrations compared to non-responders with adequate serum vedolizumab (1.04 vs 0.83 ng/mL, p = 0.03, **Figure 4**). These results suggest that in non-responders with adequate drug exposure other pathways, besides the MAdCAM-1/$\alpha_4\beta_7$ axis, might contribute to the trafficking of lymphocytes into the mucosa.

Serum sICAM-1 and sVCAM-1 concentrations in patients with adequate serum vedolizumab concentrations

As the results above suggest that other pathways, besides the MAdCAM-1/α4β7 axis, might contribute to the trafficking of lymphocytes into the mucosa, soluble ICAM-1 (sICAM-1) and soluble VCAM-1 (sVCAM-1) concentrations in serum were compared between responders and non-responders with adequate serum vedolizumab concentrations. These cell adhesion molecules have been suggested to play a role in the pathophysiology of IBD.[21,22] The sICAM-1 and sVCAM-1 measured in serum is a reflection of transmembrane ICAM-1 and VCAM-1.[23] Serum sVCAM-1 concentrations were not significantly different in non-responders with adequate vedolizumab concentrations in serum than in responders (633 *vs.* 697 ng/mL, respectively; p = 0.44), nor were serum sICAM-1 concentrations (180 *vs.* 224 ng/mL, respectively; p = 0.15).

Discussion

Some UC patients do not respond to vedolizumab treatment despite sufficient drug exposure in the blood circulation.[6] In this study, we investigated vedolizumab tissue exposure and explored whether insufficient tissue exposure could explain non-response in UC patients with adequate serum vedolizumab concentrations.

In our study, a positive correlation was observed between vedolizumab concentrations in colonic mucosal tissue and matched serum, both in inflamed and uninflamed tissue. These findings indicate that vedolizumab concentrations in colonic mucosal tissue of UC patients reflect the concentration in serum regardless of the macroscopic inflammatory state of the tissue. Moreover, vedolizumab tissue concentrations were lower in non-responders than in responders. This difference in vedolizumab tissue concentration is most probably driven by decreased systemic concentrations of vedolizumab as supported by the strong correlation between vedolizumab concentrations in matched tissue and serum. Several explanations can be put forward for low vedolizumab concentrations. A high inflammatory burden can result into a high clearance of the drug and consequently low serum drug concentrations. Alternatively, drug can be lost through leaky epithelial barriers.

The results obtained in our study are in contrast to what was reported for the anti-TNF biologicals adalimumab and infliximab in the ATLAS study by Yarur *et al.*[13] In this study, a positive correlation was observed between anti-TNF in serum and uninflamed tissue but not inflamed tissue. Furthermore, anti-TNF concentrations were higher in inflamed than uninflamed tissue. These opposing results could be explained by differences in the study design. A biological with a different mechanism of action was used (anti-TNF vs.

vedolizumab) and only 20% of the cohort consisted of UC patients. More important, inflamed and uninflamed tissue samples in the ATLAS study were collected within the same patient while the tissue samples collected in the current study were from different patients. Lastly, Yarur and colleagues corrected the tissue anti-TNF concentrations for epithelial content (determined by the human epidermal growth factor receptor 2, HER2). In our study, tissue vedolizumab concentrations were not corrected for the epithelial content because this is only appropriate for proteins that are expressed by cells.

Several studies have challenged the 'inhibition of T-cell migration concept' as the sole mechanism of action of vedolizumab. [14-17] Along these lines, we hypothesized that insufficient tissue exposure could explain non-response in UC patients with adequate serum vedolizumab concentrations. Blockade of the $\alpha_4\beta_7$ integrin could alter the mucosal immune cell's phenotype or affect the activation or differentiation of cells. In patients with adequate vedolizumab serum concentrations (>14.6 µg/mL), tissue vedolizumab was not significantly different between responders and non-responders. Hence, insufficient tissue exposure does not explain non-response in UC patients with adequate serum vedolizumab concentrations.

Additionally, serum concentrations of different soluble endothelial cell adhesion molecules were determined to identify a mechanistic explanation for non-response. We observed that serum sMAdCAM-1 concentrations were significantly higher in responders with adequate vedolizumab serum concentrations compared to non-responders with adequate serum vedolizumab. Based on these total sMAdCAM-1 values, which reflect the amount of transmembrane MAdCAM-1 on intestinal endothelium, these results suggest that in non-responders with adequate drug exposure other pathways, besides the MAdCAM-1/α4β7

axis, might contribute to the trafficking of lymphocytes into the mucosa. This suggested mechanistic explanation for non-response is supported by a recent study where it was shown that more T cells from responders than from non-responders adhered to MAdCAM-1 prior to initiation of vedolizumab therapy.[24] In contrast, a study by Paul *et al.* reported higher sMAdCAM-1 values in non-responders than in responders.[25] Nevertheless, the results of this study cannot be compared to our study because the patient population (primarily CD patients vs all UC patients), dosing regimen (dosage optimization vs no optimization) and sampling time point (maintenance vs. induction) are considerably different.

Next to the MAdCAM-1/$\alpha_4\beta_7$ axis, lymphocytes can enter the mucosa through other cell adhesion molecules.[26] In CD patients, it has been shown that T cell homing through the $\alpha_4\beta_1$-VCAM-1 is an essential and non-redundant pathway.[27] In our cohort, neither sVCAM-1 nor sICAM-1 serum concentrations were significantly different between responders and non-responders with adequate serum vedolizumab concentrations. Lymphocyte trafficking in non-responders with adequate vedolizumab concentrations in serum might not occur through one specific pathway but a combination of different pathways or other, less-characterized cell adhesion molecules might contribute to the influx of lymphocytes

The real-life setting, the use of a validated vedolizumab assay, and the sampling of paired serum and tissue samples are the main strengths of this study. Nevertheless, limitations include the lack of central reading of endoscopy and the fact that only UC patients and only one colonic mucosal tissue per patient was included and that no histological scores were used. Moreover, as inflamed tissue samples were collected in non-responders and uninflamed tissue samples in responders, a bias might be introduced. This should be kept in

mind when interpreting the results, especially when comparing inflamed versus uninflamed tissue. Lastly, the sample size is relatively small and therefore, the results should be validated in a larger cohort.

In summary, vedolizumab concentrations in colonic mucosal tissue of UC patients reflect the concentration in serum regardless of the macroscopic inflammatory state of the tissue. Moreover, our data shows that insufficient tissue exposure does not explain non-response in UC patients with adequate serum vedolizumab concentrations. More research is needed to identify a mechanistic explanation for primary non-response.

References

1. Sartor RB. Mechanisms of disease: Pathogenesis of crohn's disease and ulcerative colitis. *Nat Clin Pract Gastroenterol Hepatol* 2006;**3**:390-407.
2. Soler D, Chapman T, Yang LL*, et al.* The binding specificity and selective antagonism of vedolizumab, an anti-alpha4beta7 integrin therapeutic antibody in development for inflammatory bowel diseases. *J Pharmacol Exp Ther* 2009;**330**:864-75.
3. Feagan BG, Rutgeerts P, Sands BE*, et al.* Vedolizumab as induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2013;**369**:699-710.
4. Sandborn WJ, Feagan BG, Rutgeerts P*, et al.* Vedolizumab as induction and maintenance therapy for crohn's disease. *N Engl J Med* 2013;**369**:711-21.
5. Sands BE, Feagan BG, Rutgeerts P*, et al.* Effects of vedolizumab induction therapy for patients with crohn's disease in whom tumor necrosis factor antagonist treatment failed. *Gastroenterology* 2014;**147**:618-27.e3.
6. Peyrin-Biroulet L, Danese S, Argollo M*, et al.* Loss of response to vedolizumab and ability of dose intensification to restore response in patients with crohn's disease or ulcerative colitis: A systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2019;**17**:838-46.e2.
7. Chaparro M, Guerra I, Muñoz-Linares P, Gisbert JP. Systematic review: Antibodies and anti-tnf-α levels in inflammatory bowel disease. *Aliment Pharmacol Ther* 2012;**35**:971-86.
8. Vermeire S, Gils A. Value of drug level testing and antibody assays in optimising biological therapy. *Frontline Gastroenterol* 2013;**4**:41-3.
9. Dreesen E, Verstockt B, Bian S*, et al.* Evidence to support monitoring of vedolizumab trough concentrations in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol* 2018;**16**:1937-46.e8.
10. Yacoub W, Williet N, Pouillon L*, et al.* Early vedolizumab trough levels predict mucosal healing in inflammatory bowel disease: A multicentre prospective observational study. *Aliment Pharmacol Ther* 2018;**47**:906-12.
11. Liefferinckx C, Minsart C, Cremer A*, et al.* Early vedolizumab trough levels at induction in inflammatory bowel disease patients with treatment failure during maintenance. *Eur J Gastroenterol Hepatol* 2019;**31**:478-85.
12. Verstockt B, Mertens E, Dreesen E*, et al.* Influence of drug exposure on vedolizumab-induced endoscopic remission in anti-tumour necrosis factor [tnf] naïve and anti-tnf exposed ibd patients. *J Crohns Colitis* 2020;**14**:332-41.
13. Yarur AJ, Jain A, Sussman DA*, et al.* The association of tissue anti-tnf drug levels with serological and endoscopic disease activity in inflammatory bowel disease: The atlas study. *Gut* 2016;**65**:249-55.
14. Fischer A, Zundler S, Atreya R*, et al.* Differential effects of α4β7 and gpr15 on homing of effector and regulatory t cells from patients with uc to the inflamed gut in vivo. *Gut* 2016;**65**:1642-64.
15. Zeissig S, Rosati E, Dowds CM*, et al.* Vedolizumab is associated with changes in innate rather than adaptive immunity in patients with inflammatory bowel disease. *Gut* 2019;**68**:25-39.
16. Schippers A, Muschaweck M, Clahsen T*, et al.* B7-integrin exacerbates experimental dss-induced colitis in mice by directing inflammatory monocytes into the colon. *Mucosal Immunol* 2016;**9**:527-38.
17. Rogler G. Mechanism of action of vedolizumab: Do we really understand it? *Gut* 2019;**68**:4-5.
18. Hatanaka K, Hokari R, Matsuzaki K*, et al.* Increased expression of mucosal addressin cell adhesion molecule-1 (madcam-1) and lymphocyte recruitment in murine gastritis induced by helicobacter pylori. *Clin Exp Immunol* 2002;**130**:183-9.

19. Hokari R, Kato S, Matsuzaki K*, et al.* Involvement of mucosal addressin cell adhesion molecule-1 (madcam-1) in the pathogenesis of granulomatous colitis in rats. *Clin Exp Immunol* 2001;**126**:259-65.
20. Leung E, Lehnert KB, Kanwar JR*, et al.* Bioassay detects soluble madcam-1 in body fluids. *Immunol Cell Biol* 2004;**82**:400-9.
21. Gu P, Theiss A, Han J, Feagins LA. Increased cell adhesion molecules, pecam-1, icam-3, or vcam-1, predict increased risk for flare in patients with quiescent inflammatory bowel disease. *J Clin Gastroenterol* 2017;**51**:522-7.
22. Burns RC, Rivera-Nieves J, Moskaluk CA*, et al.* Antibody blockade of icam-1 and vcam-1 ameliorates inflammation in the samp-1/yit adoptive transfer model of crohn's disease in mice. *Gastroenterology* 2001;**121**:1428-36.
23. Patel RT, Pall AA, Adu D, Keighley MR. Circulating soluble adhesion molecules in inflammatory bowel disease. *Eur J Gastroenterol Hepatol* 1995;**7**:1037-41.
24. Allner C, Melde M, Becker E*, et al.* Baseline levels of dynamic cd4(+) t cell adhesion to madcam-1 correlate with clinical response to vedolizumab treatment in ulcerative colitis: A cohort study. *BMC Gastroenterol* 2020;**20**:103.
25. Paul S, Williet N, Di Bernado T*, et al.* Soluble mucosal addressin cell adhesion molecule 1 and retinoic acid are potential tools for therapeutic drug monitoring in patients with inflammatory bowel disease treated with vedolizumab: A proof of concept study. *J Crohns Colitis* 2018;**12**:1089-96.
26. Arseneau KO, Cominelli F. Targeting leukocyte trafficking for the treatment of inflammatory bowel disease. *Clin Pharmacol Ther* 2015;**97**:22-8.
27. Zundler S, Fischer A, Schillinger D*, et al.* The α4β1 homing pathway is essential for ileal homing of crohn's disease effector t cells in vivo. *Inflamm Bowel Dis* 2017;**23**:379-91.

Figure legends

Figure 1: Correlation between the vedolizumab concentration in serum and colonic mucosal tissue of UC patients at week 14 of vedolizumab treatment (n=36). Four tissue samples were excluded because the sample was not collected at trough. Black dots represent inflamed tissue, white dots represent uninflamed tissue. Spearman ρ = 0.84, p <0.0001

Figure 2: (A) Median vedolizumab colonic mucosal tissue concentrations in non-responders (n = 17; 0.07 µg/mg) versus responders (n = 19; 0.12 µg/mg). (B) Median vedolizumab colonic mucosal tissue concentrations in non-remitters (n = 26; 0.07 µg/mg) versus remitters (n = 10; 0.12 µg/mg). * p < 0.05. Four tissue samples were excluded because the tissue sample was not collected at trough

Figure 3: Vedolizumab concentrations in colonic tissue of non-responders (0.15 µg/mg; n = 5) and responders (0.13 µg/mg; n = 13) which are deemed to have adequate vedolizumab serum concentrations (>14.6 µg/mL). p = 0.92, not significant

Figure 4: sMAdCAM-1 concentrations in serum of non-responders (n = 7; 0.83 ng/mL) and responders (n = 13; 1.04 ng/mL) which are deemed to have adequate vedolizumab serum concentrations (>14.6 µg/mL). * p < 0.05

Tables

Table 1 - Baseline characteristics of the 40 included ulcerative colitis patients

Conflicts of Interest

B Verstockt reports financial support for research from Pfizer; lecture fees from Abbvie, Ferring, Takeda Pharmaceuticals, Janssen and R-Biopharm; consultancy fees from Janssen and Sandoz. A Gils reports financial support for research from Pfizer, MSD and Takeda; lecture fees from MSD, Janssen Biologicals, Pfizer, Takeda, Novartis and AbbVie; consultancy fees from Takeda; and advisory board fees from Takeda. KU Leuven holds a license agreement with R-Biopharm, apDia and Merck. J Sabino reports having received speaker fees from AbbVie, Nestle Health Sciences, and Takeda; consultancy fees from Janssen. M Ferrante reports research grants from Amgen, Biogen, Janssen, Pfizer and Takeda; consultancy fees from Abbvie, Boehringer-Ingelheim, Celltrion, Janssen, Lilly, Medtronic, MSD, Pfizer, Sandoz, Takeda and Thermo Fisher; and speakers fees from Abbvie, Amgen, Biogen, Boehringer-Ingelheim, Falk, Ferring, Janssen, Lamepro, MSD, Mylan, Pfizer, Sandoz, Takeda and Truvion Healthcare. S Vermeire reports financial support for research from AbbVie, MSD, Pfizer, J&J, and Takeda; received speaker fees from AbbVie, MSD, Takeda, Ferring, Dr. Falk Pharma, Hospira, Pfizer Inc., and Tillots; and served as a consultant for AbbVie, MSD, Takeda, Ferring, Genentech/Roche, Robarts Clinical Trials, Gilead, Celgene, Prometheus, Avaxia, Prodigest, Shire, Pfizer Inc, Galapagos, Mundipharma, Hospira, Celgene, Second Genome, and Janssen. The remaining authors declare no conflicts of interest.

Funding

This work was supported by the TBM Grant T003716N of the Research Foundation—Flanders (FWO), Belgium. N Van den Berghe is a Strategic Basic Research PhD fellow at FWO. S Vermeire and M Ferrante are Senior Clinical Investigators of the Research Foundation Flanders, Belgium.

Authors' contributions

N Van den Berghe: conceptualization, investigation, formal analysis, Writing - Original Draft, Writing - Review & Editing, visualization, project administration. B Verstockt: conceptualization, resources, formal analysis, Writing - Review & Editing. A Gils: conceptualization, Writing - Review & Editing. J Sabino, M Ferrante and S Vermeire: resources, Writing - Review & Editing. P Declerck: conceptualization, supervision, Writing - Review & Editing. D Thomas: conceptualization, supervision, Writing - Review & Editing, Project administration, validation.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Table 1. Baseline characteristics of the 40 included ulcerative colitis patients

	Responders	Non-responders
Number of patients, n (%)	20 (50%)	20 (50%)
Sex, women, n (%)	13 (65%)	13 (65%)
Age, median (IQR), y	45.7 (30.3-62.4)	41.5 (30.1-55.7)
Disease duration, median (IQR), y	8.9 (3.1-14.7)	7.3 (2.1-14.2)
Smoking status, n (%)		
Active smoking	0	1 (5%)
Previously smoking	7 (35%)	5 (25%)
Never smoked	13 (65%)	14 (70%)
Previous anti-TNF, n (%)	11 (55%)	13 (65%)
Concomitant corticosteroid therapy, n (%)	10 (50%)	13 (65%)
Topical (n) %	8 (80%)	8 (62%)
System (n) %	2 (20%)	5 (38%)
C-reactive protein, median (IQR), mg/L	2.0 (1.1-4.3)	3.0 (0.8-7.0)
Serum albumin, median (IQR), g/L	43.1 (39.3-45.4)	42.5 (40.1-44.2)
Disease extent		
Proctitis (E1) (n) %	4 (20%)	3 (15%)
Left-sided colitis (E2) (n) %	8 (40%)	5 (25%)
Extensive colitis (E3) (n) %	8 (40%)	12 (60%)
Mayo Endoscopic Sub-score, n (%)		
0 (n) %	10 (50%)	/
1 (n) %	10 (50%)	/
2 (n) %	/	10 (50%)
3 (n) %	/	10 (50%)

IQR, interquartile range

Figure_1

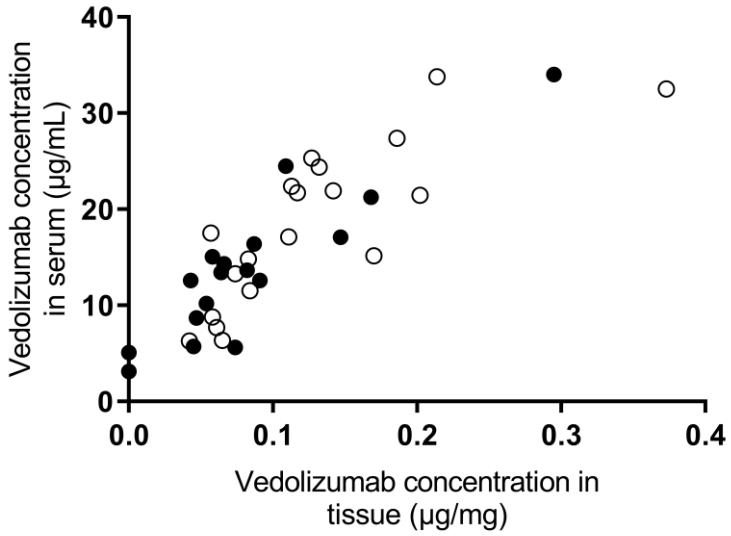

Figure_2

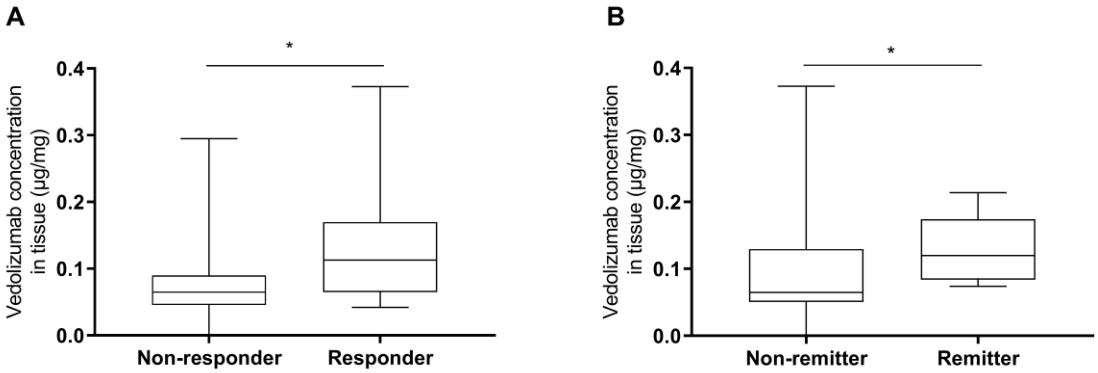

Figure_3

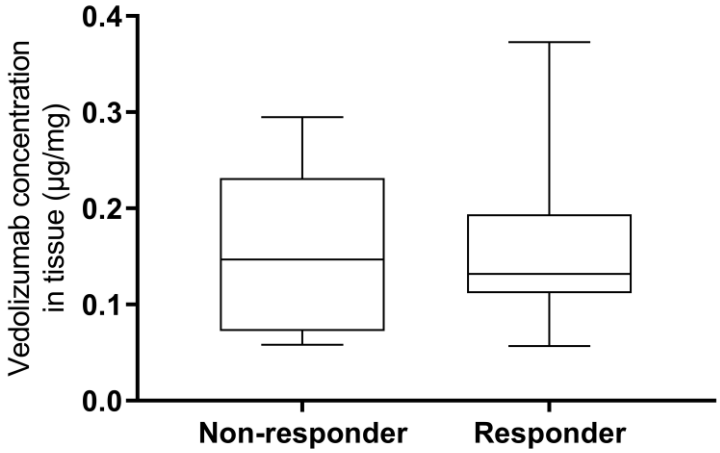

Figure_4