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Dose optimisation for Loss of Response to Vedolizumab— Pharmacokinetics and Immune Mechanisms

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Abstract

Background: Real life data regarding pharmacokinetics of vedolizumab in patients needing dose optimisation are scarce. We set to examine whether pre-optimisation vedolizumab levels associate with therapy outcomes and which mechanisms explain the associations.

Methods: A multicentre observational study assessed the outcome of dose increase in association with pre-escalation levels in vedolizumab-treated patients. Subsequently, $\alpha4\beta7$ occupancy on peripheral blood [PB] and intestinal lamina propria [LP] tissues was investigated on various cellular subsets in patients undergoing lower endoscopy on infusion day. Cellular localisation of vedolizumab-bound $\alpha4\beta7$ and effects on M1 and M2 macrophages were also explored.

Results: A total of 161 inflammatory bowel disease [IBD] patients were included. Among 129/161 patients intensified during maintenance [Week 14 onward], pre-intensification trough levels were comparable or higher among those subsequently attaining post-optimisation clinical, biomarker, and endoscopic remission, compared with non-remitting patients [$p = 0.09$, 0.25, 0.04, respectively]. Similar results were demonstrated for those dose-optimised during induction [Week 6, $n = 32$]. In the immune sub-study [$n = 43$], free $\alpha4\beta7$ receptors at trough were similarly low among patients with/without mucosal healing, on PB T cells [$p = 0.15$], LP T cells [$p = 0.88$], and on PB eosinophils [$p = 0.08$]. Integrin receptors on M1 and M2 macrophages were also saturated by low levels of vedolizumab and anti-inflammatory cytokine secretion was not increased. Co-localisation and dissociation experiments demonstrated membranal $\alpha4\beta7$ receptors of two origins: non-internalised and newly generated $\alpha4\beta7$, but re-binding was still complete at very low concentrations.

Conclusions: These results do not support pharmacokinetics as the mechanism responsible for loss of response to vedolizumab, nor do they support a need for higher drug concentration to enhance vedolizumab's immune effects. Higher pre-escalation levels may indicate less clearance [less severe disease] and higher likelihood of subsequent re-gained response, regardless of therapy escalation.

Key Words: Vedolizumab; IBD; pharmacokinetics; immunology; clinical outcome

1. Introduction

Vedolizumab is a gut-specific anti α4β7 integrin monoclonal antibody. It has been shown to induce and maintain clinical and endoscopic remission in ulcerative colitis [UC] and Crohn's disease [CD] and is currently approved for patients with moderate to severe CD and UC, either naïve or anti-TNF experienced.[1,2]

Therapeutic drug monitoring [TDM] of anti-TNF therapy has become the standard of care for many clinicians worldwide.[3] Sufficient trough levels of infliximab and adalimumab have been associated with higher rates of mucosal healing and decreased incidence of long-term complications in both UC and CD.[4–6] TDM of anti-TNFs has been shown to be beneficial and cost-effective when used reactively—upon loss of clinical response.[7] As to TDM of vedolizumab, data so far have been more inconclusive with some—but not all—studies indicating a modest correlation between drug levels and clinical outcomes.[8–12]

Upon insufficient response/loss of response to vedolizumab's standard dosing of 300 mg/8 weeks, the GEMINI non-controlled single-arm extension studies showed that roughly 40% of patients recapture response following interval shortening to every 4 weeks[13] and this has become a common practice. However, the pharmacokinetic basis for this practice, i.e. whether patients with loss of response have lower drug levels that require dose escalation to increase drug concentration into a therapeutic range, has not been hitherto explored. Thus, in the present study we aimed to explore pharmacokinetics associated with response to dose optimisation of vedolizumab and to elucidate whether reactive TDM would be beneficial among vedolizumab therapy patients.

Furthermore, although almost complete saturation of α4β7 receptors on peripheral blood and lamina propria T cells of vedolizumab-treated patients was previously demonstrated,[8] levels at trough time point and on different cell lines have not been explored. Thus, in the second part of the study we set to investigate if immune mechanisms can account for the observations of the clinical part.

2. Methods

2.1. Patient population and endpoints

The study had two parts. The first was a prospective observational study of inflammatory bowel disease [IBD] patients receiving scheduled vedolizumab therapy in six medical centres in Israel and Europe [Cohort A], who had undergone vedolizumab dose optimisation by interval shortening, due to primary non-response/secondary loss of response, as per treating physicians' judgment. All patients had signs of active inflammation [biomarker/imaging/endoscopy] upon enrolment, i.e. at the time of dose intensification. The primary endpoint was the correlation of pre-escalation baseline drug levels with clinical remission 6 months after interval shortening. Secondary outcomes included the correlation of pre-escalation baseline drug levels with biomarker remission and mucosal healing 6 months after dose optimisation, as well as the correlation of post-optimisation drug levels and these three outcomes. We excluded patients aged below 18, undetermined diagnosis of UC or CD [IBD unclassified], missing clinical/demographic data or missing evidence of active inflammation upon interval shortening, not receiving scheduled vedolizumab induction therapy, or not interval-shortened to vedolizumab every 4 weeks [q4], or if clinical outcomes 6 months after intervention were not available.

The second part [Cohort B] included IBD patients receiving scheduled vedolizumab therapy at Sheba Medical Center, who were referred by their physician for lower endoscopy for clinical reasons. Peripheral blood [10 ml] and up to eight colonic biopsies on endoscopy were obtained prospectively. Supplementary Table 1, available as Supplementary data at *ECCO-JCC* online, depicts their clinical and demographic characteristics. Endoscopies were performed just before the next scheduled infusion [trough time point]. Biopsies were also obtained from 12 [four healthy controls and eight IBD] patients not treated with vedolizumab.

2.2. Ethics

The study was approved by the medical centres' ethics committees, and all patients included gave a written informed consent.

2.3. Biomarker measurements

Serum albumin and C-reactive protein [CRP] levels were measured using the Beckman Coulter Clinical Chemistry AU5800 Analyser [Beckman Coulter, USA].

Stool calprotectin was measured using commercially available ELISA assays. Biomarker remission was defined as normalisation of previously increased biomarker [CRP/calprotectin]. Patients' pre- and post-escalation serum samples were shipped to Sheba Medical Center, where drug serum levels were measured centrally at the GI Immunology Laboratory by a previously described validated ELISA assay.[14] Because of a previous study indicating a possible role for soluble MADCAM1 for predicting response in patients starting vedolizumab,[15] we also measured sMADCAM1 in baseline pre-escalation sera of our cohort using a commercially available ELISA kit according to manufacturer instructions [abx054719 Human MAdCAM 1 ELISA Kit, Rainbow Biotechnologies, China].

2.4. Measurement of vedolizumab concentrations

Integrin alfa4 beta7 [2 μg/ml, R&D, MN, USA] was added to pre-plated anti-His tag [4 μg/ml, RD, MN, USA] wells of ELISA plates [Nunc, Roskilde, Denmark];100 μl of 1:1000 diluted serum was added and incubated for 60 min at room temperature. Plates were then washed and goat anti-human κ chain HRP-labelled antibody [Serotec, Oxford, UK] was added at a concentration of 66 ng/ml for 40 min. The results were read by an ELISA reader EL 800 [Biotek Instruments, Winooski, USA] and expressed as μg/ml.

2.5. Definition of clinical remission

Clinical status was determined by HBI [Harvey-Bradshaw Index] for Crohn's disease [CD] and by SCCAI [Simple Clinical Colitis Activity Index] for ulcerative colitis [UC] patients. Clinical remission was defined as HBI < 5 for CD patients and SCCAI ≤ 3 for UC patients.

2.6. Definition of mucosal healing

All endoscopies, performed at interval shortening and 6 months after therapy intensification at physician discretion, were reviewed for their endoscopic scores. Mucosal healing was defined as absence of ulcers/lack of inflammation on endoscopic examination, for CD and UC respectively.[5]

2.7. Fluorescence-activated cell sorting [FACS] analysis of α4β7 occupancy

Whole-blood eosinophils and monocytes and freshly obtained peripheral blood mononuclear cells [PBMC] and lamina propria [LP] T cells were isolated and prepared as previously described.[16] FACS staining and sorting was performed as described in Supplementary Document 2, available as Supplementary data at *ECCO-JCC* online.

2.8. Membranal localisation experiments

A previous study has shown α4β7 integrin internalisation after its binding.[17] To test whether seeming complete saturation is due to complete internalisation or due to blockade on the membrane at clinically relevant concentrations, the designated cell subset cultures were pre-incubated with unlabelled vedolizumab at pre-specified concentration for 24 or 72 h. Acid dissociation was then performed [Supplementary Document 2]. Staining for membranal α4β7 expression was then performed with unlabelled vedolizumab and with/without the addition of monensin, a Golgi transport inhibitor to block transport of internalised integrin back to the cell surface. To exclude permeability of the cell membrane induced by acid dissociation, permeabilising capacity was determined [Supplementary Document 2]. Intracellular TNFα content was then assessed by staining with PE-conjugated anti-TNFα and FACS analysis.

To explore the localisation of α4β7 following vedolizumab binding, multispectral imaging flow cytometry [IFC] analysis was applied [Supplementary Document 2].

2.9. Assessment of vedolizumab effects on differentiated M1 and M2 macrophages

To explore whole-blood monocytes' sub-classes, FACS analysis was performed for CD14 + and C16 + cells. To explore M1 and M2 macrophages, CD14 + magnetic bead isolation was employed in whole blood. Resulting CD14 + cells [purity > 95%] were then differentiated into M1 and M2 [Supplementary Document 2]. FACS was performed and secreted cytokines [IL-10, TNFα and TGF-β] were then tested in supernatant by ELISA.

To ascertain effects of vedolizumab on M1 and M2 macrophages derived from a second cell source, THP1 cells were differentiated into M1 macrophages [Supplementary Document 2]. Staining for CD80, CD86, CD163, and CD206 was performed using flow cytometry. Increasing concentrations of vedolizumab were added to determine the concentration required to block α4β7 receptors. The effects of different vedolizumab concentrations [0,1,3,10,40,80 μg/ml] on non-classical monocytes' cytokine secretion were tested.

2.10. Materials

For details of materials, please see Supplementary Document 1, available as Supplementary data at *ECCO-JCC* online.

2.11. Statistical analysis

Continuous variables were expressed as the median and interquartile range [IQR] and categorical variables as a percentage. Mann-Whitney testing was used to compare continuous variables and Fisher's exact test was used for categorical data. A receiver operating characteristic [ROC] analysis was performed for vedolizumab trough levels using mucosal healing as a classification variable. Sensitivity, specificity, and area under the curve [AUC] were determined for these variables. Correlations were analysed by the Spearman rank correlation test. All reported *p*-values were two-sided, and a *p*-value less than 0.05 was considered statistically significant. All statistical calculations were performed with the use of MedCalc software [version 12.2.1.0, Mariakerke, Belgium].

3. Results

3.1. Outcomes of vedolizumab therapy interval shortening

Overall, 161 IBD patients comprising Cohort A [74/161, 44% Crohn's disease] were included, of whom 32 patients underwent intensification at end of induction [Week 10] and 129 during maintenance period [Weeks 14–44, median Week 30, Figure 1]. In total, 34%, 60%, and 30% of the 161 patients reached clinical, biomarker, and endoscopic remission by 6 months after therapy intensification, respectively. Table 1 depicts the Cohort's demographic and clinical parameters.

3.2. Associations between baseline vedolizumab levels and outcomes of interval shortening

Among 129 patients intensified during maintenance period, there was no statistically significant difference in vedolizumab baseline pre-optimisation trough levels among those reaching clinical remission after dose escalation, compared with those clinically active after the intervention [34.4 vs 24.4 μg/ml, IQR 18.5–60, 10.5–51 μg/ml, respectively, $n = 129$, $p = 0.09$, Figure 2a]. In fact, patients who attained endoscopic remission following optimisation had higher vedolizumab levels before intensification compared with patients not attaining it [median vedolizumab levels 31.3 vs 17 μg/ml, respectively, $n = 49$, $p = 0.04$, Figure 2b]. There was no difference in vedolizumab trough levels before escalation among those achieving or not achieving biomarker remission [normal C-reactive protein, CRP] after dose escalation [median levels 30.7 vs 30.5 μg/ml, $n = 79$, $p = 0.25$, respectively, Figure 2c].

The same analysis was performed for patients who underwent vedolizumab therapy intensification during induction period [$n = 32$]. Among patients intensified at Week 6 by an extra dose of vedolizumab 300 mg at Week 10, those with higher levels before escalation had higher rates of clinical remission 6 months following escalation [$n = 32$, median levels 101 vs 61.5 μg/ml, $p = 0.05$, for patients with and without clinical remission at 6 months, respectively]. No statistically significant difference was observed in vedolizumab drug levels before dose intensification between patients with or without biomarker remission [100 vs 55 μg/ml, $n = 18$, $p = 0.15$, respectively] or baseline drug levels in patients with/without endoscopic remission after dose intensification [88.5 vs 43 μg/ml, $n = 12$, $p = 0.09$, for those with and without mucosal healing, respectively].

ROC curve analysis was performed for vedolizumab levels in association with mucosal healing, among maintenance period patients. As demonstrated in Figure 2d, baseline pre-escalation trough vedolizumab levels above 14.5 μg/ml identified patients who had higher rates of mucosal healing at 6 months after dose optimisation (AUC 0.69, $p = 0.02$, sensitivity 93%, specificity 43%,

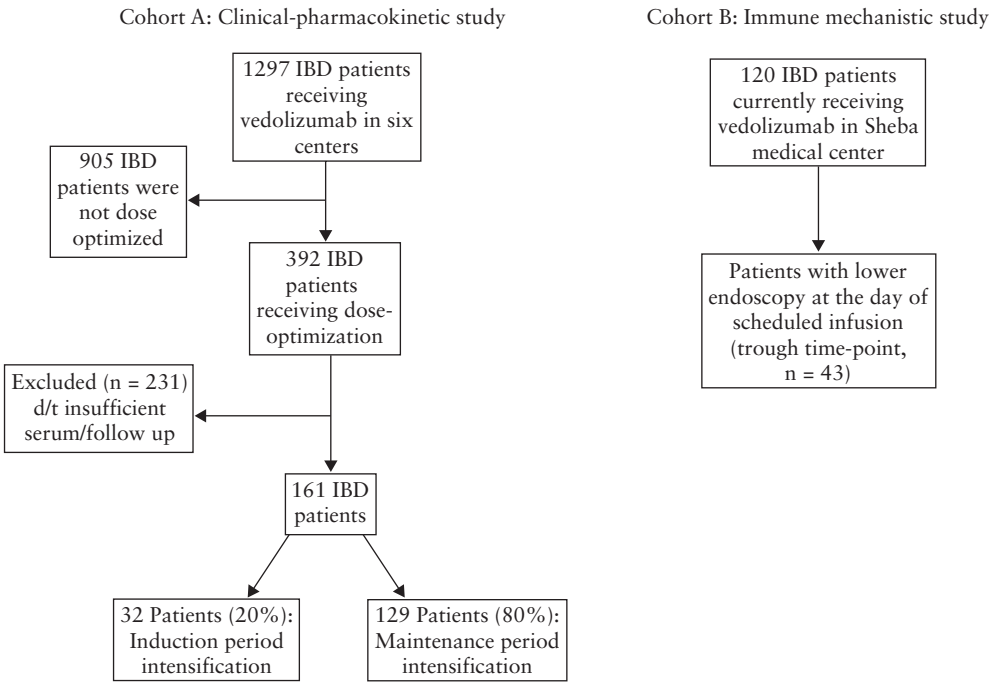

Figure 1. Study flow for both study cohorts. IBD, inflammatory bowel disease.

Table 1. Patients' demographic and clinical characteristics, Cohort A.

N		161
IBD type [CD], *n* [%]		71 [44]
Age, years [median, IQR]		39 [29-57]
Disease duration, years [median, IQR]		9 [5-15]
Female gender		41.7%
Smoking at induction, *n* [%]		21 [13]
BMI, kg [median, IQR]		23 [20-26]
Previous surgery, *n* [%]		28 [17.4]
Concomitant medical condition, *n* [%]		49 [30]
Extra-intestinal manifestations, *n* [%]		44 [27]
Concomitant immunomodulator therapy, *n* [%]		30 [18.6]
Concomitant steroids at interval shortening		76 [47]
CD behaviour	Inflammatory, *n* [%]	35 [50]
	Stricturing *n* [%]	27 [39]
	Penetrating *n* [%]	9 [11]
CD location	Ileal *n* [%]	22 [31]
	Ileo colonic *n* [%]	38 [54]
	Colonic *n* [%]	11 [15]
Perianal CD, *n* [%]		21 [29.5]
CD—upper gastrointestinal involvement, *n* [%]		7 [10]
UC location	Proctitis	5 [5]
	Left-sided colitis, *n* [%]	33 [37]
	Pancolitis, *n* [%]	51 [58]
Failure of anti-TNFα therapy, *n* [%]		74 [87]
Vedolizumab Week 6 pre-escalation trough serum level, μg/ml [median, IQR]		83 [45–107]
Vedolizumab maintenance period pre-escalation trough serum level, μg/ml [median, IQR]		27 [12–55]
HBI at interval shortening [median, IQR][a]		5 [3–9]
SCCAI at interval shortening [median, IQR][a]		4 [2–7]
CRP at interval shortening, mg/l [median, IQR]		7 [3–12]

IBD, inflammatory bowel disease; CD, Crohn 's disease; UC, ulcerative colitis; BMI, body mass index; IQR, interquartile range; HBI, Harvey- Bradshaw Index; SCCAI, Simple Clinical Colitis Activity Index; CRP, C- reactive protein.

[a]Patients who were clinically assessed using Mayo score/CDAI [Crohn's Disease Activity Index] were not included in this analysis.

positive predictive levels [PPV] = 68.5%, negative predictive levels [NPV] = 31.5%).

To further illustrate the associations between vedolizumab trough levels and ouctomes, two additional analyses were performed. The first one used quartiles of pre-escalation drug levels compared against subsequent response to escalation, and the second was an analysis of response to dose intensification in patients with levels below/above 17 μg/ml [threshold found in some previous studies to be associated with the occurrence of the non-response that prompts the dose intensification[10,11]]. The results of these two analyses are depicted in Supplementary Figures 1 and 2, available as Supplementary data at *ECCO-JCC* online and broadly corresponded to the aforementioned findings, showing that higher—rather than lower—levels before intensification were associated with favourable response to the dose intensification.

3.3. Association of post-optimisation drug concentrations with outcomes

Next, we set to determine whether vedolizumab levels after dose intensification could be associated with success of this intervention. Vedolizumab trough levels 1 month after intensification [before the second shortened dose, q4] were analysed in association with outcomes 6 months after the intervention. Trough drug levels 1 month after optimisation tended to be higher among patients with later clinical remission 6 months after optimisation compared with patients without clinical remission, but this did not reach statistical significance [median levels 55 vs 45 μg/ml, IQR 42–78, 36.6–56 μg/ml, $n = 75$, $p = 0.15$, respectively, Figure 3a]. A similar, although more pronounced finding was observed among those with and without biomarker remission [median levels 63 vs 40 μg/ml, $n = 50$, $p = 0.04$, respectively, Figure 3b] and those with mucosal healing vs endoscopically active disease [median levels of 84 vs 45 μg/ml, $n = 39$, $p = 0.007$, respectively, Figure 3c].

3.4. Albumin, sMADCAM1 and CRP levels in patients receiving dose optimisation

To assess whether trough levels before escalation are actually a harbinger for severity of inflammation, we studied albumin and CRP levels in

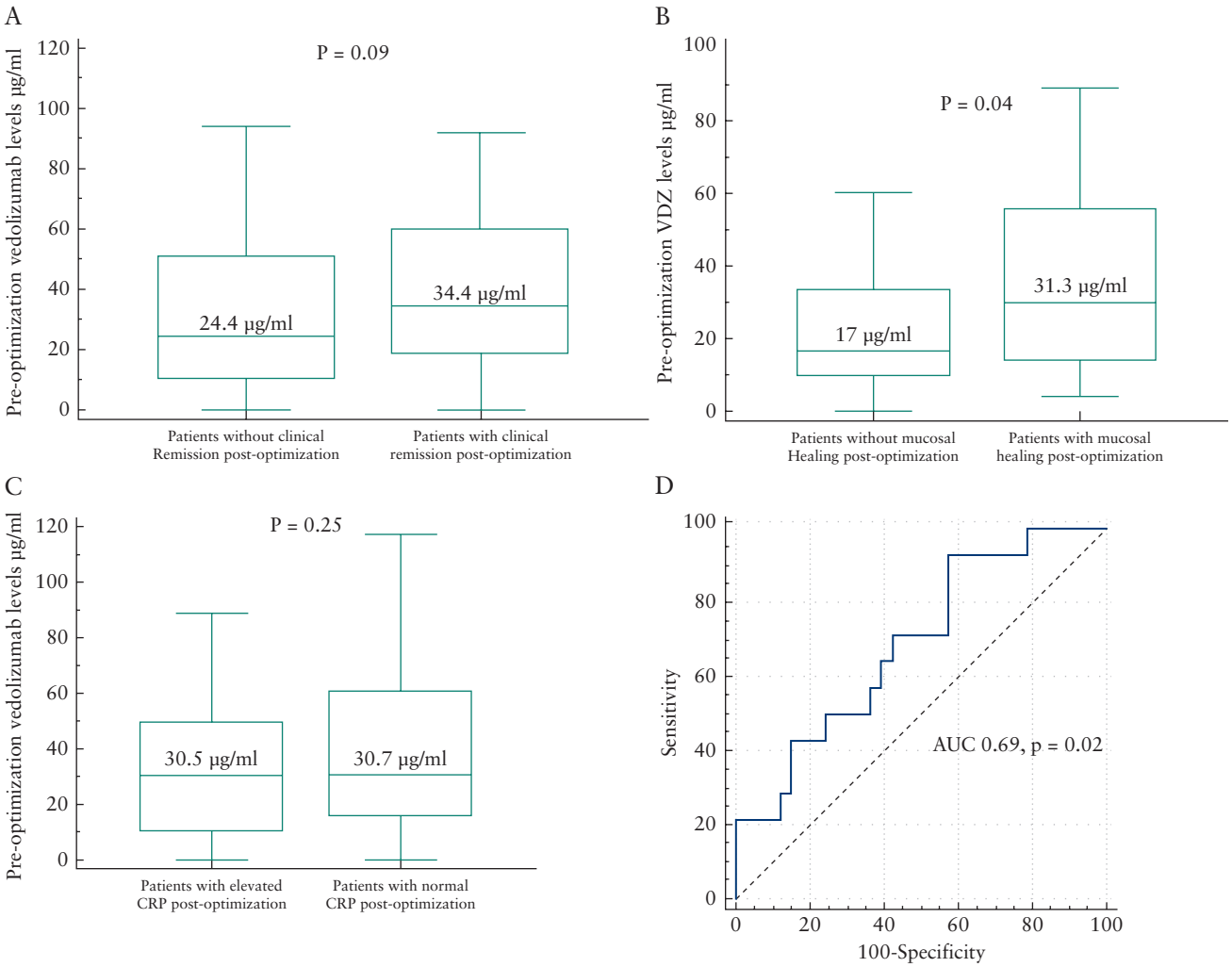

Figure 2. a. Vedolizumab levels before maintenance therapy intensification in patients with/without clinical remission by 6 months post-intensification [*n* = 129]. b. Vedolizumab levels before maintenance therapy intensification in patients with/without endoscopic remission by 6 months post-intensification. c. Vedolizumab levels before maintenance therapy intensification in patients with/without CRP remission by 6 months post-intensification. CRP, C C-reactive protein. d. Trough vedolizumab levels above 14 µg/ml were significantly associated with mucosal healing at 6 months post-intensification [AUC 0.69, *p* = 0.02, sensitivity 93%, specificity 43%]. AUC, area under the curve.

pre-escalation sera of patients in the clinical study, as well as sMADCAM levels, previously reported to correlate with response in patients starting vedolizumab.[18] Pre-escalation albumin levels tended to be higher among patients with post-escalation clinical and endoscopic remission compared with patients not attaining these outcomes [median 4.4, IQR 4.1–4.8 g/dl vs median 4.2, IQR 3.9–4.5 g/dl, *p* = 0.08 and median 4.6, IQR 4.2–4.8 g/dl, vs 4.2, IQR 3.9–4.5 g/dl, respectively, *p* = 0.06, Supplementary Figure 3, available as Supplementary data at *ECCO-JCC* online]. CRP levels were not statistically different between the two groups [data not shown]. With regard to sMADCAM, there was no association between pre-optimisation serum levels and clinical or endoscopic remission after interval shortening [clinical remission: MADCAM—median 2966, 2821 pg/ml, IQR 1772–4368, IQR 2393–3226 pg/ml, respectively, *p* = 0.9; endoscopic remission: MADCAM—median 2639, 1916 pg/ml, IQR 1772–3581, 843–3313 pg/ml, respectively, *p* = 0.6; Supplementary Figure 4, available as Supplementary data at *ECCO-JCC* online].

3.5. Association between vedolizumab trough levels and α4β7 receptor saturation

Unlike PK PD in anti-TNFs treatments, for which lower pre-optimisation drug levels are associated with higher likelihood of response to dose optimisation, the first part of this study found that vedolizumab drug levels before dose optimisation were similar or even higher among patients with higher likelihood of subsequent response to dose optimisation. Two possible explanations may account for this observation [Supplementary Figure 5, available as Supplementary data at *ECCO-JCC* online], one of which suggesting that much higher vedolizumab levels than widely accepted 'therapeutic levels' are needed. Thus in the second part of this study we set to investigate if immune mechanisms support such a hypothesis that a much higher vedolizumab concentration is needed to bind its cognate integrin antigen and/or to mediate anti-inflammatory effect.

To examine this, peripheral blood and lamina propria samples were collected exclusively from patients undergoing lower endoscopy on the day of the infusion [at trough time point, *n* = 43]. Even then, with minimal trough concentration of drug, the percentage of free α4β7 receptors was low on PB and LP CD45RO + CD3 + memory T cells [median 1.5%, IQR 0.6–4.5%, and 8.6%, IQR 3.9–26%, respectively, Figure 4a]. No statistical significant difference in free α4β7 receptors on PB memory T cells was detected between patients with and without mucosal healing [median 0.5%, IQR 0.3–1.4% vs 1.46%, IQR 0.9–3.5%, respectively, *p* = 0.15, *n* = 43] or on LP memory T cells [median 11.5%. IQR 3.5–24.2% vs 7%,

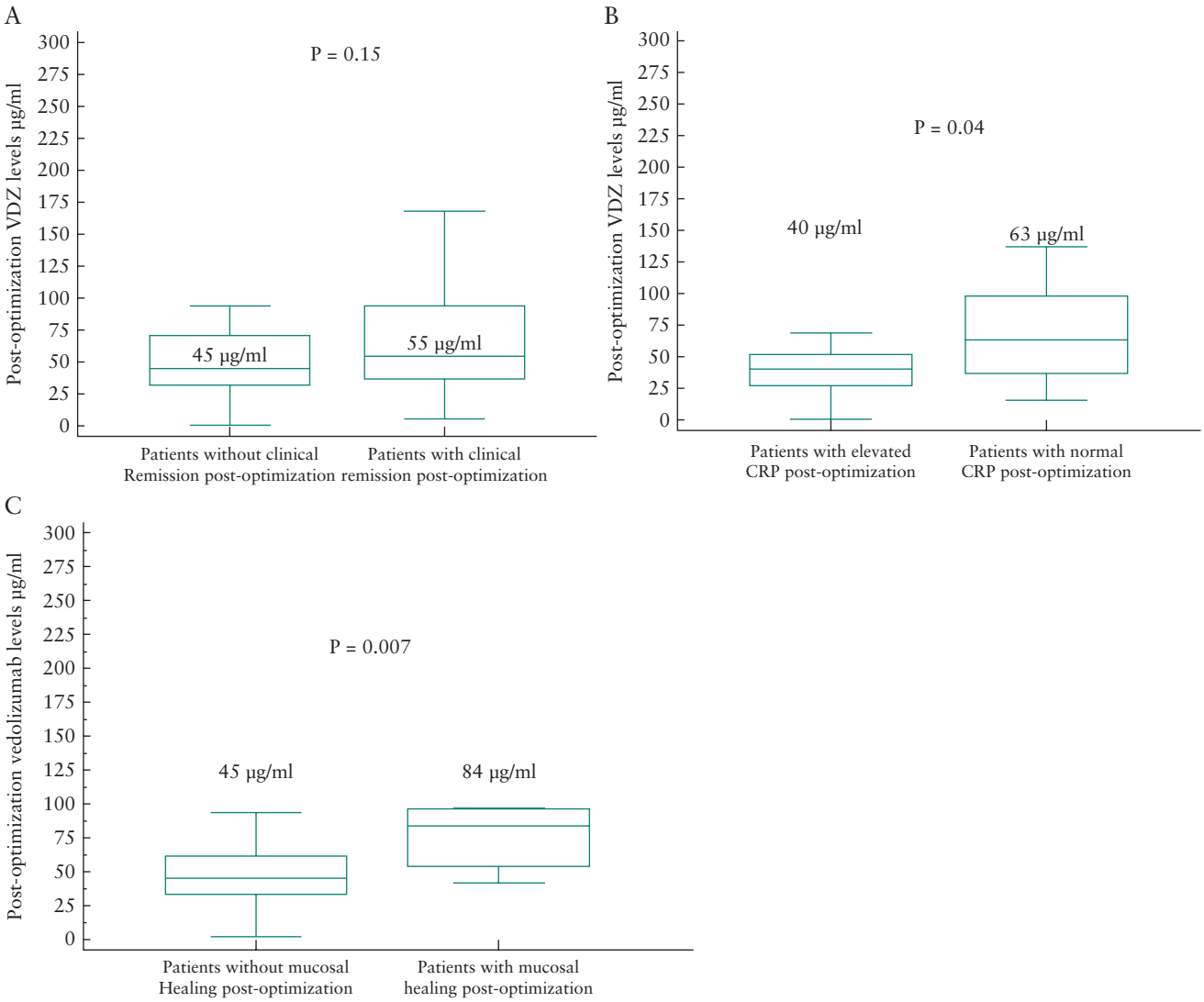

Figure 3. a. Vedolizumab levels 1 month after end of induction therapy intensification in relation to clinical remission by 6 months after the intervention. b. Vedolizumab levels 1 month after end of induction therapy intensification in relation to CRP remission by 6 months after the intervention. CRP, C -reactive protein. c. Vedolizumab levels 1 month after end of induction therapy intensification in relation to endoscopic remission by 6 months after the intervention.

IQR 5.8–20.9%, $p = 0.88$, $n = 38$]. These rates were significantly lower compared with free α4β7 in IBD patients before initiation of vedolizumab treatment [$n = 13$, $p < 0.0001$, Figure 4b] and healthy/IBD controls not treated with vedolizumab [$p < 0.0001$, $n = 12$, Figure 4c]. No difference was discerned in the percentage of free α4β7 on PB and LP T cells obtained from inflamed or uninflamed mucosa among the same patients [$n = 43$, 38, respectively, data not shown].

Receptor saturation upon vedolizumab therapy was also explored on eosinophils, previously shown to express high levels of α4β7 receptors.[19] Similarly to T cells, α4β7 receptor saturation on eosinophils was near complete at trough, with a median of only 1.5% free α4β7 receptors [IQR 1–2.6%]. Furthermore, no significant difference was detected in median percentage of free α4β7 receptors on PB eosinophils, in patients with or without mucosal healing on same day endoscopy [median 1.3%, 1.9%, IQR 0.8–2.3, 1–4.2%, $p = 0.25$, $n = 18$, for patients with and without mucosal healing, respectively, Supplementary Figure 6, available as Supplementary data at *ECCO-JCC* online].

3.6. Cell membrane localisation of bound α4β7 integrin

It was previously shown in vitro that α4β7 receptors are internalised within 24 h of binding by vedolizumab.[17] We therefore set to determine if the near complete absence of free α4β7 receptors on lymphocytes and eosinophils of vedolizumab-treated patients in vivo is due to their internalization or due to complete blockade at the cell membrane level, and whether these processes depend on very high vedolizumab concennetrations. Upon acid dissociation, a significant percentage of cells had α4β7 receptors present on the cell membrane, both for PB T cells [median 34, IQR 23–40%, Figure 5a] and for LP T cells [26%, IQR 18–39%]. Similarly, after dissociation, 82% [IQR 67–88%] of the previously completely saturated peripheral blood eosinophils had now detectable membranal α4β7 receptors. When compared with receptor saturation before dissociation, the proportion of bound receptors was considerably lower [$p < 0.0001$ for all comparisons].

To verify that acid dissociation does not cause pores in the cell membrane with resultant detection of the intracellular α4β7

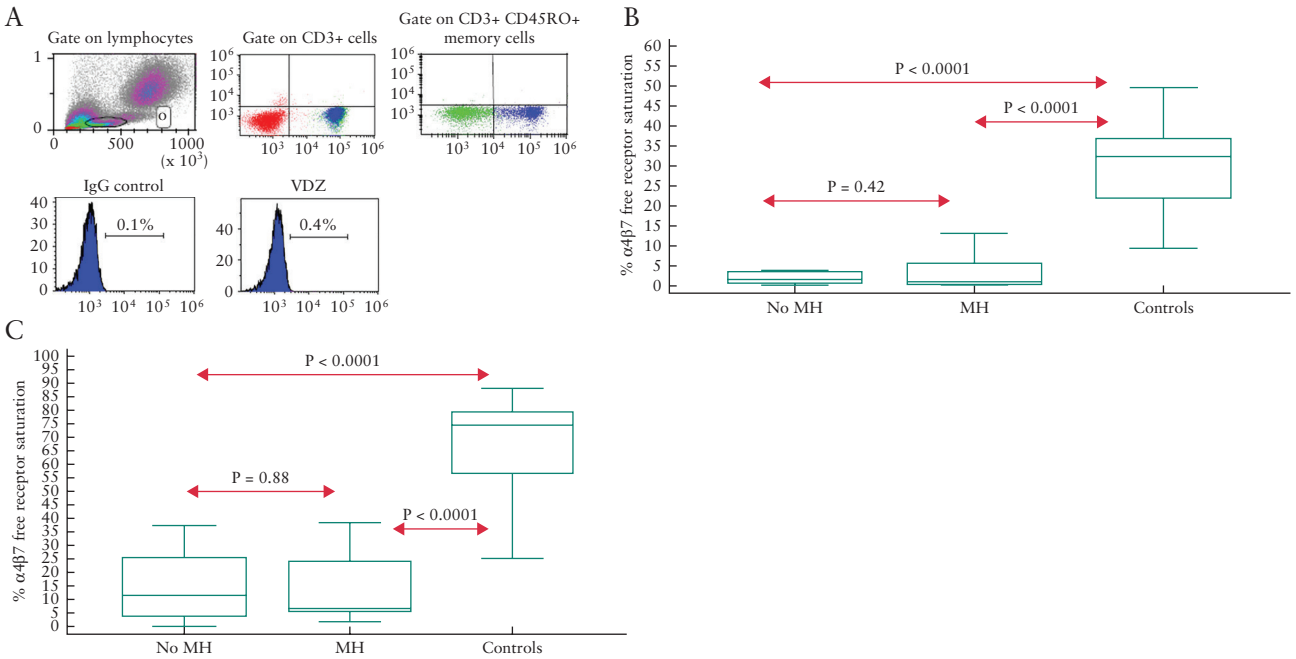

Figure 4. a. α4β7 was hardly detected on PB memory T cells of vedolizumab-treated patients. PB—peripheral blood. b. α4β7 was hardly detected on peripheral blood memory T cells of vedolizumab-treated patients at trough time point [$n = 43$], regardless of mucosal healing status. MH, mucosal healing; PBMC, peripheral blood mononuclear cells. Controls are inflammatory bowel disease [IBD] patients not receiving vedolizumab [$n = 13$]. c. There was no difference in percentage of intestinal LP α4β7 + memory T cells in vedolizumab-treated patients with/without MH. Both were significantly lower than the percentage of free α4β7 receptors among patients untreated with vedolizumab [$n = 12$]. MH, mucosal healing; LP, lamina propria.

vedolizumab complex, PBMC were activated and subjected to permeabilisation or dissociation as above. Cells were then stained by anti-TNF labelled antibody for intracellular TNF content. After cell permeabilisation, there was a significant increase in detectable intra-cellular TNF content [27.9% with permeabilisation, compared with 0.02% with no permeabilisation], but no increase was seen with acid dissociation and Golgi inhibition alone [0%, Supplementary Figure 7, available as Supplementary data at *ECCO-JCC* online]. Moreover, previously α4β7-bound cells disclosed by acid dissociation were functional as evident by TNFα secretion on the newly detectable α4β7 ± cells [Supplementary Figure 8, available as Supplementary data at *ECCO-JCC* online]. Hence, acid dissociation does not permeabilise the membrane and post-dissociation newly detected α4β7 receptors are cell surface-localised on functional T cells.

As demonstrated by multispectral imaging flow cytometry [Figure 5b] vedolizumab binding to α4β7 resulted in significant—but not complete—internalisation of complexes [by 24 h] and retention intracellularly up to 5 days. This parallelled a gradual accumulation of unlabelled receptors, i.e. newly generated free α4β7 integrin, on the cell membrane by 5 days. Significant reduction of cell surface α4β7 receptors was observed with Golgi inhibition, which was statistically significant after 5 days of incubation [$n = 6$, $p \leq 0.05$ for all comparisons, Supplementary Figure 9, available as Supplementary data at *ECCO-JCC* online], corroborating that membranal detectable receptors after vedolizumab exposure were newly generated ones. There was no statistically significant difference in the percentage of bound newly generated receptors after exposure to varying concentrations of vedolizumab from 1 µg/ml to 80 µg/ml [sSpplementary Figure 9]. Similar complete saturation of regenerated receptors was also found with very low vedolizumab concentration [1 µg/ml] in a similar experiment whereby cells were this time only briefly incubated with vedolizumab for 3h, and thereafter cultured for additional 1 to 5 days before vigorous washing and interrogation of surface receptors [Supplementary Figure 10, available as Supplementary data at *ECCO-JCC* online]. Collectively, this set of experiments indicated that cell surface α4β7 after vedolizumab exposure comprises newly generated receptors and previously ligated non-internalised receptors, but both are blocked by miniscule [1 µg/ml] vedolizumab concentrations.

3.7. Vedolizumab effects on M1 and M2 macrophages

Recent studies suggested a role for vedolizumab in modulating the innate system, in particular the M2 tissue-healing macrophages lineage.[20] Thus, we aimed to determine if differential effects of graded vedolizumab concentrations on innate mononuclear cells subsets, and specifically M2 macrophages, could explain a putative need for much higher vedolizumab concentrations than the 12-14 µg/ml cited in several publications as possible therapeutic thresholds during maintenance.

First, we explored the expression of α4β7 on circulating monocyte sub-classes. Only a minute percentage of classical [CD14 + CD16] and non-classical tissue-healing monocytes [CD14dimCD16+] were α4β7 + [Supplementary[Figure 11, available as Supplementary data at *ECCO-JCC* online].

Subsequently, PBMC from IBD patients untreated with vedolizumab [$n = 4$] were induced to differentiate into M1 [CD8 6 ± INOS ± arginase1 ± cells] and M2 [CD163 ± CD206±] macrophages. Staining of these cells with labelled vedolizumab did not show increased expression of α4β7 [Figure 6a]. Subsequently, we explored the direct effect of vedolizumab on M2 macrophages derived from THP1 as well as PB cells. We first noted that α4β7 expression was as high as 30% on THP1-derived M2 macrophages, but full saturation of the integrin receptors on these was already achieved with as low as 1 µg/ml of vedolizumab [Figure 6b]. Moreover, cytokine secretion [IL-10, TNFα and TGFβ] was measured separately in

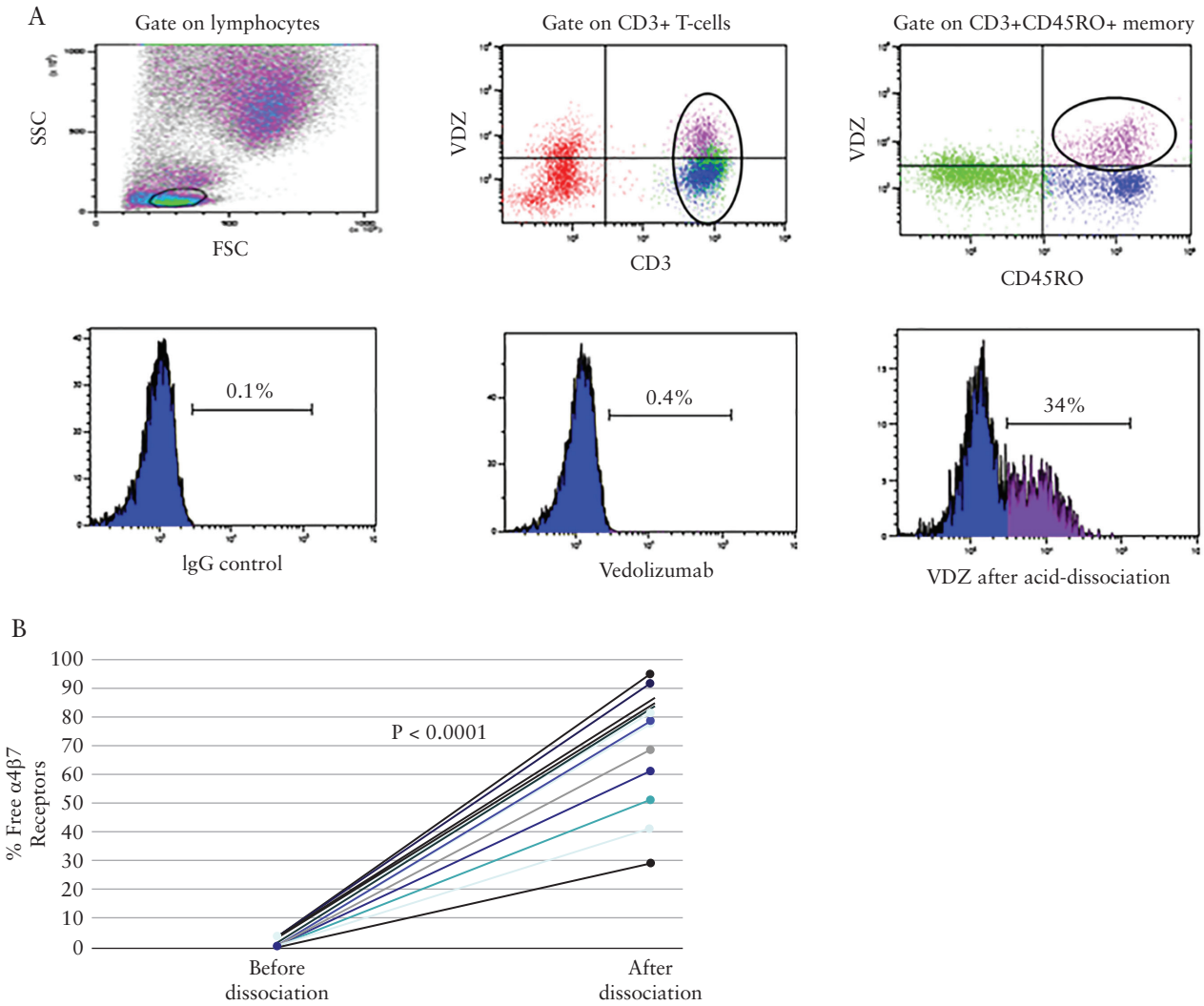

Figure 5. a. Free α4β7 becomes detectable on peripheral blood memory T cell surface after acid dissociation. b. Exploring post-binding α4β7 and α4β7 vedolizumab complex intracellular localisation using two differently labelled vedolizumab antibodies applied at different time points. On imaging flow cytometry analysis [left panel], T cell membrane was stained by anti-CD3 [PE, purple], and α4β7 integrin by both a green Alexa488 labelled vedolizumab, added at time 0 of each experiment, and a red Alexa467 labelled vedolizumab added at the designated time points for cell harvesting, showing internalisation of the majority—but not all—of initially bound α4β7 green Alexa488 labelled vedolizumab complexes, with later intracellular sequestration, in parallel with accumulation of newly generated [red stained] α4β7 on the cell membrane by Day 5. On the right panel, a FACS plot, similarly employing in parallel two differently labelled vedolizumab molecules [Alexa488, purple , Alexa647, light blue] to quantitate membrane α4β7 receptors demonstrates gradual accumulation of newly added labelled vedolizumab on the cell membrane, maximal by Day 5.

the supernatant of PB- and THP1-derived M2 differentiated macrophages, with or without incubation with graded concentrations of vedolizumab [$n = 4$, 5 for PB and THP1, respectively]. Secretion of none of the cytokines by either PB or THP1 cells was affected by vedolizumab, even at very high concentrations of up to 80 µg/ml [Figure 6c]. Collectively, these results do not support a distinct anti-inflammatory role for high vedolizumab concentration via the innate immune system axis.

4. Discussion

Previous clinical trials and 'real life' cohorts have demonstrated higher vedolizumab trough levels [above 18–37 µg/ml at Week 6, above 18 µg/ml at Week 14, and above 12–14 µg/ml during the maintenance period] to be associated with better clinical and endoscopic outcomes,[8–11,21] although other studies demonstrated conflicting findings.[20–24] Two previous small-scale studies with 20 and 23 patients each, have found trough levels to be associated with loss of response, but did not identify vedolizumab levels predicting the outcomes of dose optimisation .[25,26] In a recent publication by Vermeire and colleagues, 167 IBD patients on long-term maintenance vedolizumab therapy were de-escalated from vedolizumab q4 to q8. Clinical and inflammatory remission rates remained stable by 56 weeks of q8 therapy without need for re-escalation to q4, despite a significant decrease in trough levels.[27] Preliminary results of the ENTERPRISE study also demonstrated that an additional infusion at Week 10 had not altered outcomes in terms of perianal CD.[25] Our findings go in line with the latter study: vedolizumab levels pre-escalation were similar or even higher in patients with better subsequent post-escalation therapy outcomes compared with patients who did not respond to dose optimisation. This indicates that for most patients, pharmacokinetics pharmacodynamics [PK

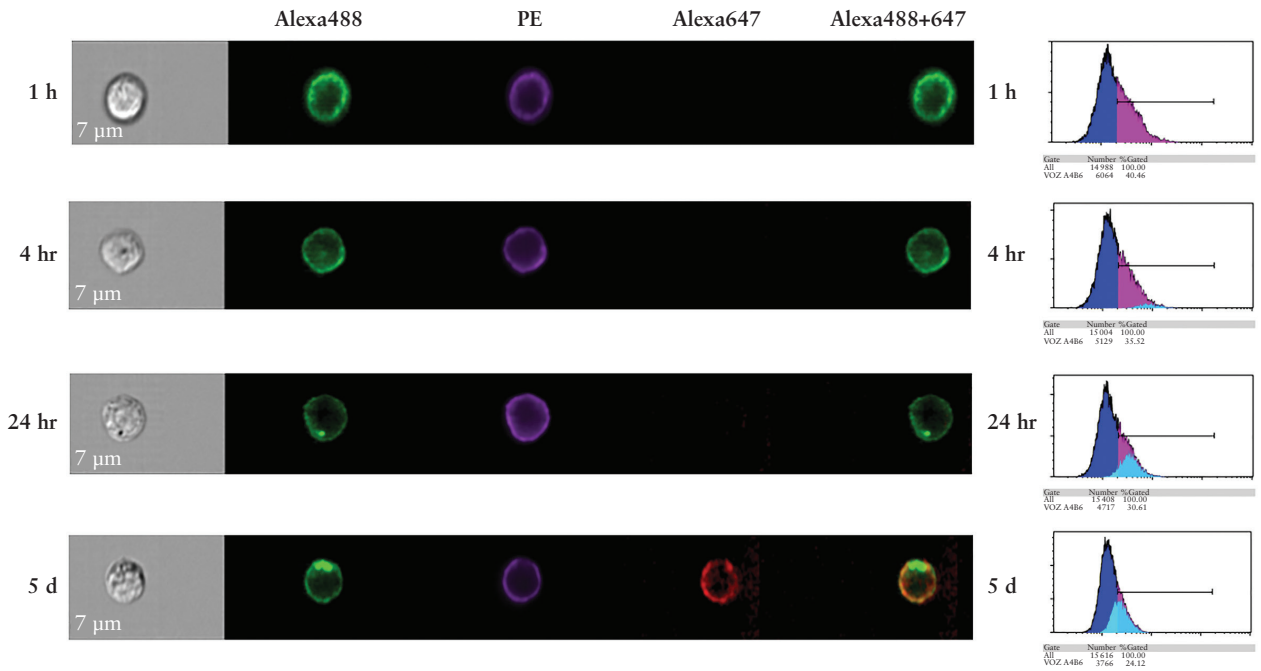

Figure 5. Continued.

PD] relationships of vedolizumab at loss of response are probably different than those of anti-TNFs, as for the latter low levels are associated with success of dose escalation [in turn implying loss of response due to sub-therapeutic anti-TNF drug levels].[4,5] A previous meta-analysis demonstrated that 50% of vedolizumab patients would clinically respond to dose escalation, although other measures of outcome have not been assessed.[26] An ongoing randomised controlled study aims to determine whether dose optimisation of vedolizumab could improve outcomes and may elucidate these questions,[28] but the present findings argue against a pharmacokinetic basis for insufficient response to vedolizumab which could be corrected by dose increase.

Arguably, the lack of association between drug blood level and response to dose intensification may indicate an alternative explanation, attributing a need for dose increase to a possible drug under exposure at the tissue level. Preliminary findings suggested lower vedolizumab levels in mucosal biopsies of clinically active UC patients, compared with those in remission.[29] Therefore, the findings described above prompted us to deepen our understanding of the mechanisms responsible for vedolizumab pharmacokinetics pharmacodynamics interplay at the peripheral circulation and tissue levels. A previous study by our group has demonstrated almost complete saturation of α4β7 receptors on PB and LP T cells, already at minimal concentrations of vedolizumab in vitro [1 µg/ml].[14] The current study adds to the previous study by showing that complete saturation on PB and LP T cells is apparent even at trough time point, when drug levels are at their lowest, and regardless of same-day endoscopic disease activity. Several recent studies have also addressed α4β7 receptor occupancy in vedolizumab therapy patients. A recent prospective study of 38 IBD patients analysed T cell-associated markers in relation to vedolizumab therapy outcome. An increase in α4β7 + TH1/17 was documented in the lamina propria of IBD patients treated with vedolizumab.[30] However, α4β7 receptor occupancy on T cells was not associated with clinical or endoscopic remission in that study. Another study showed that 14 responders to vedolizumab therapy had higher vedolizumab levels as well as higher pretreatment α4β7 expression on CD4, CD8 and NK cells.[31] Furthermore, in five patients reaching clinical remission, higher baseline numbers of α4β7 expressing mucosal cells were identified, followed by a significant reduction of α4β7 expression on Th2- and Th17-polarised CD4 + T cells.[32] However, none of these studies explored these attributes in patients with loss of response to vedolizumab, or in other cell types. Eosinophils have been shown to express high levels α4β7 and therefore could be potentially in need of higher drug concentrations for their effective blockade. However, similarly to T cells, we found that α4β7 receptor saturation on eosinophils was also maximal upon standard vedolizumab therapy, regardless of mucosal healing status.

With an aim to further understand whether any cellular effects of vedolizumab can be augmented by higher drug concentrations, we set to determine the sub-cellular localisation and the kinetics of α4β7 integrin receptors after vedolizumab binding. Using dissociation experiments we first found that non-detectability of free α4β7 on PB, LP T cells and eosinophils is due to complete binding at the cell surface level, and not exclusively due to full receptor internalisation. Subsequent experiments with vigorous washing to remove any remnant vedolizumab with or without blocking the cellular Golgi apparatus, coupled with image stream experiments, all suggested a complex α4β7 cellular localisation process, whereby a fraction of vedoliuzmab-bound integrin receptors are not internalised and remain on the cell membrane in their blocked complexed form. The rest are internalised as α4β7 drug complexes after vedolizumab exposure, and are retained in the cell. In parallel, newly formed free receptors are transported to the cell surface where they gradually accumulate within several days on the cell membrane in their free form amenable to re-binding by vedolizumab. Importantly, however, these processes took place regardless of whether cells were exposed to clinically relevant low or high drug concentrations. These results extend previous observations[17,33] and suggest that newly generated α4β7 receptors are amenable to complete re-binding with circulating

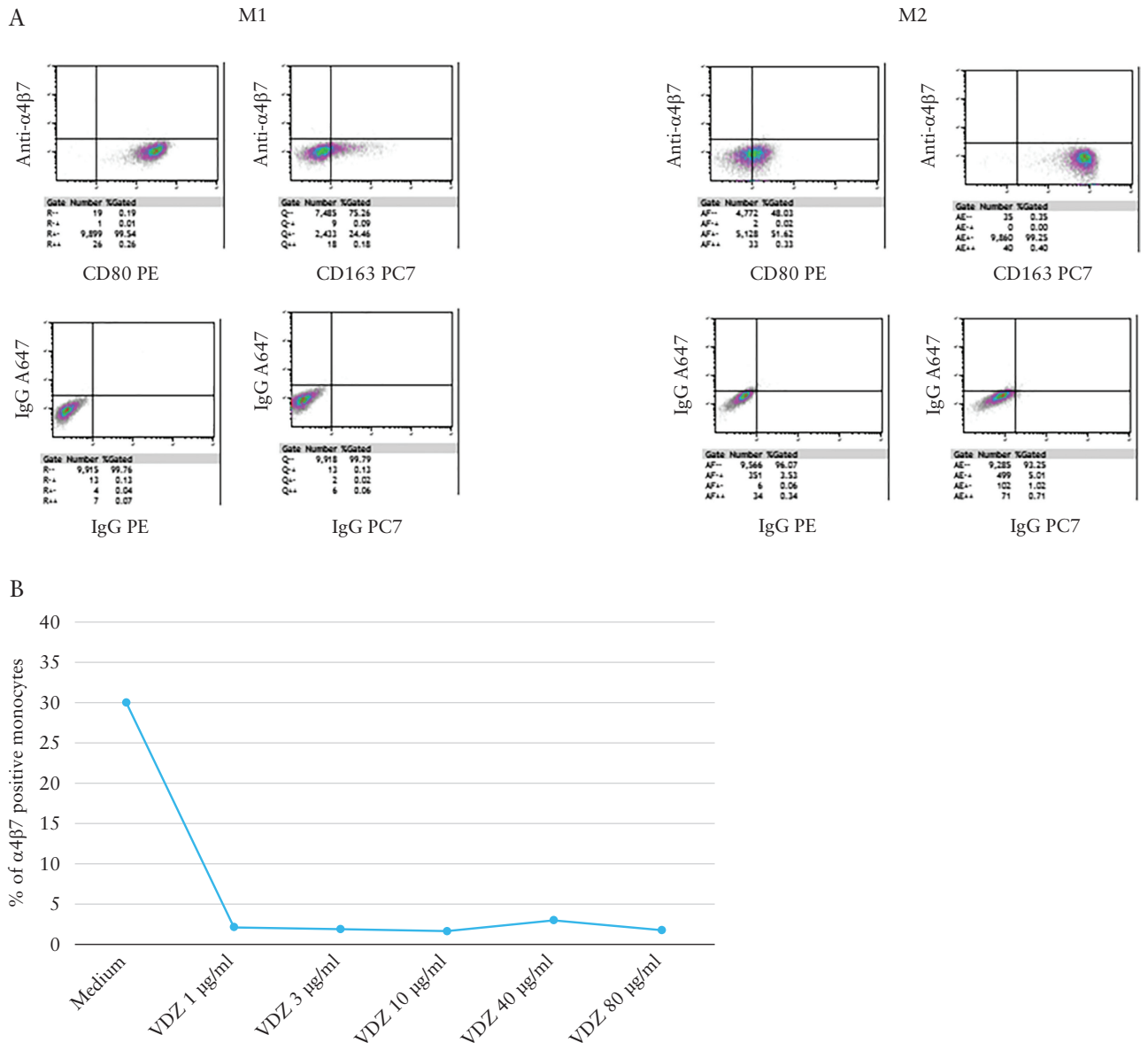

Figure 6. a. Expression of α4β7 on M1 and M2 macrophages induced to differentiate from peripheral blood mononuclear cells [$n = 4$] is demonstrated in an exemplary experiment. Whereas M1 and M2 macrophages expressed CD80 and CD163, respectively, none of them expressed increased levels of α4β7. b. Low concentration of vedolizumab is sufficient to block α4β7 integrin receptors on M2 macrophages induced from THP1. Baseline α4β7 expression on M2 macrophages was 30%. c. Secretion of cytokines [TGF-β, TNFα, IL-10, IL-15, CCL-18] by M2 macrophages induced from PB of IBD patients [upper graph, $n = 4$] and THP1 [lower graph, $n = 5$] was not affected by vedolizumab, even at high concentrations [up to 80 µg/ml]. PB, peripheral blood; IBD, inflammatory bowel disease.

vedolizumab after previous exposure at very low concentration of drug. Moreover, subsequent experiments of cytokine secretion could not show any cytokine pathway being augmented or inhibited by such binding, at the same range of vedolizumab concentrations.

The above findings cast doubt on the need for higher drug concentrations to induce any additional immune effects at the receptor or the T cell function level. In this context, a recent study implicated vedolizumab mode of action with substantial effects on innate immunity, including changes in macrophage populations, and more specifically, increased abundance of M2 tissue-healing macrophages, which was not observed in response to infliximab therapy.[20] In contrast, another recent study demonstrated that non-classical M2 monocytes expressed higher levels of α4β7 integrin receptors than classical monocytes, and found their trafficking to the gut was reduced by vedolizumab, arguing for hampered tissue healing in patients receiving vedolizumab.[34] Considering these studies, we explored if effects on M2 macrophages could be dependent on higher concentrations of vedolizumab. However, PB non-classical monocytes as well as differentiated M2 macrophages hardly expressed α4β7. We did find increased expression of α4β7 on THP1-derived macrophages, but upon a blocking experiment, full saturation of the integrin receptors was achieved with as low as 1 µg/ml of vedolizumab. Moreover, no changes in cytokine secretion were discerned on either M1 or M2 macrophages upon addition of vedolizumab at high concentrations of up to 80 µg/ml. These observations further argue against enhanced immune effect of high-dose vedolizumab on either T cells, eosinophils, or M2 macrophages in vitro, beyond a minimal 3 µg/ml concentration that confers maximal blockade of all these cellular subsets.

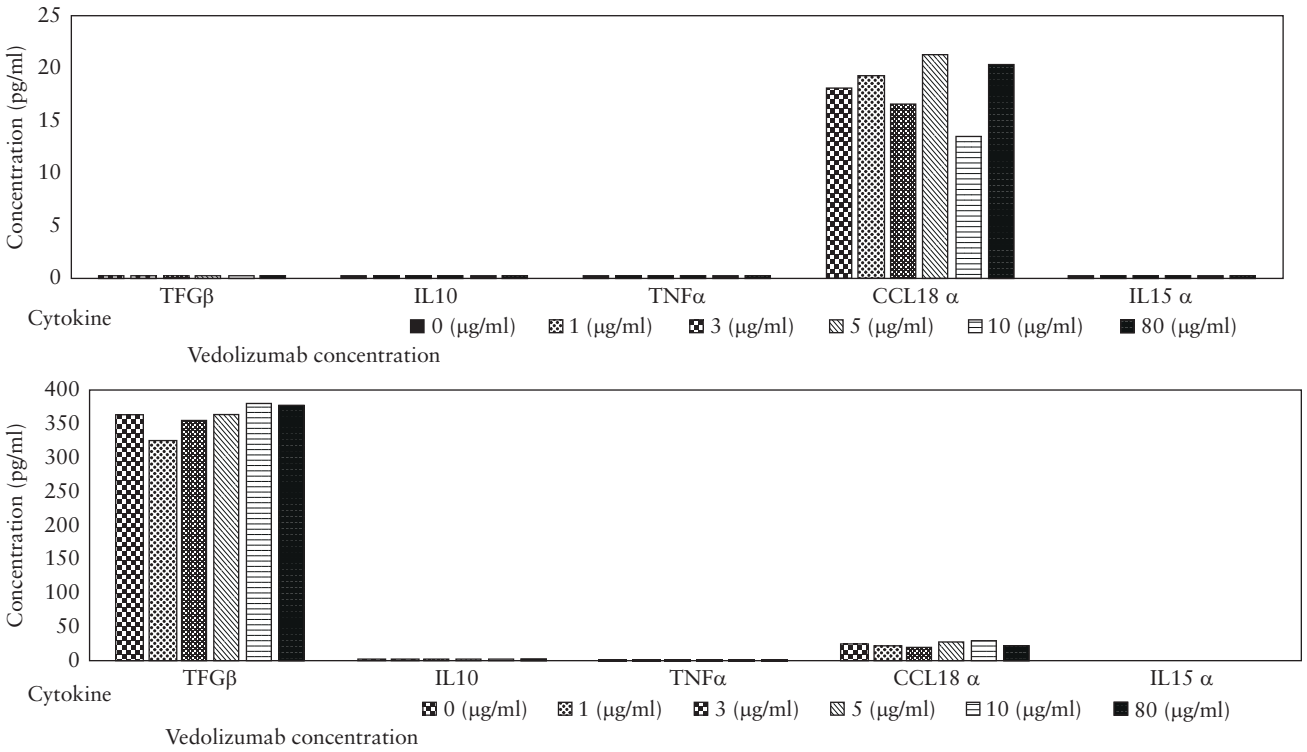

Figure 6. Continued.

Finally, we set to examine whether vedolizumab levels serve more as a biomarker of disease severity, i.e. high levels reflecting less inflammation. Indeed, in our study higher albumin levels, similarly to vedolizumab levels, were positively associated with better outcomes of dose optimisation, suggesting that it is less severe disease [with higher albumin and higher vedolizumab levels] that could be the decisive marker of those responding to dose optimisation. This is congruent with several previous studies demonstrating that less severe disease [based on higher level of albumin and/or haemoglobin and lower levels of inflammatory markers] at the start of vedolizumab therapy was associated with higher trough concentrations of vedolizumab and higher probability of achieving mucosal healing.[11,35] Possibly, higher vedolizumab levels associate with reduced inflammation and could result from reduced faecal loss and potentially lower drug catabolism.[34] Finally, to further elucidate if differing integrin blockade by various vedolizumab concentrations could explain the putative effectiveness of dose optimisation, we examined whether success of dose optimisation was linked to levels of the integrin ligand, i.e. soluble MADCAM1 [sMADCAM1]. A recent study found pre-vedolizumab sMADCAM1 levels to be associated with outcomes of therapy[15] and other investigators showed more rapid decline of this marker in responders to vedolizumab.[36] In our study, however, investigating a somewhat different population of patients with loss of response [LOR] to vedolizumab, there was no association between pre-optimisation sMADCAM levels and clinical/biomarker/endoscopic remission rates at 6 months following escalation.

Our study has several limitations. First, it included sera samples of patients from six medical centres in Israel and Europe. As this is a 'real life' study, the clinical decision making, leading to vedolizumab dose escalation, may be different between treating physicians. Nevertheless, all patients undergoing interval shortening had objective evidence of active inflammation. Furthermore, C-reactive protein values as well as vedolizumab levels were centrally measured at Sheba Medical Center. Second, although endoscopies were not centrally read but performed at each medical centre, mucosal healing was defined as 'no ulcers or active inflammation', as conventional in many studies.[37,38]

In conclusion, in this real life prospective cohort of vedolizumab-treated IBD patients, drug levels before the interval shortening were similar or even higher, but not lower, among patients with clinical, biomarker, and endoscopic remission after dose optimisation, as compared with patients not responding to dose intensification. Peripheral blood and intestinal target α4β7 integrin receptors were nearly fully occupied by vedolizumab on all cell lineages examined, even at trough time point and regardless of mucosal healing status. The localisation and transport of α4β7 to the cell surface after vedolizumab binding depends on newly generated receptors and to a smaller scale on non-internalised ones, regardless of drug concentrations. These pharmacokinetic pharmacodynamic data collectively question the putative causality underlying patient outcomes after dose optimisation of vedolizumab and call for further mechanistic and clinical corroborating studies.

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Conflict of Interest

SBH received consulting and advisory board fees and/or research support from AbbVie, MSD, Janssen, Takeda, and CellTrion. UK received speaker fees from Abbvie, Janssen, and Takeda, research support from Takeda and

Janssen, and consulting fees from Takeda and CTS. YC received grant support, and speaker and consultant fees from AbbVie, speaker and consultant fees from Janssen, grant support, speaker, and consultant fees from Takeda, speaker fees from Ferring, and consultant fees from Medtronics. RE received consultant and speaker fees from Janssen, Abbvie, Takeda, and Medtronic. BU received consultation fees from Neopharm, Takeda, Janssen, and Abbvie. FM served as speaker and received honoraria from: Abbvie, Amgen, Biogen, Falk, Ferring, Hospira, Janssen, Laboratórios Vitoria, Merck Sharp and Dohme, Pfizer, Sandoz, Takeda, UCB, Vifor. XR received consultation/speaker fees from MSD, Abbvie, Jansen, Amgen, Biogen, Ferring, and Theradiag. DD has served as a speaker, a consultant, and an advisory board member for MSD, Abbvie, Takeda, Pfizer, Janssen, Amgen, Biogen, and Krka. JH has received speaker's fees from Biogen, Janssen, Pfizer, and Takeda. None of the other authors have any conflicts to declare. Sheba Medical Center and Rambam Health Care Campus have filed intellectual property requests on the assays for vedolizumab levels.

Author Contributions

SBH conceived of the study and drafted the manuscript; BU was involved in study concept, analysis and interpretation of data, and manuscript drafting; UK, KM, JH, MAA, SP, CR, ZBS, CMA, OHN, LS, and IS participated in acquisition of data; EF, MY, and OP took part in data analysis; RE, YC, FM, XR, DD, and ML participated in data interpretation and in critical revision of the manuscript for important intellectual property. All authors have approved the final draft submitted.

Supplementary Data

Supplementary data are available at *ECCO-JCC* online.

References

1. Shelton E, Allegretti JR, Stevens B, *et al.* Efficacy of vedolizumab as induction therapy in refractory IBD patients: a multicenter cohort. *Inflamm Bowel Dis* 2015;**21**:2879–85.
2. Dulai PS, Singh S, Jiang X, *et al.* The real world effectiveness and safety of vedolizumab for moderate-severe Crohn's disease: results from the US VICTORY consortium. *Am J Gastroenterol* 2016;**111**:1147–55.
3. Kopylov U, Seidman E. Predicting durable response or resistance to antitumor necrosis factor therapy in inflammatory bowel disease. *Therap Adv Gastroenterol* 2016;**9**:513–26.
4. Yanai H, Lichtenstein L, Assa A, *et al.* Levels of drug and antidrug antibodies are associated with outcome of interventions after loss of response to infliximab or adalimumab. *Clin Gastroenterol Hepatol* 2015;**13**:522–30.e2.
5. Ungar B, Levy I, Yavne Y, *et al.* Optimising anti-TNF α therapy: serum levels of infliximab and adalimumab are associated with mucosal healing in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol* 2016;**14**:550–7.e2.
6. Paul S, Del Tedesco E, Marotte H, *et al.* Therapeutic drug monitoring of infliximab and mucosal healing in inflammatory bowel disease: a prospective study. *Inflamm Bowel Dis* 2013;**19**:2568–76.
7. Vermeire S, Dreesen E, Papamichael K, Dubinsky MC. How, when, and for whom should we perform therapeutic drug monitoring? *Clin Gastroenterol Hepatol* 2020;**18**:1291–9.
8. Rosario M, French JL, Dirks NL, *et al.* Exposure efficacy relationships for vedolizumab induction therapy in patients with ulcerative colitis or Crohn's disease. *J Crohns Colitis* 2017;**11**:921–9.
9. Osterman MT, Rosario M, Lasch K, *et al.* Vedolizumab exposure levels and clinical outcomes in ulcerative colitis: determining the potential for dose optimisation. *Aliment Pharmacol Ther* 2019;**49**:408–18.
10. 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.
11. 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.
12. Pouillon L, Vermeire S, Bossuyt P. Vedolizumab trough level monitoring in inflammatory bowel disease: a state of the art overview. *BMC Med* 2019;**17**:89.
13. Loftus EV Jr, Colombel JF, Feagan BG, *et al.* Long-term efficacy of vedolizumab for ulcerative colitis. *J Crohns Colitis* 2017;**11**:400–11.
14. Ungar B, Kopylov U, Yavzori M, *et al.* Association of vedolizumab level, anti-drug antibodies, and α4β7 occupancy with response in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol* 2018;**16**:697–705.e7.
15. 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.
16. Ben Horin S, Polak Charcon S, Barshack I, *et al.* Celiac disease resolution after allogeneic bone marrow transplantation is associated with absence of gliadin specific memory response by donor-derived intestinal T cells. *J Clin Immunol* 2013;**33**:1395–402.
17. Wyant T, Yang L, Fedyk E. In vitro assessment of the effects of vedolizumab binding on peripheral blood lymphocytes. *MAbs* 2013;**5**:842–50.
18. Williet N, Boschetti G, Fovet M, *et al.* Association between low trough levels of vedolizumab during induction therapy for inflammatory bowel diseases and need for additional doses within 6 months. *Clin Gastroenterol Hepatol* 2017;**15**:1750–7.e3.
19. Brandt EB, Zimmermann N, Muntel EE, *et al.* The alpha4bbeta7 integrin is dynamically expressed on murine eosinophils and involved in eosinophil trafficking to the intestine. *Clin Exp Allergy* 2006;**36**:543–53.
20. 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.
21. Sandborn WJ, Colombel JF, Panaccione R, *et al.* Deep remission with vedolizumab in patients with moderately to severely active ulcerative colitis: a GEMINI 1 post hoc analysis. *J Crohns Colitis* 2019;**13**:172–81.
22. Plevris N, Jenkinson PW, Chuah CS, *et al.* Association of trough vedolizumab levels with clinical, biological and endoscopic outcomes during maintenance therapy in inflammatory bowel disease. *Frontline Gastroenterol* 2020;**11**:117–23.
23. Dulai PS, Amiot A, Peyrin Biroulet L, *et al.* A clinical decision support tool may help to optimise vedolizumab therapy in Crohn's disease. *Aliment Pharmacol Ther* 2020;**51**:553–64.
24. Gouynou C, Pouillon L, Rousseau H, Zallot C, Baumann C, Peyrin Biroulet L. Early changes in the pharmacokinetic profile of vedolizumab treated patients with IBD may predict response after dose optimisation. *Gut* 2019;**68**:178–9.
25. Schwartz DA, Peyrin Biroulet L, Lasch K, Adsul S, Danese S. Efficacy and safety of 2 vedolizumab IV regimens in patients with perianal fistulizing Crohn's disease: results of the ENTERPRISE study. *Gastroenterology* 2020;**158**:S193-4.
26. 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.
27. Vermeire S, Lukáš M, Magro F, *et al.* Vedolizumab efficacy, safety, and pharmacokinetics with reduced frequency of dosing from every 4 weeks to every 8 weeks in patients with Crohn's disease or ulcerative colitis. *J Crohns Colitis* 2021;**14**:1066–73.
28. Takeda. *Vedolizumab Intravenous [IV] Dose Optimisation in Ulcerative Colitis*.2020. https://www.clinicaltrials.gov/ct2/show/NCT03029143 Accessed May 21, 2020.
29. Van den Berghe N, Verstockt B, Gils A, Ferrante M, Vermeire S, Thomas D. Vedolizumab concentrations in colonic mucosal tissue of ulcerative colitis patients inversely correlate with the severity of inflammation. *Gastroenterology* 2020;**158**:S47.

30. Coletta M, Paroni M, Alvisi MF, *et al.* Immunological variables associated with clinical and endoscopic response to vedolizumab in patients with inflammatory bowel diseases. *J Crohns Colitis* 2020;**14**:1190–201.
31. Boden EK, Shows DM, Chiorean MV, Lord JD. Identification of candidate biomarkers associated with response to vedolizumab in inflammatory bowel disease. *Dig Dis Sci* 2018;**63**:2419–29.
32. Rath T, Billmeier U, Ferrazzi F, *et al.* Effects of anti integrin treatment with vedolizumab on immune pathways and cytokines in inflammatory bowel diseases. *Front Immunol* 2018;**9**:1700.
33. Lichnog C, Klabunde S, Becker E, *et al.* Cellular mechanisms of etrolizumab treatment in inflammatory bowel disease. *Front Pharmacol* 2019;**10**:39.
34. Ward ES, Ober RJ. Targeting FcRn to generate antibody based therapeutics. *Trends Pharmacol Sci* 2018;**39**:892–904.
35. Yarur AJ, Bruss A, Naik S, *et al.* Vedolizumab concentrations are associated with long term endoscopic remission in patients with inflammatory bowel diseases. *Dig Dis Sci* 2019;**64**:1651–9.
36. Battat R, Dulai PS, Vande Casteele N, *et al.* Biomarkers are associated with clinical and endoscopic outcomes with vedolizumab treatment in ulcerative colitis. *Inflamm Bowel Dis* 2019;**25**:410–20.
37. De Cruz P, Kamm MA, Hamilton AL, *et al.* Efficacy of thiopurines and adalimumab in preventing Crohn's disease recurrence in high risk patients: a POCER study analysis. *Aliment Pharmacol Ther* 2015;**42**:867–79.
38. Ungar B, Engel T, Yablecovitch D, *et al.* Prospective observational evaluation of time dependency of adalimumab immunogenicity and drug concentrations: the POETIC study. *Am J Gastroenterol* 2018;**113**:890–8.