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ORIGINAL ARTICLE

Identification of Candidate Biomarkers Associated with Response to Vedolizumab in Inflammatory Bowel Disease

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Abstract

Background/Aims Vedolizumab is an anti-α4β7 monoclonal antibody approved for the treatment of inflammatory bowel disease (IBD). This exploratory study aimed to identify biomarkers associated with vedolizumab response.

Methods Twenty-six IBD patients (15 with Crohn's, 11 with ulcerative or indeterminate colitis) initiating vedolizumab at a single center between 2014 and 2016 underwent sampling of serum and peripheral blood mononuclear cells (PBMCs) before and during vedolizumab therapy. Response was defined as steroid-free improvement in endoscopic score or Harvey–Bradshaw index/simple clinical colitis activity index (reduction greater than 3 or total less than 3). PBMCs were evaluated for immunophenotype and expression of α4β7 integrin on lymphocytes before and during vedolizumab therapy. Serum vedolizumab levels and α4β7 saturation were measured serially after induction.

Results Fourteen out of 26 (54%) patients treated with vedolizumab responded to therapy. Pretreatment α4β7 expression was higher in responders on multiple subsets of T, B, and NK cells, with terminal effector memory ($p = .0009$ for CD4 and .0043 for CD8) and NK cells ($p = .0047$) best discriminating between responders and nonresponders. During therapy, $\log_{10}$ serum vedolizumab levels at trough were higher in responders than nonresponders ($p = .0007$). Conversely, the percentage of effector memory T cells with free α4β7 at trough was lower in responders than nonresponders ($p < .0001$). However, loss of α4β7 saturation with vedolizumab was more sensitive to low serum vedolizumab in nonresponders.

Conclusions Pretreatment α4β7 expression and α4β7 receptor saturation during maintenance therapy were identified as candidate biomarkers for vedolizumab response.

Keywords Anti-integrin · Biomarkers · Inflammatory bowel disease · Personalized medicine

Abbreviations

IBD	Inflammatory bowel disease
UC	Ulcerative colitis
CD	Crohn's disease
IC	Indeterminate colitis
MAdCAM-1	Mucosal addressin cell adhesion molecule
PBMC	Peripheral blood mononuclear cell
NK	Natural killer
HBI	Harvey–Bradshaw index
SCCAI	Simple clinical colitis activity index
APC	Allophycocyanin
Tregs	Regulatory T cells
IC50	Half maximal inhibitory concentration
IQR	Interquartile range
MFI	Mean fluorescence intensity
T_{EM}	T effector memory
T_{EMRA}	Terminal effector memory T cells
AUC	Area under the curve
tTreg	Thymically derived Treg
pTreg	Peripherally derived Treg
MAIT	Mucosal-associated invariant T cells
T_{FH}	T follicular helper

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Introduction

Vedolizumab is a monoclonal antibody directed against the gut-homing integrin, α4β7, approved for the treatment of inflammatory bowel disease (IBD). Integrin α4β7 is expressed on T cells, B cells, and NK cells as well as subsets of innate immune cells [1, 2] and binds to mucosal addressin cell adhesion molecule (MAdCAM-1) expressed on the endothelium of gastrointestinal and gut-associated lymphoid tissue [1]. Blockade of α4β7-MAdCAM-1 interactions prevents migration of memory and effector T cells to the gut in murine models of colitis [3]. In clinical trials, vedolizumab was effective in both anti-TNF naïve and experienced patients with moderate to severe Crohn's disease and ulcerative colitis [4, 5].

The ideal placement of vedolizumab within the treatment paradigm for Crohn's disease (CD) and ulcerative colitis (UC) is unclear. No comparative efficacy studies are currently available to guide management of drug-naïve patients with moderate to severe disease. Vedolizumab is often given to patients after anti-TNF failure. However, TNF-experienced patients have decreased response rates to vedolizumab [6] and its safety profile may be superior to anti-TNF therapy [7, 8]. Biomarkers that could predict an individual patient's likelihood of response to vedolizumab therapy would assist in clinical decision making by increasing use of vedolizumab as the first-line therapy in patients likely to respond and preventing a delay in effective therapy in patients unlikely to respond.

In addition to predicting response to therapy, biomarkers may offer improved outcomes during vedolizumab treatment by identifying patients in whom therapeutic optimization is possible. There is data for anti-TNF antibodies that trough serum drug levels and antidrug antibodies assist in therapeutic optimization and may reduce overall costs [9, 10]. In clinical trials, serum vedolizumab levels at week 6 after induction correlated with sustained response to vedolizumab, although there was substantial variability in serum levels across responders and nonresponders [5]. The relationship of trough vedolizumab levels to response during the maintenance phase of therapy remains unclear.

Animal studies suggest that inhibition of memory T cell homing to the bowel via α4β7-MAdCAM-1 interactions prevents experimental models of colitis [11]. However, it is not known what specific cell types are responsible for the effect of vedolizumab in humans. Integrin α4β7 is expressed on many cell types including T cells, B cells, and NK cells, all implicated in the pathophysiology of IBD [1] [2]. It is known that α4β7 is variably expressed on different cell types with the highest expression on memory T cells [1]. Expression is bimodal in this population, with high expression presumably reflecting T cells that have encountered antigen in the mesenteric lymph nodes in the presence of retinoic acid-producing dendritic cells that imprint for intestinal homing [12, 13]. Conversely, the majority of naïve T cells express low levels of α4β7 on the cell surface [1].

In an attempt to identify candidate biomarkers for vedolizumab response, patients undergoing vedolizumab induction as standard of care were followed at a single center. Patients were sampled before their first dose of vedolizumab and serially after initiating therapy, to evaluate the immunophenotype, integrin expression, and α4β7 receptor saturation with vedolizumab in a variety of lymphocyte populations. Serum vedolizumab levels were also measured serially during the maintenance phase of therapy. We studied the relationship of these biomarkers to clinical response to vedolizumab.

Materials and Methods

Patients

This was an exploratory observational cohort study with subjects recruited from among enrollees in an IBD biorepository at the Benaroya Research Institute at Virginia Mason Medical Center. Consecutive patients with well-characterized IBD initiating vedolizumab therapy at Virginia Mason Medical Center between 7/2014 and 2/2016 were reviewed for study inclusion. Inclusion criteria were:

1. an evaluable baseline peripheral blood mononuclear cell (PBMC) sample drawn prior to vedolizumab initiation or at least one PBMC sample drawn after vedolizumab administration.
2. pretreatment endoscopic evaluation within 180 days and posttreatment endoscopic evaluation within 270 days of vedolizumab initiation OR pretreatment symptom score within 90 days and posttreatment symptom score within 180 days of vedolizumab initiation.

Patients were excluded if they required chronic steroids for a diagnosis other than IBD. The dose of vedolizumab in all patients was 300 mg IV given at weeks 0, 2, 6, and subsequently every 8 weeks.

Data Collection

PBMCs and serum were collected from patients during routine clinic visits and were cryopreserved in aliquots for batched processing, using established techniques [14]. In addition, patients prospectively provided data on symptoms during clinic visits via disease activity assessments: Harvey–Bradshaw index (HBI) [15] for subjects with CD and simple clinical colitis activity index (SCCAI) [16] for

subjects with UC or indeterminate colitis (IC). Demographic and clinical data including age, gender, disease duration and distribution, smoking history, concurrent medications, endoscopic activity, histology, albumin, and serological inflammatory marker levels were obtained retrospectively through chart review.

Determination of Response

Response to vedolizumab was defined by the ability to achieve steroid-free endoscopic or clinical response. Endoscopic response was defined as an improvement from baseline moderate or severe inflammation to no more than mild inflammation as documented by the treating provider on endoscopic evaluation within 270 days after vedolizumab induction while the patient remained on vedolizumab. A reduction in disease extent of more than 50% was also considered an endoscopic response. For patients who underwent pretreatment and posttreatment endoscopy within the specified time period, endoscopic outcome was utilized to determine response. For patients who did not have endoscopic data available, clinical criteria were used to determine response. Clinical response was defined as a decrease in HBI or SCCAI of at least three points or HBI or SCCAI of three or less measured within 180 days after induction therapy while remaining on vedolizumab. Nonresponse was considered failure to wean steroids completely during follow-up evaluation or to meet criteria for response as outlined above.

FACS Analysis of Immunophenotypes and Integrin Saturation

Frozen PBMCs were thawed in batches and stained extracellularly with panels of antibodies designed to identify subsets of T, B, and NK cells, as well as intracellularly to identify regulatory T cells (Tregs) by the transcription factors FOXP3 and Helios (Supplemental Table 1). Each panel used antibodies that bind to alternative regions of α4 and β7 than vedolizumab and were not hindered from binding by in vivo-bound vedolizumab. This allowed α4β7+ cells to be quantified in the presence of vedolizumab therapy. To normalize for the effect of in vivo vedolizumab exposure on integrin staining in subsequent comparisons between pre- and posttreatment integrin expression, samples were pretreated with a saturating dose of unlabeled vedolizumab prior to staining. For naïve T and B cells, that lack clear bimodal expression of α4β7, mean fluorescence intensity (MFI) of integrin β7 staining was used as a measure of α4β7 expression, as virtually all β7-expressing lymphocytes in the peripheral blood are α4β7+ [17, 18]. For NK cells, as well as memory T and B cells, where the bimodal distribution of α4β7 provided a clear positive and negative gate, the percentage of cells expressing α4β7 was used to quantify integrin expression. Gating strategies for panels are exhibited in Supplemental Figure 1.

In order to determine the effects of vedolizumab on immune cell phenotypes, PBMCs were analyzed serially during vedolizumab treatment for changes in expression of α4β7 and cell surface markers (Supplemental Table 1). The first available sample for each patient drawn between day 42 and day 105 after vedolizumab initiation was compared to the pretreatment sample. For the majority of subjects (69% of responders, 72% of nonresponders) this sample was drawn 42–56 days after vedolizumab initiation.

To determine α4β7 saturation with vedolizumab on different cell types, a fraction of PBMCs were pretreated with or without a saturating dose of unlabeled vedolizumab. Cells were subsequently stained with a panel containing markers for T, B, and NK cell subsets, antibodies to integrin α4 and β7 chains, as well as biotinylated vedolizumab, followed by fluorophore-conjugated streptavidin (Supplemental Table 1). Vedolizumab biotinylation was performed according to the manufacturer's instructions (Invitrogen, catalogue #F6347). Gating on α4β7+ cells in each of several defined lymphocyte subpopulations, the percent, and mean fluorescent intensity (MFI) of vedolizumab staining in cells pretreated with unlabeled vedolizumab was subtracted from that of cells not pretreated with vedolizumab, to determine integrin receptor saturation in each sample (Supplemental Figure 2). Trough drug levels were defined to be between 50 and 68 days after last vedolizumab dose.

All data were collected by a blinded technician on a single Fortessa flow cytometer (Beckton Dickenson) and evaluated by a blinded analyst (JDL) with FlowJo software.

Quantitation of Serum Vedolizumab Level

Serum from vedolizumab-exposed subjects and a known concentration of vedolizumab were each serially diluted in the serum of a single untreated healthy control subject and used to pretreat the thawed PBMC of a single healthy donor. The donor cells were then washed and labeled with antibodies to CD4, CD45RA, integrin β7, and biotinylated vedolizumab, followed by allophycocyanin (APC)-conjugated streptavidin. Cells were analyzed on a FACSCaliber flow cytometer (Beckton Dickenson), and data were analyzed with FlowJo software. The APC (vedolizumab) MFI of CD4, $CD45RA^{-}$, and integrin $\beta7^{+}$ cells pretreated with known concentrations of vedolizumab was plotted as a standard dose-response curve using GraphPad Prism software to determine the half maximal inhibitory concentration (IC_{50}) for integrin saturation. APC MFI was then plotted for cells pretreated with subject serum dilutions to determine the dilution at which an IC_{50} was seen for each serum sample. The starting concentration of vedolizumab in serum samples was then calculated by dividing the IC_{50} of the known

vedolizumab concentrations by the dilution at which a serum sample demonstrated an IC_{50} (Supplemental Figure 3):

$$\text{Serum [vedolizumab]} = \frac{\left(IC_{50} \text{ of known [vedolizumab]}\right)}{\left(\text{dilution of serum producing an } IC_{50}\right)}.$$

Statistical Analysis

Dichotomous variables were presented as frequencies and percentages, and p value associations were determined with χ^2 or Fisher exact tests. For continuous variables, data were presented as mean ± standard error of the mean (SEM) and comparisons on normally distributed data were made using an unpaired sample t test. Paired sample t tests were used to compare immune subsets before and after vedolizumab therapy. All t tests were two-sided. No adjustments for multiple comparisons were performed, as this was a hypothesis-generating study and many of the outcomes measured were biologically related. Statistical analyses were performed using Excel (Microsoft) and GraphPad Prism (GraphPad Software Inc.). All analyses were reviewed by a biostatistician at Benaroya Research Institute (see "Acknowledgments").

Ethical Considerations

All study subjects provided written informed consent, and human subjects research was approved by the institutional review board of the Benaroya Research Institute at Virginia Mason Medical Center.

Results

Patient Demographics and Baseline Characteristics

Thirty-one patients with IBD initiating vedolizumab were evaluated for inclusion in the study. Five patients were excluded from analysis. Reasons for exclusion were missing baseline or posttreatment endoscopy or symptom score evaluation ($n = 3$), requirement for chronic steroids related to a non-IBD indication ($n = 1$), and nonevaluable PBMC sample ($n = 1$). Thus, twenty-six patients were included in the study, with a vedolizumab response in 14/26 patients (54%). Response was defined by endoscopic criteria in 7/14 patients and clinical criteria in 7/14 patients. Nonresponse was determined by failure to wean steroids in 6/12 of the patients and a lack of endoscopic improvement in 6/12 patients. All nonresponders had follow-up evaluations between 90 and 180 days after vedolizumab induction that failed to meet criteria for response.

In the overall cohort, 58% had Crohn's disease and 42% had ulcerative colitis or indeterminate colitis. The vast majority of patients (88%) were TNF-experienced. Half of patients were on combination therapy with a thiopurine or methotrexate during induction with vedolizumab. Responders and nonresponders had very similar demographic and clinical characteristics with no significant differences in reported variables including age, gender, race, IBD subtype, disease distribution, disease duration, prior anti-TNF use, baseline disease activity, CRP, albumin, concurrent smoking, or concomitant medication use (Table 1).

Pretreatment α4β7 Expression on Multiple Cellular Subsets Correlates with Response to Vedolizumab

The peripheral blood of responders collected prior to treatment with vedolizumab demonstrated a significantly higher per-cell expression of integrin α4β7 on naïve ($CD45RA^+CCR7^+$) CD4 and CD8 T cells as well as naïve ($CD19^+CD20^+CD27^-CD38^-IgD^+$) B cells (Fig. 1a). Patients who responded to vedolizumab also had a higher percentage of α4β7-expressing CD4 and CD8 effector memory T (T_{EM}) cells ($CD45RA^-CCR7^-$) (Fig. 1b). Effector memory T cells are differentiated from central memory T cells ($CD45RA^-CCR7^+$) by lack of expression of CCR7, and unlike central memory T cells maintain constitutive expression of effector functions. Central memory ($CD45RA^-CCR7^+$) CD4 and CD8 T cells did not significantly differ in α4β7 expression between responders and nonresponders ($p = .771$ in CD4 and .586 in CD8 T cells, data not shown). A subset of highly differentiated effector CD4 and CD8 T cells called terminal effector memory (T_{EMRA}) T cells ($CD45RA^+CCR7^-$) showed even more significant differences in percentage of α4β7 expression at baseline between responders and nonresponders (mean CD4: 41 ± 2.6% versus 25 ± 3.3%, $p = .0009$, mean CD8: 43 ± 3.5% versus 28 ± 3.2%, $p = .0043$; Fig. 1c). T_{EMRA} cells are armed to secrete cytokines and for cell killing and are present in the human intestine in small numbers (data not shown). In addition, the percentage of NK cells ($CD56^+CD161^+$) expressing α4β7 prior to treatment with vedolizumab was significantly increased in responders compared to nonresponders (mean = 34 ± 3.2% versus 17 ± 4.4%, $p = .0047$; Fig. 1d). These data demonstrate that pretreatment α4β7 expression is higher across multiple cellular subsets in patients who respond to vedolizumab.

Other than α4β7 expression, few significant immunophenotypic differences were found between responders and nonresponders prior to treatment among a diverse array of cellular subsets evaluated in PBMC by flow cytometry. However, expression of the ectonucleoside triphosphate diphospohydrolase, CD39, was seen among significantly more thymically derived ($Helios^+FOXP3^+$) regulatory T cells (tTregs) in PBMC from responders than nonresponders prior to vedolizumab therapy (Supplemental Figure 4).

Table 1 Clinical characteristics of patients according to response

	Response ($n = 14$)	Nonresponse ($n = 12$)	p value
Age at first infusion	41.4 ± 3.6	43.1 ± 4.2	.75
Gender			
Male	6 (43%)	4 (33%)	.70
Female	8 (57%)	8 (67%)	
Race			
White	12 (86%)	10 (83%)	.99
Nonwhite	2 (14%)	2 (17%)	
IBD type			
Crohn's disease	8 (57%)	7 (58%)	.95
Ileal	1 (14%)	1 (14%)	.99
Colonic	2 (25%)	3 (43%)	.99
Ileocolonic	5 (71%)	4 (57%)	.99
Ulcerative colitis/indeterminate colitis	6 (43%)	5 (42%)	.95
Extensive	5 (83%)	4 (80%)	.99
L sided	1 (17%)	1 (20%)	.99
HBI[a]	7.7 ± 1.6	8.6 ± 1.7	.71
SCCAI	3.4 ± 1.6	4.6 ± .93	.54
CRP	13.5 ± 4.9	18.6 ± 6.5	.53
Albumin	3.7 ± .07	3.5 ± .12	.12
Disease duration (years)	12.3 ± 2.4	13 ± 2.8	.85
Prior anti-TNF use	13 (93%)	10 (83%)	.58
Smoking	2 (14%)	0 (0%)	.48
Concomitant treatment			
Steroids	9 (64%)	8 (67%)	.99
Aza/6-MP/MTX	6 (43%)	7 (58%)	.70

[a]Two patients with ileostomies in the nonresponder group were excluded due to the inability to calculate HBI

Otherwise, the frequency and phenotype of Tregs and other major lymphocyte populations did not differ between cohorts (Supplemental Table 2).

Changes in α4β7 Expression During Vedolizumab Therapy

There were few significant changes in the evaluated immunophenotype and percentage of cellular subsets of T cells, B cells, or NK cells over time, regardless of patient response (Supplemental Table 3), except for an increase in circulating plasmablasts ($CD19^+IgD^-CD27^+CD20^-CD38^+$) in both responders and nonresponders ($p = .00042$ overall, Supplemental Figure 5). However, vedolizumab therapy did have an effect on the expression of integrin α4β7 on T cells. Both CD4 and CD8 naïve T cells showed a significant decline in the amount of α4 and β7 expression per cell as determined by a decrease in MFI for α4 and β7 on both responders and nonresponders (Fig. 2a, b). In contrast, the overall percentage of CD4 T_{EM} cells expressing α4β7 increased after therapy regardless of response, whereas no change was seen among CD8 T_{EM} cells (Fig. 2c).

Both Trough Serum Vedolizumab Concentrations and Vedolizumab Receptor Occupancy Are Associated with Response to Therapy

The relationship of trough vedolizumab concentrations to response during the maintenance phase of therapy remains poorly defined. While increasing vedolizumab dose results in increased serum levels, α4β7 saturation has been reported to be nearly complete at low doses [19] and to remain 100% saturated for more than 100 days after dosing [20]. Thus, it is possible that α4β7 receptor saturation on particular cell subsets might provide a more specific biomarker for vedolizumab response than serum levels. In order to better understand the relationship of α4β7 receptor saturation to serum levels of vedolizumab and clinical response, we devised an assay to measure both parameters during vedolizumab therapy in our cohort (Supplemental Figures 2, 3).

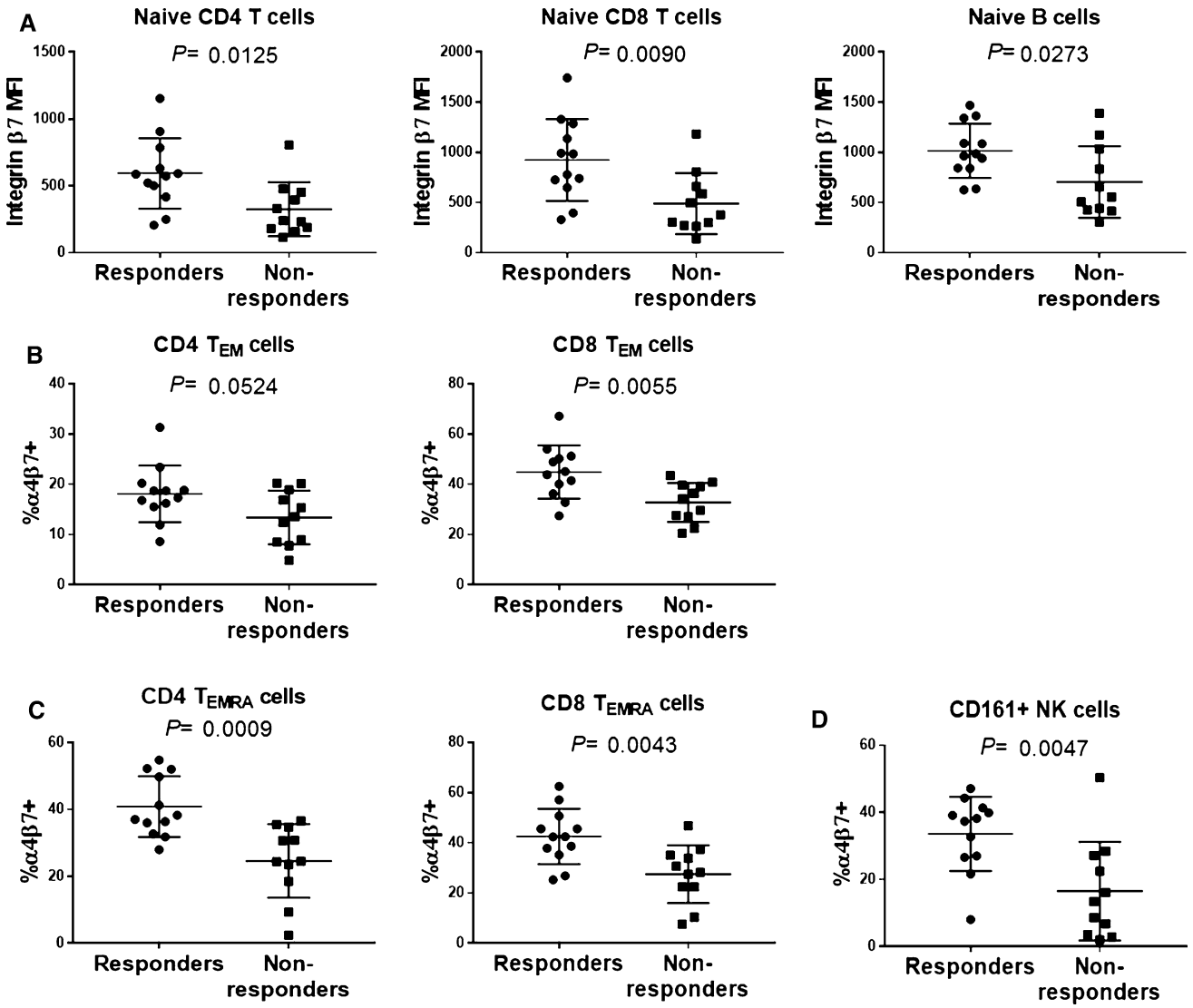

Fig. 1 Pretreatment α4β7 expression on multiple immune cell subsets is higher on subjects who respond to vedolizumab. Prior to therapy with vedolizumab, PBMCs were analyzed by flow cytometry for expression of cell surface markers identifying immune cell subsets and α4β7 expression. **a** Mean fluorescence intensity (MFI) of β7 expression on $CD45RA^{+}CCR7^{-}$naïve CD4 and CD8 T cells, and $CD19^{+}CD20^{+}CD27^{-}CD38^{-}IgD^{+}$ naïve B cells, among responders and nonresponders to vedolizumab. **b** Percent α4β7-expressing $CD45RA^{-}CCR7^{-}$ effector memory (T_{EM}) CD4 and CD8 T cells among responders and nonresponders to vedolizumab. (C) Percent α4β7-expressing $CDR5RA^{-}CCR7^{+}$ terminal effector memory (T_{EMRA}) CD4 and CD8 T cells among responders and nonresponders to vedolizumab. **d** Percent α4β7-expressing $CD56^{+}CD161^{+}$ NK cells among responders and nonresponders to vedolizumab. *p* values reflect unpaired two-way *t* tests

Serum levels of vedolizumab and concurrent α4β7 saturation with vedolizumab were determined for patients in our cohort at various times from their last vedolizumab dose. Serum vedolizumab levels decreased over time in both responders and nonresponders, consistent with prior reports of serum vedolizumab clearance [20], although this decrease occurred more rapidly in nonresponders (Fig. 3a). Conversely, α4β7 lost saturation with vedolizumab in T_{EM} CD4 and CD8 T cells over time in nonresponders, but not responders (Fig. 3b, c). When examined at trough, nonresponders had significantly lower $\log_{10}$ vedolizumab levels than responders (mean = −.04 ± 0.3 versus 1.2 ± 0.17, $p = .0007$; Fig. 3d). Nonresponders also demonstrated a significantly higher percent of T_{EM} cells with free α4β7 at trough (mean CD4: 24 ± 4.9% versus 4 ± .98%, $p < .0001$, CD8: 16.6 ± 4.2% versus 2 ± .65%, $p < .0001$; Fig. 3d).

When plotted against simultaneous serum vedolizumab concentrations for each PBMC sample, there was a clear correlation between low serum concentrations and receptor

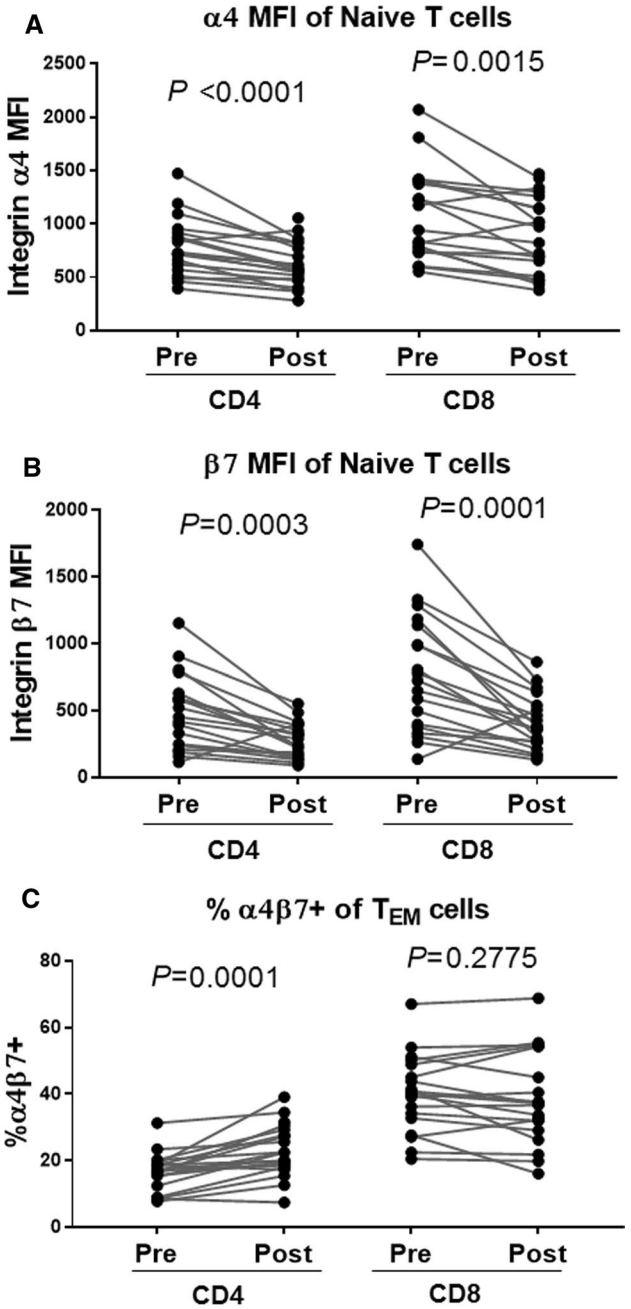

Fig. 2 Changes in α4β7 expression after vedolizumab treatment. PBMCs were serially sampled in patients undergoing vedolizumab therapy and assessed by flow cytometry for expression of α4β7 before and after exposure to therapy (first draw at day 42–105). Mean fluorescence intensity (MFI) of β7 (**a**) and α4 (**b**) expression on naïve CD45RA+ CCR7− CD4 and CD8 T cells before (pre) and after (post) vedolizumab exposure is shown. **c** The percentage of $CD45RA^{-}CCR7^{-}$ T_{EM} CD4 and CD8 T cells expressing α4β7 before (pre) and after (post) vedolizumab exposure is shown. *p* values reflect paired two-way *t* tests

desaturation of T_{EM} cells in nonresponders (CD4: Pearson $r^2 = 0.59$, $p < 0.0001$ and CD8: Pearson $r^2 = 0.271$, $p = 0.0032$), but not responders. Nonreponders demonstrated α4β7 receptor desaturation at serum concentrations that did not result in receptor desaturation in responders (Fig. 4a, b).

Discussion

In this exploratory study of inflammatory bowel disease patients undergoing therapy with vedolizumab, we identified several candidate biomarkers for treatment response. Pretreatment α4β7 expression on multiple T, B, and NK subsets was higher in responders than nonresponders to vedolizumab. In addition, both serum vedolizumab levels and α4β7 receptor saturation at trough were associated with response to therapy.

While pretreatment α4β7 expression correlated with subsequent treatment response among multiple lymphocyte subsets in our study, expression on T_{EMRA} and NK cells provided the best discrimination between responders and nonresponders to vedolizumab. These data support an important role for both effector memory T cells and NK cells in the pathogenesis of IBD. One explanation for the lower baseline expression of α4β7 on the peripheral blood lymphocytes of nonresponders is that these cells are robustly recruited to the intestines, effectively eliminating these cells from the peripheral blood. Alternatively, it is possible that baseline expression of α4β7 is globally reduced on the lymphocytes of nonresponders, regardless of anatomic location. In this case, nonresponders might preferentially utilize an α4β7-independent mechanism for lymphocyte migration to the intestine, rendering them intrinsically resistant to anti-integrin therapy. Future studies will focus on characterizing integrin expression in the intestinal tissues of patients before and during therapy with vedolizumab. These studies may provide further insight into the mechanisms that result in vedolizumab failure.

While the factors influencing α4β7 expression in vivo remain incompletely characterized, it is possible that integrin expression or turnover is affected by inflammation or cytokine milieu. While there were no statistically significant differences in baseline albumin, CRP, or clinical score (HBI/SCCAI) in our cohort, there was a trend toward factors associated with more inflammation in nonresponders (low albumin, high CRP, and higher clinical scores). This raises the possibility that the observed pretreatment differences in α4β7 expression and/or α4β7 saturation were the result of higher inflammatory burden among nonresponders. While it will be important to address this in future studies, it should be noted that pretreatment α4β7 in this study was a superior biomarker for vedolizumab response than any reported outcomes associated with disease severity (CRP, albumin, and clinical scores).

Vedolizumab treatment did appear to have an effect on α4β7 expression during therapy, with the percentage of

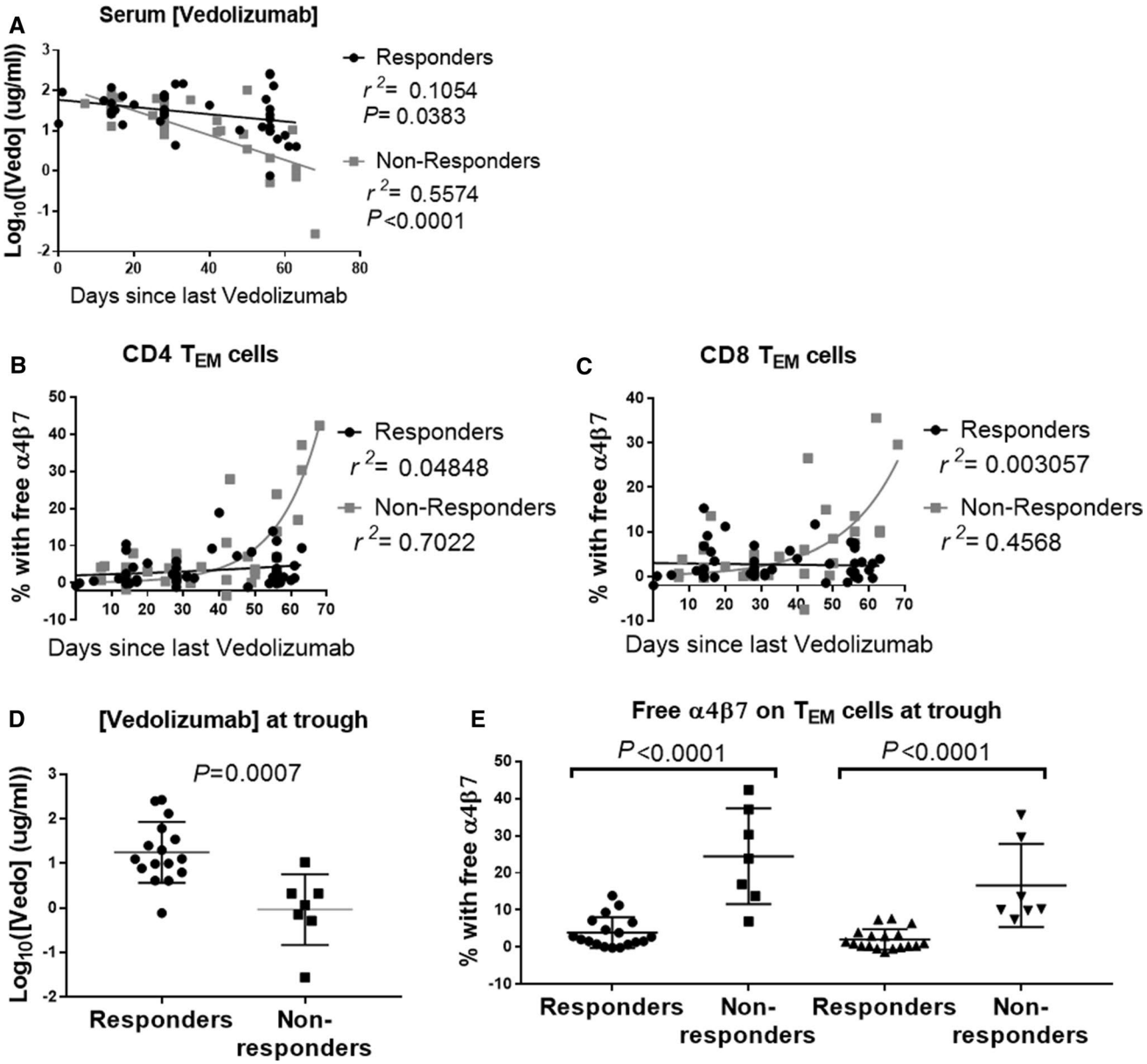

Fig. 3 High trough serum vedolizumab concentrations and saturated α4β7 on effector memory T cells are associated with response to vedolizumab. PBMCs and serum from patients undergoing treatment with vedolizumab were serially evaluated after drug initiation for serum vedolizumab concentration and presence of free α4β7 on CD45RA$^-$CCR7$^-$ T_{EM} cells. Each subject was sampled an average of three different times (range 1–5, SD 1.06) after starting vedolizumab. **a** Log_{10} serum vedolizumab concentration (μg/ml) plotted as a function of time since last vedolizumab dose (days) in responders and nonresponders. The squared Pearson's correlation coefficient (r^2) and the two-tailed p value of the Spearman's coefficient are shown for each linear regression. **b** Percent of CD4 and CD8 CD45RA$^-$CCR7$^-$ T_{EM} cells with free α4β7 as a function of time since last vedolizumab dose (days) in responders and nonresponders. The goodness of fit (r^2) of the semilog nonlinear regression is shown. Log_{10} serum vedolizumab levels (μg/ml) (**c**) and percent of CD4 and CD8 T_{EM} (CD45RA$^-$CCR7$^-$) with free α4β7 (**d**) at trough (50–68 days following last vedolizumab dose) in responders and nonresponders. p values reflect two-way, unpaired t tests

α4β7-expressing T_{EM} CD4 cells increasing during the maintenance phase of therapy. The mechanism of action of vedolizumab would suggest that this finding results from blockade of intestinal trafficking, thus sequestering these cells in the peripheral blood. Vedolizumab treatment also resulted in a decreased per-cell expression of α4β7 on naïve CD4 and CD8 T cells, suggesting an effect of vedolizumab binding on α4β7 integrin turnover or expression. These effects occurred independent of response to the drug.

This study provides novel information regarding the relationship of vedolizumab serum levels to α4β7 receptor saturation in responders and nonresponders to therapy. The

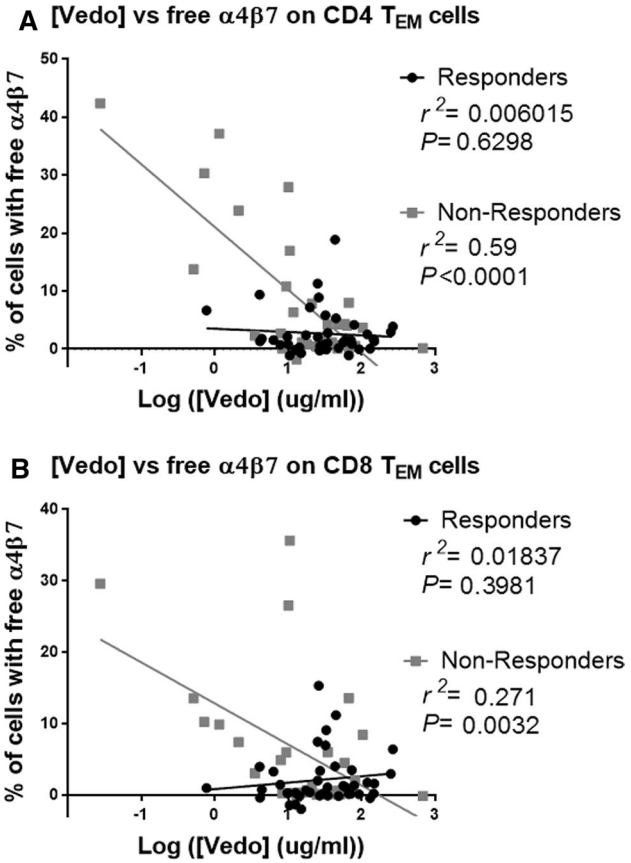

Fig. 4 Serum vedolizumab levels are inversely related to free α4β7 in nonresponders but not responders to vedolizumab therapy. Percent of $CD45RA^-CCR7^-CD4$ (**a**) and CD8 (**b**) T_{EM} cells with free α4β7 is inversely related to $\log_{10}$ serum vedolizumab levels in nonresponders, but not in responders to vedolizumab. The squared Pearson's correlation coefficient (r^2) and the two-tailed p value of the Spearman's coefficient are shown for each linear regression

pharmacodynamics of vedolizumab differ from other biologic medications used in the treatment of IBD including anti-TNF-α monoclonal antibodies such as infliximab. The latter bind cytokines, which have a short half-life and rapid turnover in blood and tissues. While infliximab may also bind transmembrane TNFα, it induces antibody or complement-mediated cytotoxicity [21]. Thus, bound TNFα-anti-TNF complex is rapidly cleared from the patient, so that the residual unbound infliximab remains available to quench additional TNFα. Consequently, serum levels of infliximab are closely associated with response to therapy [22–24]. In contrast, vedolizumab binds to α4β7 on lymphocytes, but does not cause lymphocyte depletion [25]. Because lymphocyte turnover is slow, α4β7 has been reported to remain almost completely saturated on memory T cells for over 100 days, while serum levels slowly decrease after infusion [20, 26]. In our cohort, serum vedolizumab levels declined more rapidly in nonresponders than responders to vedolizumab. While the majority of cells had complete receptor saturation during the maintenance phase, even a small recovery of free α4β7 over time was associated with nonresponse to vedolizumab. Thus, any lapse in complete integrin blockade may undermine the clinical efficacy of anti-integrin therapy.

Interestingly, responders were able to maintain complete receptor saturation at lower serum concentrations of vedolizumab than nonresponders. This points to intrinsic differences in the pharmacodynamics of vedolizumab binding between the two cohorts. That nonresponders lose saturation of α4β7 receptors at vedolizumab levels that maintain saturation in responders suggests that nonresponders have either a higher rate of T cell turnover from the bone marrow or increased α4β7 turnover, perhaps via differences in integrin internalization and intracellular trafficking. Indeed, if nonresponders have intrinsically faster integrin turnover, it could also explain the lower α4β7 expression at baseline.

This study did not address whether nonresponders with low serum vedolizumab levels and free α4β7 on lymphocytes may benefit from vedolizumab dose escalation. Some patients with secondary loss of response to vedolizumab do appear to benefit from escalation to every 4-week dosing [27]. Whether these patients are effectively identified by vedolizumab serum levels or free α4β7 remains an important area for future research.

With the recent proliferation of novel biologic medications with efficacy in the treatment of IBD, biomarkers that can predict primary response and facilitate optimization of therapy will help improve patient outcomes. The candidate biomarkers identified in this study present significant promise. However, this was an exploratory study with small numbers of patients. Statistical analyses did not adjust for multiple comparisons which raises the possibility that some of the less significant differences identified may represent false-positive findings. However, the fact that pretreatment α4β7 expression was consistently increased in responders on multiple cell populations and across multiple investigational panels is encouraging. Further limitations of the study including nonstandardized timing of data acquisition and variability in the parameters used to determine response to therapy reflect that the study was performed during routine care in a clinical practice setting rather than in the context of a clinical trial. The fact that clear differences between responders and nonresponders were identified despite heterogeneity in data collection suggests promise for the translation of these assays to clinical practice.

Future prospective studies are needed to validate the biomarkers identified in this cohort. It will be important in these studies to determine the stability of α4β7 expression in a single patient over time, which was not addressed in this study. In addition, future studies including anti-TNF naïve patients are warranted to determine if the findings in this study apply to this population, as nearly all of our

subjects were anti-TNF-exposed. We believe that these investigations may lead to the development of an assay that could be utilized in clinical practice to identify patients likely to respond to vedolizumab. Such biomarkers would allow for a personalized assessment of the risks and benefits of vedolizumab therapy that would aid in clinical decision making. In addition, further study of the relationship of vedolizumab serum levels and α4β7 saturation to response could assist in optimization of vedolizumab therapy once initiated.

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Compliance with ethical standards

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Author's contribution JDL contributed to study concept. EKB and JDL helped in study design. EKB, MVC, and JDL participated in clinical care and subject recruitment. EKB contributed to clinical data acquisition. DMS and JDL contributed to scientific data acquisition. EKB, DMS, and JDL involved in data analysis and interpretation. EKB and JDL drafted the manuscript and made critical revision for important intellectual content. All authors contributed to the critical revision of the manuscript and have read and approved the final version of this manuscript.

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