Title: Defining Predictors of Radiological Transmural Response to Vedolizumab in Small Bowel Crohn's disease through Serum Proteomic Biomarkers

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B. Background and rationale

VEDOLIZUMAB IN CROHN'S DISEASE: Crohn's disease (CD) is characterized by a dysregulation of both the innate and adaptive immunity responses.[1] Vedolizumab is a novel therapeutic monoclonal antibody that binds to the $\alpha_4\beta_7$ integrin which is expressed specifically by a subset of gastrointestinal-homing T lymphocytes.[2] The binding of $\alpha_4\beta_7$integrin to mucosal addressin cell adhesion molecule-1 (MAdCAM-1) expressed on the surface of mucosal endothelial cells is a crucial component of the gut-selective homing mechanism for lymphocytes. It is currently approved for both induction and maintenance of clinical response and remission in moderate-severe CD who have failed at least one conventional therapy.[3]

FACTORS IMPACTING CLINICAL USE OF VEDOLIZUMAB IN CROHN'S DISEASE: Small bowel CD has no durable surgical cure and, when moderate to severe, typically requires long-term maintenance therapy. Unfortunately, small bowel CD activity is poorly reflected by clinical symptoms indices alone.[4,5] If left untreated, inflammation is primary predictor of disease complications. In clinical practice, the safety profile of vedolizumab makes it an appealing choice to both clinicians and patients for maintenance therapy. However, clinician confidence in using vedolizumab for small bowel CD is not as high as one may predict it would be. Several reasons may underlie this.

Pivotal trials for vedolizumab demonstrated its effectiveness in CD; however, the onset of benefit was less robust and slower than in UC.[3,6] This slower onset, combined with the clinical challenge of determining disease response in Crohn's has prompted some physicians to either avoid use or

abandon vedolizumab as a therapy prematurely. However, the trial had clinical disease activity indices as their primary endpoint. Clinical status and disease activity indices such as the CD Activity Index (CDAI) and the Harvey Bradshaw Index (HBI) correlate poorly with objective markers of inflammation in the small bowel, such as C-reactive protein (CRP), endoscopy, or radiological disease activity parameters.[5] The use of imprecise and flawed clinical disease activity endpoints may explain the GEMINI II trial data in the subset of patients with small bowel CD (Supplementary Figure 2 A and B) which has led some physicians to assume that the role for vedolizumab is predominately for UC and colonic CD and more limited in small bowel CD.[3]

ENDOSCOPIC REMISSION WITH VEDOLIZUMAB IN CD: Recently the VICTORY consortium reported the rates of mucosal healing (MH) across 141 patients with moderately to severe CD cohort.[7] In this cohort, 76.4% of the patients had either ileal or ileocolonic CD. Despite this, 121 out of the 141 individuals had mainly endoscopic follow-up.

CROSS-SECTIONAL IMAGING AS A PREDICTOR OF VEDOLIZUMAB RESPONSE IN SMALL BOWEL CD: In population-based cohorts, up to one-third of patients with CD have evidence of bowel damage with stricturing or penetrating complications at diagnosis, findings which are underdiagnosed without the aid of cross-sectional imaging.[8] This bowel damage at diagnosis identified by cross-sectional imaging is an independent prognostic factor associated with 3-fold increase in intestinal surgery and a 2-fold increase in CD-related hospitalization during patient follow-up.[9] CTE and MRE can additionally detect inflammation in regions inaccessible to standard endoscopy (proximal small bowel or stricturing phenotype), and those

CD patients with isolated intramural disease.[4] Therefore, radiological response has emerged as an important non-invasive treatment target in small bowel CD, associated with decreased long-term need for corticosteroid usage, hospitalization, and surgery.[10-12] Additionally, the EMBARK study has demonstrated that a combined scoring of disease activity using ileocolonoscopy (SES-CD) and CTE correlated much better with biomarkers of inflammation (fecal calprotectin, IL-22, and serum matrix metalloproteinase-9) than ileocolonoscopy alone.[13] Furthermore, a recent multicenter study (N = 214 CD patients) by the Grupo de Estudos de Doença Inflamatória Intestinal compared outcomes with a target of transmural healing (TH) on MRE compared to MH on colonoscopy.[14] At 12 months, TH compared to MH, showed lower rates of therapy escalation (15.2% vs 36.5%, p=0.03) and surgery (0% vs 11.5%, p=0.047), and longer times to therapy escalation and surgery (p=0.046 and p=0.045, respectively. **There is an unmet need for assessing objective response to vedolizumab in small bowel CD using cross-sectional imaging that reflects transmural healing compared to detection of MH with endoscopy**.

PREDICTORS OF REMISSION AND NON-RESPONSE TO VEDOLIZUMAB IN CROHN'S DISEASE: Finally, while objective and complete small bowel assessment of response to therapy is important, the ability to predict early who is likely to achieve response to vedolizumab at 6 and 12 months is equally critical to optimize medical therapy within the ideal window of 'early CD'.[15] In the VICTORY consortium a week 6 clinical remission was achieved in 10.9% with a median time to achieve a clinical remission of 25 weeks, though this increased to 18% at 6 months and 35% at 12 months.[7]

A posthoc analysis of GEMINI II trial data was undertaken to construct a predictive model for week 26 clinical remission using clinical predictors.[16] In this model, an elevated baseline CRP was incrementally associated with a reduction in the probability of achieving clinical remission in anti-TNF-naïve participants (OR 0.94 per mg/L increase; P-value < 0.05), but not anti-TNF-exposed/failure participants (OR 1.00; 95% CI, 0.98–1.02). The model had limited performance in the validation cohort for Week 26 clinical remission (AUC 0.69), which was not significantly improved by adding baseline CDAI into the model (AUC 0.73). This suggests that a model incorporating only clinical predictors is not highly discriminatory for clinical remission. **Hence, in small bowel CD, there is a knowledge gap in the ability to predict and assess response/remission. This unmet need may be addressed by demonstrating the value of baseline radiological and endoscopic features of disease severity and burden to improve prediction of clinical remission with vedolizumab**.

Early identification of primary non-response (PNR) is equally critical to optimize medical therapy within the ideal window of 'early CD'.[15] CD patients initiated on a TNF-alpha antagonist (anti-TNF) exhibit PNR ranging from 36–40% in clinical trials and 13–33% in clinical practice.[17] **There is an additional knowledge gap of clinically relevant serum biomarkers that can predict PNR with vedolizumab therapy in small bowel CD assessed by objective radiological criteria**. This would allow early detection of patients that are less likely to achieve long term response to vedolizumab. This is important given the longer time to response (median 19 weeks) observed in the real-world usage of vedolizumab (VICTORY consortium).[7] In IBD, proteomic approaches have shown promise with regards to identifying active disease and predicting response to anti-

TNF.[18-20] A serum proteomic analysis of patients who received ustekinumab in UNITI-1, UNITI-2 and IM-UNITI identified IFN γ as a pharmacodynamic marker, and Serum Amyloid A and IL-6 as early biomarkers of response to ustekinumab.[21,22]

SUMMARY: **The overall goal of the study is to develop data that can convincingly guide clinicians on the use and efficacy of vedolizumab in patients with small bowel CD.** There is an unmet need to identify response to vedolizumab in small bowel CD using objective endpoints. Current data suggest that MR enterography may meet this unmet need. There is an additional unmet need to develop predictive models incorporating both clinical and baseline radiological and endoscopic variables with higher discriminatory performance in identifying longer term clinical remission with vedolizumab. Finally, this proposal is strengthened by the exploratory studies which may identify new proteomic biomarkers that correlate with longer term radiological response with vedolizumab reflecting its latency of response. If successful, these serum biomarkers may guide a personalized approach to the treatment of small bowel CD with vedolizumab, allowing early identification of PNR, monitoring disease activity and the pharmacodynamics of vedolizumab.

C. HYPOTHESIS

We hypothesize that radiological response to vedolizumab will predict a reduction in need for corticosteroids, hospitalizations and/or surgeries in small bowel CD. We also hypothesize that predictive models incorporating baseline radiological variables in addition to clinical variables will improve model performance in predicting remission with vedolizumab at 6 months.

We plan to test our central hypothesis by pursuing the following specific aims:

Aim 1: To prospectively determine if short term (4 months) radiological TR on MRE in small bowel CD patients starting vedolizumab predict clinically relevant outcomes at 12 months;

Aim 2: To prospectively determine if inflammatory severity defined by the length of active small bowel lesion at baseline enterography improves the performance of model incorporating clinical variables in predicting clinical remission at 4 months;

Aim 3: To assess if quantitative or qualitative change in proteomic serum biomarkers (using SOMAscan™ assay) is a relevant surrogate biomarker for radiologic TR on MRE at 4 months after starting vedolizumab.

The work proposed in Aim 1 would establish radiological response as a treatment endpoint with vedolizumab for small bowel CD. The work proposed in Aim 2 would allow improved prediction of remission with vedolizumab in small bowel CD. The work proposed in Aim 3 would allow the development of a serum biomarker that correlates with radiological response at 4 months. After completion of the current study aims, the next step using this study cohort would be to compare radiological versus endoscopic response at week 52. Future directions would include validating the findings of this study by performing an interventional study to personalize the dosing of vedolizumab starting at induction (based on markers of baseline inflammatory severity using MRE and cytokine burden) to achieve clinical and radiological response at 4 and

12 months. We would also seek to validate the proteomic biomarkers of primary non-response identified in the current study cohort, in the dose optimization study.

D. Pilot Data

Retrospective study of treating to radiological response

Our recent retrospective study demonstrated that treating to a target of radiological transmural response (TR) at initial follow-up computed tomography enterography (CTE) or magnetic resonance enterography (MRE) in small bowel CD is associated with an improvement in relevant long-term outcomes.[10] In this cohort of 150 small bowel CD patients who were longitudinal followed with serial CTE and/or MRE, radiological TR was examined as a decrease in radiological findings of inflammation, in up to five small bowel lesions per patient, over follow-up CTE/MRE. In this study, 55 patients (37%) were complete radiologic responders, 39 partial (26%), and 56 non-responders (37%), at the 1st follow-up CTE/MRE.

In multivariable Cox models, complete and partial response were associated with a decreased risk for corticosteroid usage by over 50% (hazard ratio (HR): 0.37 (95% confidence interval (CI), 0.21–0.64); 0.45 (95% CI, 0.26–0.79)), and complete response decreased the risk of subsequent hospitalizations (**Figure 1)** and surgery (**Figure 2**) by over two-thirds (HRs: HR, 0.28 (95% CI, 0.15–.50); HR, 0.34 (95% CI, 0.18–0.63)). Prior work has demonstrated that small bowel CD inflammatory severity is highly correlated with disease length, and that changes in the length of small bowel CD inflammation itself is a highly accurate

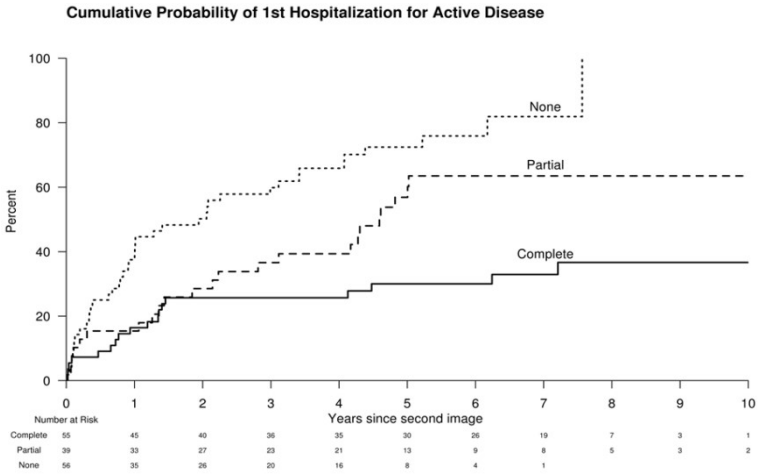

Figure 1: Patients who demonstrated partial (p =0.04) or complete response (p < 0.001) at 2nd CTE/MRE demonstrated improved survival free of hospitalizations for CD, compared to those with non-response

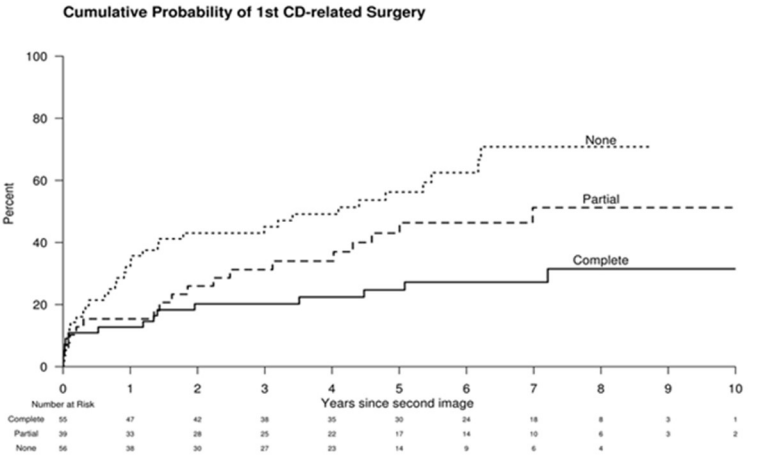

Figure 2: Complete response at 2nd CTE/MRE demonstrated improved survival free of CD-related surgeries compared to those with non-response.

SOMAscan™ in biomarker exploration

SOMAscan™ (SomaLogic, Inc., Boulder, CO, USA) is a novel high-throughput analysis of proteins through Slow Off-rate Modified DNA Aptamer (SOMAmer)-based capture array. SOMAscan™ has enabled the simultaneous measurement of a large number of proteins to identify biomarkers in lung cancer, cardiovascular disease and inflammatory arthritis.[25-29] SOMAscan™ has also previously identified 18 proteins responsive to both prednisone and infliximab in pediatric IBD patients, with gene promoters regulated by nuclear factor-κB.[30] Of these, five proteins with known functions associated with inflammation, including α-1-antitrypsin, insulin-like growth factor binding protein 1 and 2, resistin, and C-C motif chemokine 23, showed a significant decrease in response to treatment.

The SOMAscan™ panel also contains serum proteins that have been identified in previous studies as serum biomarkers correlating with a combination of endoscopic and cross-sectional imaging findings of inflammation in CD including IL-22, and serum matrix metalloproteinase-9.[13] Additional proteins in the panel have been identified in prior studies as potential biomarkers of infliximab (platelet factor 4, soluble CD40 ligand and IL-6) and ustekinumab response (IFN γ, serum amyloid A, IL-6, interleukin-17A and creatine kinase M-type: creatine kinase B-type heterodimer.[19,21,22]

The mechanism of action of vedolizumab involves vedolizumab selectively inhibition of adhesion of alpha-4 beta-7 expressing cells to MAdCAM-1 and fibronectin but not to vascular cell adhesion molecule 1 (VCAM-1).[2] While a prior study has explored serum MAdCAM-1 as a

possible marker of response to therapy, the SOMAscan™ panel contains fibronectin and VCAM-1, which could be explored as mechanistic biomarkers of response with vedolizumab therapy.[31]

E. Study design

Total Study Duration:

- Final protocol to IEC/IRB/HA approval: Approved
- IEC/IRB/HA approval to First Patient, First Visit: 2 month
- First Patient, First Visit to Last Patient, Last Visit: 48 month
- Treatment period: 12 months
- Final study report timing after Last Patient, Last Visit: 6 months
- Publication submission timing after final study report:3 months

Primary endpoint:

1. Radiological response at 4 months (16±2 weeks)

Radiological TR will be defined per-lesion and per-patient (**Appendix A**). Per-lesion TR will be defined as a decrease in the length of inflamed small bowel lesion from baseline or improvement in any imaging findings associated with severe mural inflammation, i.e., restricted diffusion,

mural thickness, intramural T2 hyperintensity, peri-enteric T2 signal, and luminal ulcerations, without development of a new penetrating or stricturing complication. Worsened lesions will be fined as those with an increased score of any imaging parameter associated with severe mural inflammation. Unchanged lesions will be defined as those with unchanged (without worsening or improving) inflammatory parameters associated with severe mural inflammation. Patients will be then classified for TR as responders if all individual lesions improved and non-responders if any of the lesions worsened, a new lesion developed at the 4 month MRE and all other scenarios not meeting definition of response. Illustrative example of a radiological complete responder is shown in **Figure 3.**

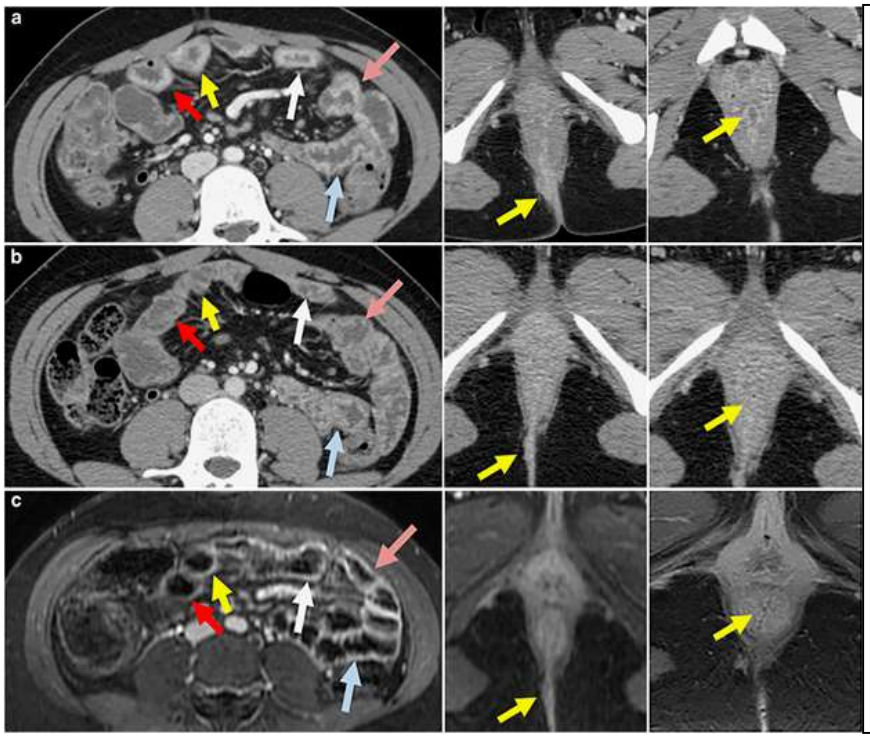

Figure 3: Illustrative example of a radiological complete responder where colored arrows indicate corresponding bowel loops on follow-up CTE or MRE. (a) CTE 7/26/04 demonstrating multifocal jejunal CD with rectovaginal and transsphincteric fistulae. (b) CTE 10/30/2006 demonstrating radiological response in the jejunal and pelvic CD after starting on combination therapy with anti-TNF along with azathioprine. (c) MRE 10/19/2007 demonstrating healed jejunal and pelvic fistulizing CD

Secondary endpoint:

1. Clinical response (compared to baseline) at 6 weeks (±1 week), 4 months (16±2 weeks) study visit, clinical visit prior to 3rd cycle of 4 weekly dosing of vedolizumab among patients who dose-escalated after week 14 dose, and 1 year (52±4 weeks)
   - 100 points reduction in CDAI or
   - ≥3 point decrease in HBI or
   - ≥5 point reduction in Patient reported outcome - 2 (PRO-2)
2. Clinical response (compared to baseline) at 6 weeks (±1 week), 4 months (16±2 weeks) study visit, clinical visit prior to 3rd cycle of 4 weekly dosing of vedolizumab among patients who dose-escalated after week 14 dose, and 1 year (52±4 weeks)
   - CDAI score ≤150 points or
   - HBI ≤ 4 points or
   - PRO-2 ≤8 points
3. Endoscopic disease activity at 1 year (52±4 weeks): SES-CD.
4. Endoscopic remission at 1 year (52±4 weeks): SES-CD 0 to 2.

Other endpoints:

5. EQ 5-D: EuroQol instrument for recording self-reported health status
6. Disease progression including:
   - Internal penetrating disease at MRE at 1 year (52±4 weeks)
   - Stricturing disease at MRE at 1 year (52±4 weeks)

- Hospitalization for flares or complications of the disease after MRE at 4 months (16±2 weeks) and till MRE at 1 year (52±4 weeks)
- Need for surgery related to CD after MRE at 4 months (16±2 weeks) and till MRE at 1 year (52±4 weeks)
- Need for rescue corticosteroids after MRE at 4 months (16±2 weeks) and till MRE at 1 year (52±4 weeks)

Study population:

Patients with known or suspected CD identified at the IBD Clinic in the Division of Gastroenterology and Hepatology at Washington University, St. Louis, MO will be recruited if they meet inclusion/exclusion criteria. Please see **Appendix B** for details on the study visits. Patient who have had a standard of care ileocolonoscopy and MRE will be recruited if they meet the following:

Key inclusion criteria:

- Moderate to severe disease activity small bowel CD (small bowel only or ileocecal only) visible on MRE
- Initiated on Vedolizumab with/without thiopurines or methotrexate
- ≥18 years old

Key exclusion criteria:

- Known pregnancy on study entry. Subjects will not be tested for pregnancy on protocol outside of standard of care, and is usually done prior to clinical radiological and endoscopic testing for patients of childbearing potential per standard of care standard operating procedures. If any subject is found to be pregnant during the study they will be discontinued from the study visit protocol and followed per the pregnancy follow up, as outlined below under "Procedures for Reporting Drug Exposure during pregnancy and Birth Events". Any pregnant partner of study subjects reported during this study will be followed also as below.
- Age <18 years old
- Planned surgery prior to the first follow-up MRE
- Inability to provide informed consent.
- Perianal CD, since assessment requires performance of additional MRI of the pelvis.
- Individuals with colonic involvement other than involvement of the ascending colon and cecum.
- Inpatient scans will only be included if this is an MRE and adequate small bowel distension with appropriate contrast has been achieved, in the opinion of the radiology co-investigator.
- Contraindications for MRE, including chronic kidney disease that precludes contrast administration, and implanted medical devices that are contraindicated for MRE.
- Any subject condition or situation which, in the opinion of the Investigator or regulatory authorities, interferes with optimal study participation of the participant or produces/could produce significant risk to the subject.

Treatment regimen:

Vedolizumab will be initiated at 300 mg intravenous (IV) dosing at 0, 2 and 6 weeks followed by the 1st maintenance with 300 mg IV at week 14. Patients who have not achieved clinical response at week 14 will be eligible to undergo dose escalation to 4 weekly dosing of vedolizumab depending on the judgement of the treating gastroenterologist. The precedent for this is observations from the open-label GEIMINI long-term safety (LTS) trial where patients who received vedolizumab every 8 weeks during GEMINI 2 maintenance were escalated to every 4 weeks during GEMINI LTS.[32] Specifically, among the 57 patients receiving vedolizumab every 8 weeks during GEMINI 2 and withdrew from the study early (sustained non-response, disease worsening, or need for rescue medication), clinical response increased from 39% (22/57) at the onset of LTS trial to 54% (n=31/57) at week 28 and 35% (20/57) at week 100. Similar trends were seen in clinical remission, with increase from 4% (2/57) at the LTS trial onset to 23% (13/57) at week 28 and 19% (11/57) at week 100. A similar trend in efficacy was observed with increased dosing frequency regardless of prior TNF antagonist exposure. Similar data was shown in the study by Wiliet et al. with dose escalation at week 10 in patients without clinical remission at week 6.[33]

Study Visits:

Patients entering the study will be evaluated by the investigator for all inclusion and exclusion criteria. Once inclusion and exclusion criteria have been determined, all subjects will sign an IRB/IEC approved consent document, which complies with regulatory requirements, prior to any study activities. Subjects will be given adequate time to read and consider the consent document

and all study information. They will have the opportunity to ask questions of the investigator(s) and the study team about the study, as well as risks and benefits, prior to signing the document.

Baseline Visit

Baseline assessments will be collected from the clinical care record from the standard of care visit(s) done most prior to the subject receiving their first dose of vedolizumab. All assessments will be done/collected prior to the subject's first does of vedolizumab. The baseline visit will consist of the following:

- Demographic information: Date of birth, gender, and race will be collected.
- Clinical assessment: These assessments will be done by the study team prior to the subject receiving the first dose of vedolizumab.
    - CDAI
    - Harvey Bradshaw Index
    - PRO-2/3
    - EQ-5D
    - IBD Disability Index
- Physical Exam Findings: Physical exam findings will be taken from the patient's clinical care record from the standard of care visit most recently preceding the initial dose of vedolizumab. If this data is not available from the visit, the information will be obtained from the subject during a separate visit to the clinic by the study team, and prior to the first dose of vedolizumab.

  - Height/Weight
  - Waist/Hip Ratio
- Blood Tests:
  - C-reactive Protein (taken from the clinical care record as described below)
  - Albumin (taken from the clinical care record as described below)
  - Hematocrit (taken from the clinical care record as described below for assessment of CDAI)
  - SOMAscan panel (drawn for protocol as described below)
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- Stool Collection: At the time of consent, participants will be provided a stool collection kit and asked to collect a small amount of stool when they have a bowel movement, prior to their first dose of vedolizumab, for genetic, microbiome, or biomarker analysis. If the patient is unable to provide a stool sample, permission may be asked to obtain a rectal swab, or a sample collection kit will be mailed to them and asked to mail the stool sample back to us in a prepaid envelope. If the patient is in the hospital and tests have been ordered on their stool, stool may be collected from what is left over after the tests have been completed. The stool will be assigned a unique specimen code. The mRNA in the stool would be extracted and the genetic expression profile of this sample would be determined. All participants consent to having details from their medical records (age, ethnicity, medications, surgery, tobacco use, family history, disease activity, disease history) at the Washington University Medical Center, and entered into a database

associated with a patient code. If the patient's records do not include all relevant information, a study team member may obtain this information directly from the patient. The specimen(s) will not have the participant name or address on it; it will be labeled with a code in order to protect the patient's health information and identity. The specimen(s) will be stored in locked freezers in a locked laboratory at WUSM. All coded specimens will be linked to study participants on a separate master list, which will be stored in a locked cabinet (the study coordinators have the only key) or a password protected file. This information will be kept separate from the clinical data.

- Endoscopic Assessment: Subjects will have endoscopic assessment as baseline prior to initiation of vedolizumab, as a part of their standard of care treatment. Subjects who have had endoscopic assessment with biopsies from the ileum confirming small bowel Crohn's disease as a part of their standard of care treatment in the six (6) months prior to study enrollment may be used for the baseline study assessment, at the investigator's discretion. If a more recent endoscopic assessment is done with this reporting, the more recent assessment will be used.
- Radiological Assessment: Radiological assessment will be performed on all patients as a part of their clinical standard of care prior to initiating vedolizumab. Clinical read will be evaluated using the assessment tool in Appendix B and guidance from Appendix A.
    - MR Enterography

Week 6 Visit (+/- 1 week)

Week 6 Visit assessment will be done 6 weeks (+/- 1 week) after initiating vedolizumab as a part of clinical standard of care treatment as described in this protocol. The visit will consist of the following:

- Clinical assessment:
    - CDAI
    - Harvey Bradshaw Index
    - PRO-2/3
    - EQ-5D
    - IBD Disability Index
- Blood Tests:
    - C-reactive Protein (taken from the clinical care record as described below)
    - Albumin (taken from the clinical care record as described below)
    - Hematocrit (taken from the clinical care record as described below for assessment of CDAI)
    - SOMAscan panel (drawn for protocol as described below)

Week 16 Visit (+/- 2 weeks)

Week 16 Visit assessment will be done 16 weeks (+/- 2 weeks) after initiating vedolizumab as a part of clinical standard of care treatment as described in this protocol. The visit will consist of the following:

- Clinical assessment:
    - CDAI
    - Harvey Bradshaw Index
    - PRO-2/3
    - EQ-5D
    - IBD Disability Index
- Physical Exam Findings: Physical exam findings will be taken from the patient's clinical care record from the most recent standard of care visit. If this data is not available from the visit, the information will be obtained from the subject during a separate visit to the clinic by the study team.
    - Height/Weight
    - Waist/Hip Ratio
- Blood Tests:
    - C-reactive Protein (taken from the clinical care record as described below)
    - Albumin (taken from the clinical care record as described below)
    - Hematocrit (taken from the clinical care record as described below for assessment of CDAI)
- Stool Collection: As above referenced in the Baseline Visit.
- Radiological Assessment:
    - MR Enterography

Clinical Visit

The Clinical Visit assessment will only be done if a dose escalation is needed for vedolizumab treatment in patients who do not achieve clinical response at week 14, per investigator discretion.

- Clinical assessment:
    - CDAI
    - Harvey Bradshaw Index
    - PRO-2/3
    - EQ-5D
    - IBD Disability Index
- Blood Tests:
    - C-reactive Protein (taken from the clinical care record as described below)
    - Albumin (taken from the clinical care record as described below)
    - Hematocrit (taken from the clinical care record as described below for assessment of CDAI)

Week 52 Visit (+/- 4 weeks)

Week 52 assessments will be done 52 weeks (+/- 4 weeks) after initiating vedolizumab as a part of clinical standard of care treatment as described in this protocol. The visit will consist of the following:

- Clinical assessment:

  - CDAI
  - Harvey Bradshaw Index
  - PRO-2/3
  - EQ-5D
  - IBD Disability Index

- Physical Exam Findings: Physical exam findings will be taken from the patient's clinical care record from the most recent standard of care visit. If this data is not available from the visit, the information will be obtained from the subject during a separate visit to the clinic by the study team.
  - Height/Weight
  - Waist/Hip Ratio
- Blood Tests:
  - C-reactive Protein (taken from the clinical care record as described below)
  - Albumin (taken from the clinical care record as described below)
  - Hematocrit (taken from the clinical care record as described below for assessment of CDAI)
- Endoscopic Assessment: Subjects will have endoscopic assessment as a part of their standard of care treatment, and this report may be used for the study assessment.
  - SES-CD

- Radiological Assessment: Radiological assessment will be performed on all patients as a part of their clinical standard of care. Clinical read will be evaluated using the assessment tool in Appendix B and guidance from Appendix A.
    - MR Enterography
- Disease Progression Assessment: In order to assess the Disease Progression endpoint the following will be assessed for the time point between the Week 16 Visit MRE and the Week 52 Visit MRE:
    - Any hospitalization for disease flare or complications of the disease
    - Need for surgery related to CD
    - Need for rescue corticosteroids

Withdrawal Criteria:

Subjects are free to withdraw their consent from study participation at any time, for any reason, without interference to their continued standard medical care. Subjects will be withdrawn should the any of the following occur:

- The subject develops, or has an exacerbation of, any medical condition, which the investigator believes would preclude, interfere, or otherwise make the subject's continued participation in the study unsafe.
- The subject withdraws consent.
- Any confirmation of pregnancy.
- Any regulatory request of subject withdrawal

- Any subject non-compliance with study procedures in the opinion of the investigator.
- The subject experiences a severe or serious adverse event which would, in the opinion of the investigator, preclude the subject from continued participation.

Safety Reporting

Institution/Investigator is solely responsible for reporting all Adverse Events and Serious Adverse Events to regulatory authorities, investigators, IRBs or IECs and Takeda, as applicable, in accordance with national regulations in the countries where the study is conducted.

Regardless of expectedness or causality, all SAEs and pregnancy reports must also be reported in English by facsimile to Takeda Pharmacovigilance or designee:

Fatal and Life Threatening SAEs *within 24 hours of the sponsor-investigator's observation or awareness of the event*

All other serious (non-fatal/non-life threatening) events *within 4 calendar days of the sponsor-investigator's observation or awareness of the event*

Takeda Safety Reporting Contact Information

Takeda requires that all information be communicated to Takeda's Pharmacovigilance Department as outlined in the study contract.

All reported adverse drug reactions and safety issues related to Takeda compound must be included in the final study report.

Describe procedures for reporting Adverse Events and Serious Adverse Events.

Definitions:

Adverse event (AE) means any untoward medical occurrence in a patient or subject administered a medicinal product; the untoward medical occurrence does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product whether or not it is related to the medicinal product. This includes any newly occurring event, or a previous condition that has increased in severity or frequency since the administration of study drug.

An abnormal laboratory value will not be assessed as an AE unless that value leads to discontinuation or delay in treatment, dose modification, therapeutic intervention, or is considered by the investigator to be a clinically significant change from baseline.

An adverse drug reaction (ADR) is a response to a medicinal product which is noxious and unintended. Response in this context means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility. This includes adverse reactions which arise from: use of a medicinal product within the terms of the marketing authorization;

use outside the terms of the marketing authorization, including overdose, misuse, abuse and medication errors; and occupational exposure.*

** This corresponds to the exposure to a medicinal product for human use as a result of one's occupation, such as nurses who may handle products routinely in their occupational setting.*

Serious AE (SAE) means any untoward medical occurrence that at any dose:

1) *results in **death**,*
2) *is **life-threatening**,*

Note: The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

3) *requires inpatient **hospitalization or prolongation of present hospitalization**,*
4) *results in **persistent or significant disability/incapacity**,*
5) *leads to a **congenital anomaly/birth defect**,*
6) *may require intervention to prevent one of 1)-5) above or may expose the patient to danger, even though the event is not immediately life-threatening or fatal or does not result in hospitalization.*

Clarification should be made between a serious AE (SAE) and an AE that is considered severe in intensity (Grade 3 or 4), because the terms serious and severe are NOT synonymous. The general term severe is often used to describe the intensity (severity) of a specific event; the event

itself, however, may be of relatively minor medical significance (such as a Grade 3 headache). This is NOT the same as serious, which is based on patient/event outcome or action criterion described above, and is usually associated with events that pose a threat to a patient's life or ability to function. A severe AE (Grade 3 or 4) does not necessarily need to be considered serious. For example, a white blood cell count of 1000/mm^3 to less than 2000 is considered Grade 3 (severe) but may not be considered serious. Seriousness (not intensity) serves as a guide for defining regulatory reporting obligations.

Procedures for Reporting Drug Exposure during Pregnancy and Birth Events

If a woman becomes pregnant or suspects that she is pregnant while participating in this study, she must inform the investigator immediately and permanently discontinue study drug. The sponsor-investigator must fax a completed Pregnancy Form to the Takeda Pharmacovigilance or designee immediately. The pregnancy must be followed for the final pregnancy outcome (i.e., delivery, still birth, miscarriage) and Takeda Pharmacovigilance or designee will request this information from the sponsor-investigator. Please refer to study contract for Takeda pharmacovigilance contact information.

If a female partner of a male patient becomes pregnant during the male patient's participation in this study, the sponsor-investigator must also immediately fax a completed Pregnancy Form to the Takeda Pharmacovigilance or designee. Every effort should be made to follow the pregnancy for the final pregnancy outcome. Please refer to study contract for Takeda pharmacovigilance contact information.

Product Complaints and Medication Errors

A product complaint is a verbal, written, or electronic expression that implies dissatisfaction regarding the identity, strength, purity, quality, or stability of a drug product. Individuals who identify a potential product complaint situation should immediately contact Takeda and report the event.

A medication error is a preventable event that involves an identifiable patient and that leads to inappropriate medication use, which may result in patient harm. While overdoses and underdoses constitute medication errors, doses missed inadvertently by a patient do not. Individuals who identify a potential medication error situation should immediately contact Takeda (see below) and report the event.

Phone: 1-877-TAKEDA7 (1-877-825-3327)

E-mail: medicalinformation@tpna.com

FAX: 1-800-247-8860

Product complaints and medication errors in and of themselves are not AEs. If a product complaint or medication error results in an SAE, an SAE form should be completed and sent to Takeda Pharmacovigilance.

Serum proteomic analysis with SOMAscan™

Serum protein concentration in AIM 3 will be as measured using SOMAscan™ at the certified Genome Technology Access Center (GTAC) microarray facility at Washington University using Agilent microarray readout.[34] This will be drawn prior to initiation of therapy and at week 6. This proteomic platform has enabled the simultaneous measurement of a large number of proteins to identify biomarkers in lung cancer, cardiovascular disease, inflammatory arthritis and inflammatory bowel disease.[25-30,35]

The GTAC lab has been certified in SomaScan™ technology from the SomaLogics company. The SOMAscan assay quantitatively transforms the proteins present in a biological sample into a specific SOMAmer-based DNA signal. A SOMAmer-protein binding step is followed by a series of partitioning and wash steps that converts relative protein concentrations into measurable nucleic acid signals that are quantified using DNA detection technology, which for the SOMAscan Assay with 1,310 SOMAmer reagents is by hybridization to custom DNA microarrays. The assay offers exceptional dynamic range, quantifying proteins that span over 8 logs in abundance (from femtomolar to micromolar) and excellent reproducibility (4.6% median %CV). Agilent SOMAScan slides will be processed through the Agilent scanner and image data will be extracted using Agilent Feature Extraction software. Extracted initial raw signal intensity data will be uploaded onto the SOMAScan server for data QC and standardization. The final data set will include SOMAscan generated .adat file, a reformatted text file with standardized relative fluorescent unit (RFU) for ~1.3K proteins, SOMAscan Quality Statements (SQS) and basic differential expression. The technology has been well validated by mass-spec here at Washington University.

Other laboratory tests

Serum will be collected at all 5 time points with CRP, hematocrit (as part of CBC) and serum albumin prior to medication initiation, week 6, 4 months (16±2 weeks), prior to the 3rd 4 weekly cycle of vedolizumab after dose escalation in patients who failed to achieve clinical response (CDAI 100 point decrease) at week 14 and at 52 ± 4 weeks.

F. Statistical plan

Statistical analysis

AIM 1: Response at 4 month MRE will be categorized as non-response versus response to biologic. Association between response status at 4 month MRE and clinically relevant outcomes will be assessed at week 52 in logistic regression model adjusting for variables include gender, age at disease onset/disease duration, smoking status and presence of stricturing/fistulizing disease at baseline MRE. Additional time-to clinically relevant outcomes after 6 month MRE depending on response status will be assessed using Cox proportional hazards model adjusting for prior mentioned variables

AIM 2: Multivariate logistic regression analyses and backward model selection will be performed for baseline clinical, radiological (length of disease) and endoscopic variables (SES-CD score) and variable interactions that are found to be significant ($P < 0.05$) on the initial univariate analyses. Adjusted odds ratio (OR) will be presented for predictors included in the model, with OR > 1 indicating an increased probability of achieving clinical remission at Week

26. Discrimination performance of the model will be presented as area under the curve (AUC) from receiver operating characteristic curves.

AIM 3: Normality of protein values will be determined by the Kolmogorov Smirnov and Shapiro-Wilk normality tests. Statistical techniques will include Wilcoxon signed-rank test (comparing baseline to posttreatment values in the same individual), Mann-Whitney U test (comparing change in individual proteins in SOMAscan^{TM} between the response categories at week 14 MRE), linear regression analysis (SOMA scan^{TM} vs. CRP) and logistic regression analysis (change in levels of identified proteins in SOMAscan^{TM} versus response categories, adjusting for patient and disease characteristics). Spearman rank correlation analysis will be applied to assess correlations. The alpha-level will be set at 0.05 for statistical significance. Bonferroni correction will be applied for multiple testing.

Sample size analysis

We estimate 37% of CD patients with complete, 26% partial and 37% non-response at 14 week MRE based on prior data.[10] At a power of 80% and a type-1 error (alpha) of 0.05 to detect a hazards ratio of 2.5 or more, we estimate that 38 or more patients will transition from partial/non-response to complete response based on prior data. In a year, we expect 50% of the patients making such a transition. Hence, we plan to recruit at least 76 patients to this cohort. Alternately, 130 CD patients would be estimated to detect an HR of 2.0 assuming 50% transition within 1 year.

G. Scientific team collaborators

This project is collaboration between the Division of Gastroenterology, Mallinckrodt Institute of Radiology and the Biomarker core at Washington University, St. Louis, MO. Dr. Deepak has joined the faculty at Washington University. He has extensive research experience in the field of the use of diagnostic imaging in IBD, mentored by Dr. David H. Bruining and Dr. Joel G. Fletcher.[4,10-12,36,37] Dr. Ciorba has published extensively on biomarkers in IBD[18,38-41] and on MRE imaging for IBD with the Mallinckrodt group.[42]

H. Limitations of study design

Our study will utilize a scoring system with MRE with components derived from previously validated methodology utilizing both CTE and MRE. While several MR based scoring systems are currently available, these have largely been modeled after and validated against endoscopic scoring systems, with only a modest weight given to small bowel inflammation (eg, only 1 of 5 bowel segments scored in the Magnetic Resonance Index of Activity Score as part of the small bowel). [36] These have additional limitations in describing overall disease burden and reproducibility.

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Appendix A: Reader's manual and sample data collection sheet for individual scan

Overview:

1. Scoring to be done per patient and per lesion, of **not more than 5** of the longest lesions identified on MR enterography (MRE).
2. Lesion to be identified on index MRE.
3. Skip lesion defined as two areas of active Crohn's disease that must be separated by circumferential normal intestine.
4. Location of segment involved(free text or selected): Series and image #
5. Length of active disease segment in centimeters.
6. Mural thickness small bowel:
   - 0 = <3 mm
   - 1 = >3–5 mm
   - 2 = >5–7 mm
   - 3 = >7 mm
7. Mural T2 Signal:
   - 0 = Equivalent to normal bowel wall
   - 1 = Minor increase in signal: bowel wall appears dark grey on fat-saturated images
   - 2 = Moderate increase in signal: bowel wall appears light grey on fat-saturated images
   - 3 = Marked increase in signal: bowel wall contains areas of white high signal approaching that of luminal content
8. Peri-mural T2 signal:
   - 0 = Equivalent to normal mesentery
   - 1 = Increase in mesenteric signal but no fluid
   - 2 = Small fluid rim (≤2 mm)
   - 3 = Larger fluid rim (>2 mm)
9. Presence of small bowel ulcerations: Wall defect that extends from the lumen intramurally, but not beyond the outside bowel wall.
   - 0 = No
   - 1 = Yes
10. Stricture (obstruction) defined as luminal narrowing in area of Crohn's disease with unequivocal proximal dilation, or that is persistent over two time points (e.g., multiple pulse sequences or delayed series)
    - 0 = none,
    - 1 = yes without upstream dilation (< 3 cm)
    - 2 = mild upstream dilation 3- 4 cm,
    - 3 = moderate-severe upstream dilation > 4 cm
11. Presence of new internal penetrating disease: Sinus, simple fistula or complex fistulae, abscess or phlegmon
    - 0 = None
    - 1 = Yes
12. Qualitative diffusion weight imaging (DWI) analysis evaluated as absence or presence of hyperintensity suggestive of active disease on DWI with b-value of 800 s/mm^2.
13. For quantitative assessment, the apparent diffusion coefficient (ADC) will be calculated for each active small bowel lesion with a region of interest area between 10 and 30 mm^2 was drawn in the area of highest signal intensity in the bowel wall.

Lesion Response Definition:

Qualitative per-lesion analysis

- Improved lesion: Decrease in length of active segment or improvement in other morphological features listed below, without new internal penetrating disease.

  - Mural thickness of small bowel segment
  - Mural T2 signal
  - Perimural T2 signal
  - Qualitative or quantitative DWI analysis
  - Luminal ulceration
  - Stricture

- Worsened lesion: Increase in any of the above imaging parameters including new internal penetrating disease.

- Unchanged lesion: Without worsening parameters of any of the above and not classified as an improved lesion.

Per patient response definition

- Response: All lesions improved.
- Non-response: Existing lesion(s) worsened, new lesion was detected and all other scenarios not meeting definition of response.

Sample data collection sheet for all 5 lesions per scan for the radiologist

MRN _____ Case Number _____

Patient Last Name _____ Date of study _____

Lesion	1	2	3	4	5
Location: 1 = Distal Ileum 2 = Proximal ileum 3 = Jejunum					
Length (record in cm)					
Mural thickness small bowel: • 0 = <3 mm • 1 = >3–5 mm • 2 = >5–7 mm • 3 = >7 mm					
Mural T2 Signal: • 0 = Equivalent to normal bowel wall •1 = Minor increase in signal •2 = Moderate increase in signal •3 = Marked increase in signal					
Peri-mural T2 signal: • 0 = Equivalent to normal mesentery • 1 = Increase in mesenteric signal but no fluid • 2 = Small fluid rim (≤2 mm) • 3 = Larger fluid rim (>2 mm)					
Small bowel ulcerations: • 0 = No • 1 = Yes					
Stricture (obstruction): • 0 = none, • 1 = yes without upstream dilation • 2 = upstream dilation 3- 4 cm • 3 = upstream dilation > 4 cm					
Presence of new sinus, simple fistula or complex fistulae, abscess or inflammatory mass • 0 = None • 1 = Yes					
Qualitative DWI analysis: 0 = No change 1 = Improved 2 = Worsened					
ADC value					
Radiologist impression: 1=improved 2=unchanged 3=worsened					

If 5 lesions on index, new inflammatory lesions appearing on f/u scans? 0 = no 1 = yes

Appendix B: Study visit with details of symptomatic, serological, endoscopic and radiological assessment at each visit.

Baseline visit

- Demographic Information
- Clinical assessment:
  - CDAI
  - Harvey Bradshaw index
  - PRO-2/3
  - EQ-5D
  - IBD Disability Index
- Physical examination:
  - Height/weight
  - Waist/hip ratio
- Blood tests
  - C-reactive protein
  - Albumin
  - Hematocrit
  - SOMAscan™: panel~1310 proteins €
- Stool Sample
- Endoscopic assessment
- Radiological assessment: MR enterography, see Appendix B

6±1 week visit

- Clinical assessment:
  - CDAI
  - Harvey Bradshaw index
  - PRO-2/3
  - EQ-5D
  - IBD Disability Index
- Blood tests
  - C-reactive protein
  - Albumin
  - Hematocrit
  - SOMAscan™: panel~1310 proteins €

16±2 week visit

- Clinical assessment:
  - CDAI
  - Harvey Bradshaw index
  - PRO-2/3
  - EQ-5D
  - IBD Disability Index
- Physical examination:
  - Weight
  - Waist/hip ratio
- Blood tests
  - C-reactive protein
  - Albumin
  - Hematocrit
- Stool Sample
- Radiological assessment: MR enterography, see Appendix B

Clinical visit§

- Clinical assessment:
  - CDAI
  - Harvey Bradshaw index
  - PRO-2/3
  - EQ-5D
  - IBD Disability Index
- Blood tests
  - C-reactive protein
  - Albumin
  - Hematocrit

52±4 week visit

- Clinical assessment:
  - CDAI
  - Harvey Bradshaw index
  - PRO-2/3
  - EQ-5D
  - IBD Disability Index
- Physical examination:
  - Weight
  - Waist/hip ratio
- Blood tests
  - C-reactive protein
  - Albumin
  - Hematocrit
- Endoscopic assessment: SES-CD
- Radiological assessment: MR enterography, see Appendix B
- Disease Progression Assessment

§ If dose escalated to q 4 weekly dosing due to lack of clinical response (CDAI-100 point decrease) at week 14. The clinical visit will coincide with trough measurement prior to 3rd cycle of vedolizumab after dose escalation.

€ SOMAscan™ measures ~1310 proteins including Tumor Necrosis Factor receptor superfamily, IL-6, IL-17A, IL-22, serum amyloid A-1 protein, chemokines, fibronectin, VCAM-1, platelet factor-4, CD40 ligand, creatine kinase M-type: creatine kinase B-type heterodimer.