Official Title: **Induction Of Response And Remission Of Vedolizumab Monotherapy Vs. Combination Therapy With Tacrolimus In Patients With Moderately To Severely Active Ulcerative Colitis**

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INDUCTION OF RESPONSE AND REMISSION OF VEDOLIZUMAB MONOTHERAPY VS. COMBINATION THERAPY WITH TACROLIMUS IN PATIENTS WITH MODERATELY TO SEVERELY ACTIVE ULCERATIVE COLITIS

I) Research question:

Does combination therapy of vedolizumab and tacrolimus increase the rate of clinical response and remission in patients with ulcerative colitis (UC) when compared to vedolizumab as monotherapy?

II) Background:

Anti-tumor necrosis factor (anti-TNF) agents have been approved by the FDA and proven to be efficacious in the induction and maintenance of both Crohn's disease (CD) and ulcerative colitis (UC). They have also significantly changed the prognosis and natural history of the disease[1]. Unfortunately, a significant number experience primary or secondary failure of therapy.

Natalizumab (NTZ), an alternative therapy for CD is a humanized monoclonal antibody against α4-β1 and α4-β7 integrin, which prevents migration of leukocytes into tissue. NTZ was shown to be more efficacious than placebo in inducing clinical response and remission in patients with CD[2]. Unfortunately, NTZ has been associated with an increased risk of developing progressive multifocal leukoencephalopathy (PML), limiting its use to patients with refractory disease.

More recently, a new drug with a mechanism similar to NTZ has been studied and approved for the treatment of patients with CD and UC. Vedolizumab is a humanized monoclonal antibody to α4-β7 integrin that modulates intestinal (but not brain) lymphocyte trafficking.

Phase III studies have shown that patients with UC who received vedolizumab had a higher rate of clinical response, clinical remission and mucosal healing when compared to placebo[3]. Nevertheless, while clinical response rate was almost 50%, the rate of clinical remission at 6 weeks was only 16.9. In comparison, in the ACT trials almost 40% of patients achieved remission at week 8[4]. The delayed onset of action of vedolizumab monotherapy in patients with UC may lead to a higher colectomy rate and limit the use of vedolizumab in patients with active disease who require rapid induction of remission. Corticosteroids are used as a bridging agent to rapidly induce remission. However, we commonly see steroid refractory or dependent disease and steroid intolerance. Furthermore, steroids have devastating side effects.

Tacrolimus inhibits the complexion of calcineurin with its respective cytoplasmic receptors cyclophilin and FK-binding protein 12 (FKBP-12), both of which regulate a calmodulin dependent-phosphatase. Tacrolimus has been found to be efficacious in

the treatment of patients with moderate to severe UC[5]. Unfortunately, because of the safety profile with long term use, the drug is mostly used as an induction agent.

While switching to vedolizumab from another drug that has not been efficacious or has lost effectiveness (or starting vedolizumab as a first agent) can be beneficious in the long term, patients need an induction agent in order to achieve remission in a short period of time. Tacrolimus is a widely used drug to prevent implant rejection after a transplant. Randomized controlled trials have shown that is highly effective with good response rates even after 2 weeks of therapy[5]. In order to avoid side effects, tacrolimus is usually used for a limited amount of time (12-14 weeks), which is sufficient time to induce remission of disease. Unfortunately, as other inflammatory bowel diseases, UC recurs and patients also require a maintenance therapy. While tacrolimus has been used with good results as a long term agent, the ideal scenario is to avoid its long term use as there is still a potential for side effects and a need for a very strict close monitoring [6]. This is why a long term maintenance agent is needed to keep the patient in remission. Until recently, no ideal agent was available for this purpose as while anti-tumor necrosis factor agents (infliximab and adalimumab) have been approved for ulcerative colitis, its combination with another agent that induces systemic immunosuppression (in this case, tacrolimus) could potentially increase the risk of infections and/or malignancies. Because vedolizumab is gut selective, does not affect the entire immune system and post-marketing studies have confirmed its safety profile[7]. This makes it a perfect combination agent to tacrolimus, theoretically decreasing the potential side effect while increasing its efficacy.

Our hypothesis is that the addition of tacrolimus as an induction agent to a standard regimen of vedolizumab increases the efficacy of the drug, decreasing the rate of need for colectomy and other complications while quickly improving the patients' quality of life without significantly increasing the risk of adverse events.

III) Study Objectives

The aim of this study is to assess if a combination therapy of tacrolimus and vedolizumab is superior to vedolizumab monotherapy for induction of remission in moderate to severe UC, and its effect on long and short-term outcomes including colectomy rate. Secondary aim of this study is to assess the safety of tacrolimus as an induction agent in patients with UC.

IV) Relevance of the study

Investigating concomitant agents to be used for a limited amount of time to rapidly induce remission may improve short and long-term outcomes, changing how we position vedolizumab in the treatment of UC and having it available as an equivalent (or potentially better) alternative to anti-TNFs. The use of an anti-TNF in combination with a calcineurin inhibitor is not feasible due to safety concerns. The exceptional

safety profile of vedolizumab and its gut selectivity makes it an ideal combination agent with tacrolimus. The proposed regimen combines the benefit of the rapid induction of remission effect that a limited course of tacrolimus has with the long term safety of vedolizumab, offering a considerable benefit over an anti-TNF.

V) Study Population

Inclusion criteria UC

1. Patients aged 18 to 65 years with a confirmed diagnosis of UC.
2. Patients with UC disease extent beyond 15 cm will be enrolled (must involve at least the sigmoid colon).
3. Diagnosis of UC established at least 6 months before enrollment or evidence of chronicity in colonic biopsies.
4. In female patients:
    - Post-menopausal for ≥1 year before screening, or
    - Surgically sterile, or
    - Agree to be on two contraceptive methods from the baseline visit through 4 weeks after discontinuing tacrolimus (or placebo), or
    - Completely abstain from heterosexual intercourse.
5. In male patients:
    - Agreement not to father a child through 4 weeks after discontinuing tacrolimus (through contraception or abstinence).
    - If contraception is used by the patient, he will need to agree to be on two contraceptive methods from the baseline visit through 4 weeks after discontinuing tacrolimus (or placebo).
6. Moderate to severe UC
    - Screening sigmoidoscopy with a Mayo endoscopic sub-score of 2 or 3, disease that extends beyond 15 cm from the anal verge; **and** one of the following:
        - Mayo Clinic partial UC score of 6 to 12 (Appendix 1).
        - Steroid dependent patients (Appendix 2).
        - Steroid intolerant (Appendix 2).
7. Patients are planned to start vedolizumab as part of their clinical care.
8. 5-aminosalicilates (oral or topical) are permitted as long as the dose is stable for at least 2 weeks before baseline
9. Patients with or without previous exposure to anti-TNF agents can be included. Patients with previous exposure to anti-TNF must be off infliximab for 8 weeks and off adalimumab for 4 weeks or have an undetectable infliximab or adalimumab serum drug level with or without anti-drug antibody.
10. Anti-diarrheals (eg, loperamide, diphenoxylate with atropine) for control of chronic diarrhea are not permitted in order to avoid confounding the evaluation of the patient. The patient must be off anti-diarrheal at the time of baseline visit.

11. The patient must have been off immunosuppressants (thiopurines or methotrexate) four weeks prior to baseline.
12. Patients with previous Clostridium Difficile infection can be included as long as they received a full course of therapy and have a negative Clostridium Difficile PCR test and are infection free for 60 days prior to screening.

Exclusion criteria UC

1. Positive stool test for parasites or stool culture for pathologic bacteria within 30 days prior to enrollment.
2. Evidence or history of Clostridium Difficile infection within 60 days prior to enrollment.
3. Active Cytomegalovirus (CMV) infection evidenced by a positive CMV PCR in serum and/or positive immunohistochemistry stain in colonic tissue.
4. Uncontrolled hypertension.
5. Chronic kidney disease (defined as a glomerular filtration rate < 60 mL/min, calculated using the Modification of Diet in Renal Disease (**MDRD**) formula)
6. Chronic liver disease.
7. A refractory electrolyte disorder (e.g. hypomagnesemia).
8. Persistent hypomagnesemia that does not respond to oral magnesium supplementation defined as a value <1.3 mEq/L in two separate readings, despite the administration of oral magnesium [10 meq of slow-release magnesium chloride three times per day for 48 hours] within a week of baseline.
9. Persistent hypophosphatemia defined as levels <2.2 mg/dL in two separate readings, 48 hours apart despite phosphate supplementation (sodium phosphate/potassium phosphate 500 mg up to three times daily for 48 hours) within a week of baseline.
10. Creatinine values of 1.5 mg/dL in 2 separate readings within a week of baseline.
11. Established diagnosis of diabetes mellitus.
12. Clinical or radiological evidence of megacolon.
13. Intestinal perforation, or abdominal abscess within 3 months prior to baseline.
14. Active clinically significant bacterial infection within 30 days of baseline.
15. Personal history of total or sub-total colectomy.
16. Current pregnancy or lactation.
17. Unstable or uncontrolled medical disorder.
18. Personal history of malignant neoplasm.
19. Inability to give informed consent.
20. History of alcohol or illicit drug abuse within 6 months of baseline visit.
21. Patient that have received any experimental drug within 6 months of baseline visit.
22. Patients with previous exposure to vedolizumab, cyclosporine or tacrolimus.

23. Personal history of congenital or acquired immunodeficiency (eg, common variable immunodeficiency, human immunodeficiency virus [HIV] infection, organ transplantation) excluding pharmacologic immunosuppressant.
24. Any of the following laboratory abnormalities during the screening period:
    a. Hemoglobin level <9 g/dL
    b. WBC count $<3 \times 10^9$/L
    c. Lymphocyte count $<0.5 \times 10^9$/L
    d. Platelet count $<100 \times 10^9$/L or $>1200 \times 10^9$/L
    e. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >2 × the upper limit of normal (ULN)
25. To avoid interactions, patients on medications that induce or inhibit the Cytochrome p450 family 3, subfamily A (CYP3A) will be excluded. CYP3A inducers and inhibitors are shown in Appendix 3.

VI) Study Setting and Procedures

Study setting

Medical College of Wisconsin (WI, USA). This center sees a large volume of patients with IBD, including UC, and has an excellent structure to perform clinical research in IBD patients. This institution actively participates in several clinical trials, including multi-site studies.

Study Procedures

Pre-enrollment assessment / Screening (standard of care and within 2 weeks of enrollment)

- The primary investigator will review the clinical, laboratory and endoscopic data for each candidate before enrollment.
- The patient will be consented for the study
- Urine Pregnancy test (women)
- Sigmoidoscopy will be done within 2 weeks of starting vedolizumab. Biopsies will be obtained for immunohistochemistry for staining for CMV.
- Extent of the disease should be documented, based on a full colonoscopy performed within the last 18 months.
- In patients who are not on a stable dose of corticosteroids or who recently initiated corticosteroids (changes in dose or started steroids less than two weeks of screening), the sigmoidoscopy should be done 2-3 days prior to starting the investigational drugs to provide a more accurate assessment of disease severity at baseline entry into the trial. Both males and females will need to use two effective methods of contraception.
- Demographics:
    - Age
    - Gender
    - Race
    - Ethnicity
    - Body Mass index recorded at admission.
- Comorbidities
- Prior exposure to medications
    - Prednisone
    - 5-Aminosalicylates
    - Use of Azathioprine /6MP (and phenotype).
    - Use of Methotrexate
    - Use of anti-tumor necrosis factor agents
- Exposures
    - Smoking status, classified as follow:
        - Current (at the time of screening)

    - Remote
    - Never
- Partial UC Mayo score (Appendix 3)
- Blood tests including biochemical and serum inflammatory markers (all studies are standard of care. They are routine labs ordered in the clinic):
    - Complete blood count
    - Electrolytes
        - Sodium
        - Potassium
        - Magnesium
        - Chloride
        - Phosphorus
    - Total protein and albumin
    - Liver enzymes
        - Aspartate transaminase (AST)
        - Alanine transaminase (ALT)
        - Alkaline phosphatase (ALP)
        - Total bilirubin
    - Kidney function tests
        - Creatinine
        - Blood urea nitrogen (BUN)
    - C-Reactive protein (CRP)
    - Infliximab levels and antibodies when available
    - Adalimumab levels and antibodies when available.
- Stool studies
    - Stool culture and ova and parasite
    - Clostridium Difficile PCR

Baseline assessment (Week 0) (all studies are standard of care. They are routine labs ordered in the clinic)**:**

- Vital signs
- Medications
- CBC
- Comprehensive Metabolic Panel (liver enzymes, kidney function tests and electrolytes)
- Phosphorus
- Magnesium
- Total Cholesterol
- Urine Pregnancy test (women)
- Stool sample for fecal calprotectin
- C-reactive protein (CRP)

- Partial and total UC Mayo score (Appendix 3) (endoscopic score calculated from screening sigmoidoscopy)
- Ulcerative Colitis Disease Activity Index (UCDAI) (Appendix 4)
- Begin vedolizumab (standard of care)
- Begin study drug

Week 2:

- Vital signs
- Urine Pregnancy test (women)
- Medications
- Comprehensive Metabolic Panel
- Magnesium
- Phosphorus
- Tacrolimus levels
- UC partial Mayo score
- UCDAI
- Adverse events
- Study drug adjustment; maintenance phase starts upon site receipt of levels from this visit

Week 6: (week 6 is a standard of care visit and CBC, CMP, and CRP at those visits are standard of care)

- Sigmoidoscopy
- UC endoscopic Mayo score (if applicable)
- Vital signs
- Medications
- Urine Pregnancy test (women)
- Fecal calprotectin
- CBC
- CRP
- Comprehensive Metabolic Panel
- Magnesium
- Phosphorus
- Tacrolimus levels
- UC partial Mayo score
- UCDAI
- Adverse events

Week 8:

- Vital signs
- Medications
- Comprehensive Metabolic Panel
- Magnesium

- Phosphorus
- Tacrolimus levels
- UC partial Mayo score
- UCDAI
- Adverse events

Week 12 (phone call):

- Stop study medication
- Adverse events

Week 14: (week 14 is a standard of care visit and CBC, CMP, and CRP at those visits are standard of care)

- Vital signs
- Medications
- Urine Pregnancy test (women)
- Fecal calprotectin
- CBC
- CRP
- Comprehensive Metabolic Panel
- Magnesium
- Phosphorus
- Tacrolimus levels
- UC partial Mayo score
- UCDAI
- Adverse events

Week 30 (phone call):

- Adverse events (including colectomy and c diff status)
- Concomitant medications

When needed:

Laboratories, tacrolimus levels, vital signs and other variables will be monitored in between the study visits as needed. For example, always after a change in tacrolimus dose, a tacrolimus level, magnesium, phosphorus and comprehensive metabolic panel will be performed.

Weekly phone call will be made to patients in order to assess for any new symptoms, including but not limited to:

- Nausea/dizziness
- Headaches
- Seizures
- Tremors
- Hearing loss
- Skin rash and/or pruritus
- Respiratory symptoms including cough, shortness of breath and wheezing
- Chest pain
- Urinary symptoms
- Decrease in urinary output
- Arthralgias and/or myalgias
- Jaundice

Of note, the investigator performing dose adjustments for tacrolimus will not only receive laboratory values, but also a report of side effects. If he/she deems necessary, the patient will be called for a hospital visit to further assess symptoms independently of the decision made by the primary investigator.

During the weekly call, the patient will also be asked regarding the start of any new drug and over the counter medication including herbs.

Study arms

- Patients who meet inclusion criteria will be randomized 1:1 to receive:
  I) Treatment arm: vedolizumab at standard regimen with concomitant induction treatment of tacrolimus (starting 0.05 mg per Kg twice daily).
  II) Placebo arm: vedolizumab at standard regimen with placebo.

- Randomization will be concealed. When a patient is enrolled, the pharmacy at each clinical center will be contacted and randomization will be done in blocks of 4. The patient, treating physician and investigator adjusting the dose of tacrolimus will be blinded to the therapy assignment.

- The study will be blinded and neither the physician nor the patient will know to which arm the patients were randomly assigned.

- All patients will receive intravenous vedolizumab 300 mg, at week 0 (baseline), 2 and 6 followed by the same dose every 8 weeks. The drug will be given as per standard of care.

- Tacrolimus (or placebo) will be started the day of the first vedolizumab infusion (+/- 24 hours).

- Tacrolimus and placebo will be provided free of charge for the patient. Patients requiring magnesium and or phosphorus supplementation will also receive oral supplement free of charge.

- 1 mg tacrolimus tabs will be provided to patients and adjustments will be performed over the phone. The target tacrolimus trough levels will be 10 to 15 ng/ml in the first two weeks but can be reduced to 5-10 if the patient has side effect including tremors, low magnesium, headaches or abnormal kidney tests. After 14 days, the goal will be 5-10 ng/ml until the patient completes 12 weeks of tacrolimus (or placebo).

- Both trial arms will come for lab work. The laboratory will communicate the results of tacrolimus levels, phosphorus, and magnesium kidney, liver tests, and electrolyte levels (comprehensive metabolic panel,), fecal calprotectin and CRP when applicable, to the study coordinator. The labs reported will depend on the visit. Because of the time some laboratories need to be processed, a 24 hour window will be permitted for reporting.

- Tacrolimus levels will be tested 2 days (+/- 24 hours) after starting the drug and then 2 days (+/- 24 hours) after every dose adjustment, or as per discretion of the investigator performing dose adjustment. Then, patients will be tested once a

week (+/- 24 hours) unless a change is made in dose, where a 2-day test will be performed.

- Both study arms will have blood drawn for tacrolimus serum trough level. The un-blinded research coordinator will submit the blood samples of the treatment group and control group for determination of tacrolimus level determined at the local lab. The research coordinator will then determine a hypothetical tacrolimus trough level for patients in the control group, using a tacrolimus dosing model, based on a steady-state infusion model, using clinical factors (age, ethnicity [African American or non-African-American), use of calcium channel blockers)[8]. The model will be programed in an Excel sheet and run by the coordinator.

- The research coordinator will provide the investigator adjusting the tacrolimus level with the lab work results and relevant clinical information including blood pressure readings and a complete report of side effects (infection, hypertension, headaches, tremor, paresthesia, insomnia, diarrhea, nausea, vomiting, hyperkalemia, and hyperglycemia). The investigator will adjust the dose of tacrolimus based on these parameters. The investigator performing the adjustments is also responsible for determining the need for supplementation of magnesium or phosphorus.

- The study site will have one investigator with experience in the management of tacrolimus and not involved in the care of these patients adjusting the tacrolimus dose based on the information above, which will be supplied by the local research coordinator. A backup investigator for dose adjustments will also be made available.

- Because of the studies have shown that higher levels are associated with a much higher rate of response, the target tacrolimus level in the first 14 days (10–15 ng/mL) then switch to maintain a level of 5–10 ng/mL for the rest of the 12 weeks.

- As a general rule, tacrolimus dose will be adjusted as follows in the first 14 days:
    - Tacrolimus whole blood concentration <5 ng/mL, 35% dose increase;
    - Tacrolimus whole blood concentration 5–9 ng/mL, 20% dose increase
    - Tacrolimus whole blood concentration 10–15 ng/mL, no change
    - Tacrolimus whole blood concentration 16–20 ng/mL, 40% dose decrease
    - Tacrolimus whole blood concentration 21–30 ng/ mL, hold the next dose of tacrolimus and then continue with a 60% dose decrease;
    - Tacrolimus whole blood concentration >30 ng/mL, hold tacrolimus until the next measurement (48 hours)

- If serum creatinine values increase by 30% of the baseline, dose will be decreased by 35%.

- In the maintenance phase, levels will can be maintained between 5 and 10 ng/ml if there are any side effects while on higher serum concentrations using the following strategy:
  - Tacrolimus whole blood concentration <5 ng/mL, 25% dose increase;
  - Tacrolimus whole blood concentration 5–10 ng/mL, no change
  - Tacrolimus whole blood concentration 11–15 ng/mL, 25% dose decrease
  - Tacrolimus whole blood concentration 16–20 ng/mL, hold the next dose of tacrolimus and then continue with a 60% dose decrease;
  - Tacrolimus whole blood concentration >20, hold tacrolimus until the next measurement (48 hrs.)
  - If serum creatinine values increase by 30% of the baseline, dose will be decreased by 35%.

- Tacrolimus will be discontinued at week 12 (levels will still be measured up to week 14).

Concomitant Medications

- Steroids will be tapered immediately upon starting vedolizumab and tacrolimus. From 40 mg to 30 mg at week 1, then to 20 mg at week 2, to 15 mg at week 3, to 10 mg at week 4 and 5 mg at week 5. Patients should be off steroids by **week 6**. This does not apply to patients not receiving steroids at the time of enrollment.
- CYP3A inducers and inhibitors should not be taken. (Appendix 3)
- The investigator performing study drug adjustments is also responsible for determining the need for supplementation. Complete stopping criteria are found in section IX, guidelines as follows
  - Persistent hypophosphatemia defined as levels <2.2 mg/dL in two separate readings, 48 hours apart despite phosphate supplementation (sodium phosphate/potassium phosphate 500 mg up to three times daily).
  - Persistent hypomagnesemia that does not respond to oral magnesium supplementation defined as a value <1.3 mEq/L in 2 separate readings in two separate readings, 48 hours apart and while on oral magnesium supplementation [10 meq of slow-release magnesium chloride three times per day].

Vaccination considerations

Non-live vaccines including parenteral influenza vaccine, hepatitis A and B and pneumonia vaccines can be received. The use of live vaccines should be avoided during treatment with tacrolimus; examples include (not limited to) the following:

intranasal influenza, measles, mumps, rubella, oral polio, BCG, yellow fever, varicella, and TY21a typhoid vaccines.

Duration of the study

- The patient will be considered to complete the study at week 14 during the visit. No intervention will be made after week 14 except for monitoring. Nevertheless, will continue to be monitored as per standard of care and at week 30 assessment will also be performed including clinical disease activity, need for colectomy, UC medications.

VII) Statistical analysis

Sample size

This study will include 10 patients per arm (for a total of 20 patients). This is a pilot study to assess different response rates. Sample size was calculated assuming that 80% of patients will achieve the primary outcome at week 6 in the tacrolimus and vedolizumab group and 20% will achieve the outcome in placebo and vedolizumab group.

Analyses

Descriptive statistics (mean or median ± standard deviation for continuous variables and count/percentages for categorical variables) will summarize baseline characteristics (demographic and clinical) in aggregate as well as across arms.

Primary outcome analyses will employ an independent two-sample test for binomial proportions to compare (a) the rate(s) of remission at week 6 and the secondary outcomes. Each of these outcomes will be analyzed separately and we will not adjust for multiple tests as each outcome carries equal weight. Analyses will use the intent-to-treat dataset, but sensitivity analyses will be performed on the per protocol dataset. Additional analyses will employ a log-rank test to compare time-to-remission across arms. Analyses will explore relationship between baseline characteristics (age, gender, CRP, smoking history, etc.) and outcome(s) in order to evaluate potential confounding or interaction effects via a series of logistic regression models.

Secondary outcome analyses will further utilize the independent two-sample test for binomial proportions for categorical variables, and the independent two-sample t-test for continuous variables (CRP, UC Mayo score, IBDQ, etc.) across arms at relevant study time points. Nonparametric methods and/or transformation of variables may be used as appropriate (i.e., when statistical assumptions of parametric tests on untransformed variables are not met or questionable).

VIII) Risks

Safety

Safety assessments included vital signs, physical examinations and laboratory analysis during each visit. Patients who discontinue the treatment will continue to be followed as per protocol. Follow-up will be discontinued in patients who withdraw informed consent or are lost for follow-up.

- For adverse reactions: the following items will be analyzed:
  - The incidence and type of any clinical adverse reaction.
  - The incidence and type of known clinical adverse reactions
  - The incidence and type of any non-clinical adverse reaction
  - The incidence and type of known non- clinical adverse reactions.

Both vedolizumab and tacrolimus have warnings in the label for the risk of progressive multifocal leukoencephalopathy (PML). There have been some potential reports of PML associated with immunosuppressant drugs including tacrolimus. There is also a potential risk with vedolizumab, even though to the date this protocol was written, no cases have been reported. Patients will be monitored for any new onset, or worsening, of neurological signs and symptoms, including the typical signs and symptoms associated with PML that are diverse, progress over days to weeks, and include progressive weakness on one side of the body or clumsiness of limbs, disturbance of vision, and changes in thinking, memory, and orientation leading to confusion and personality changes. Since the progression of deficits usually leads to death or severe disability over weeks or months, the study drug(s) will be stopped immediately if PML is suspected, and the patient will be referred to a neurologist**Patient confidentiality:**

To protect patient confidentiality, databases with personal identifiers will be stored on secure servers with restricted and audited access.

IX) Regulatory

Institutional review board (IRB) approval will be acquired from the Medical College of Wisconsin(MCW) prior to commencement of study activities. Guidelines for Good Clinical Practice (GCP), as described in ICH Guideline E6, will be followed. The investigator will conduct this study in accordance with applicable national, state, and local laws of pertinent regulatory authorities, and FDA's regulations 21 CFR 312, part 50, 54, and 56.

The Investigator will be responsible for regulatory compliance and ongoing timely reporting of annual reports, protocol deviations and serious adverse events to the respective IRB. The Principal Investigator at Medical College of Wisconsin will comply with any necessary reporting to the FDA and study sponsor within the timelines suggested by FDA.

This study has the benefit of testing a drug that has been approved by the federal drug agency (FDA) for the treatment of UC. Tacrolimus, while not FDA approved for the treatment of UC, was shown to be efficacious in prior clinical trials[5].

Quality Control and Data Management

A data safety monitoring board will be assigned. The committee members will include participants who are not involved in the study and will overlook the safety of the study agent. For that purpose, un-blinded safety data will be available to the committee.

The investigator is responsible for ongoing review of the data quality and compliance to protocol. The PI will conduct ongoing quality checks or assign study team members for ongoing reviews to ensure data integrity and accuracy. Any unexpected deviations will be corrected and reported in a timely manner per IRB requirements.

X) Outcomes

Primary Outcome

- Steroid-free clinical response at week 6 after starting vedolizumab, defined as a reduction in the Mayo Clinic score of at least 3 points and a decrease of at least 30% from the baseline score with a decrease of at least 1 point on the rectal bleeding subscale or an absolute rectal bleeding score of 0 or 1 while off steroids. Oral budesonide will be considered steroid use. If the patient is steroid-dependent by week 6 (Appendix 1), will be considered to have failed the primary outcome.

Secondary Outcomes

- Steroid-free clinical response at week 14 after starting vedolizumab.
- Steroid-free Clinical remission at week 6 defined as either:
  - Mayo clinic score <3 with:
    - Stool Frequency sub-score <2
    - Rectal Bleeding sub-score <2
    - Endoscopy sub-score = 0 or 1 (on Mayo Score)
- Steroid-free Clinical remission at week 14 defined as:
  - Mayo clinic score <3 with:
    - Stool Frequency sub-score <2
    - Rectal Bleeding sub-score <2
- Change in CRP at week 6 and 14.
- Change in fecal calprotectin at week 14.
- Endoscopic remission evaluated between weeks 12 and 16 defined as an endoscopic Mayo score of 0 or 1.

- Colectomy-free survival at week 14 and 30.
- Clostridium Difficile infection.
- Any infection including bacterial, viral or fungal as per investigator.
- Kidney injury defined as a 30% increase in creatinine.
- Other side effects (tremor, headaches, etc.)

Exploratory Outcomes

- Starting another therapy for UC by week 30.
- Durable clinical remission measured at week 30 and defined as:
  - Continuation of vedolizumab
  - Colectomy-free
  - Steroids-free
  - No need for salvage therapy with cyclosporine/tacrolimus

- Durable endoscopic remission measured at week 30 and defined as:
  - Continuation of vedolizumab
  - Colectomy-free
  - Endoscopic remission (Mayo endoscopic score ≤1).
  - Steroids-free
  - No need for salvage therapy with cyclosporine/tacrolimus

Consideration about outcomes

- Recurrence of symptoms with a negative C. Diff PCR stool test while off steroids will be considered as a failure of therapy.
- The need for a course of steroids, inability to taper off steroids due to symptoms or a colectomy due to active disease will also be considered a treatment failure. The decision to change this management will be perform per standard of care by the patient's primary gastroenterologist (who will be blinded to the drug/placebo assignment).

Follow-up and assessment of outcomes (Appendix 5)

- Patients will be evaluated at baseline, and then at week 0, 2, 6, 8 and 14.
- A long term follow-up (week 30) will also be done.
- Visits at week 6 and 14 will be done as part of the standard of care. The research coordinator will extract laboratory and clinical information from that visit to populate the database and communicate it to the external investigator adjusting doses.
- Additional laboratory visits may be needed. An average of 32 total lab assessment are estimated per patient.

XI) Safety and Data Monitoring

The Investigator will monitor the conduct of protocol on a continuous basis for expected and unexpected patient's safety related issues. The PI will appoint a Data Safety Monitoring Board. Members of this board will be independent of study team and will overlook the safety of the study agent. For that purpose, un-blinded safety data will be available to the committee.

Annual reports will be submitted to the IRB. Severe or unanticipated adverse events will be reported immediately to the IRB, DSMB and FDA within timelines suggested by each of these regulatory bodies.

Risks of the Study Medication:

Tacrolimus (formerly known as FK506) was described in the treatment of various immune-mediated diseases including Crohn's disease, rheumatoid arthritis, uveitis, nephrotic syndrome, psoriasis, and lupus membranous nephritis, among others. It is also widely used to prevent post-transplant allograft rejection. While the drug is associated with several side effects, these are usually seen in transplant patients. Previously described potential side effects by system:

- Nephrotoxicity: patients may develop a raise in serum creatinine, which is usually reversible when decreasing or discontinuing the drug. Chronic renal damage is almost never seen in patients on short courses of therapy. In a study done in patients with UC for 29 weeks, none developed elevation of the creatinine. In a randomized controlled trial looking at the efficacy of tacrolimus in UC, the authors reported that 4-5% of patients receiving the drug developed an elevation of creatinine >30% of the baseline. No one developed severe kidney injury. In our study, renal function will be monitored carefully, and doses will be decreased if there is an increase of 30% or more of the baseline creatinine or discontinued if the creatinine reaches 1.5 mg/dL.
- Hypertension: tacrolimus may raise the blood pressure. Patients with a known diagnosis of hypertension at baseline, will not be included. Blood pressure will be monitored throughout the study and on every visit. If blood pressure reaches 140/90 mmHg (sustained, appropriately measured during 2 consecutive visits), the drug will be adjusted. If the blood pressure at any time is higher than >160/100 mmHg, the drug will be decreased without waiting for a second reading. If despite the dose reduction, blood pressure continues to be >160/100 and there is no alternative plausible etiology, the drug will be discontinued due to side effects. A reading of >160/100 was set as a limit as it is considered "moderate hypertension"[9].
- Neurological side effects. Tremors are a common side effects of tacrolimus, and are usually transient, even without dose adjustment. Rarely, patients may develop headaches. If patients in our study develop headaches or tremors, the severity of the symptoms will be assessed with an appropriate

dose reduction. Studies have shown mild tremors in up to 20% of patients and headaches in about 5%[5]. Seizures were previously reported, but they are usually seen with IV administration of the drug and patients with previous history of seizures will be excluded from the study.

- Metabolic abnormalities. Tacrolimus may cause hyperglycemia. Patients with diabetes mellitus will be excluded from our study, and glucose levels will be measured every follow-up. No severe cases of hyperglycemia have been reported.
- Rare side effects: anorexia, nausea, vomiting, diarrhea, and abdominal discomfort. Increased liver enzymes are reversible with dose reduction or drug discontinuation. Patients with elevation of AST, ALT or ALP more than two times the upper limit of normal will be excluded.
- Infections: there is a theoretical risk of infections in patients on immunosuppressants. If the patient develops any infection that warrants admission, the drug will be discontinued until the infection is cleared.
- Reproductive side effects: tacrolimus is a FDA category C drug and while it could be used in pregnant patients in some cases, for the purpose of this study, pregnant patients or those planning for pregnancy in 6 months following enrollment will be excluded.

Any of the above mentioned side effect for event grade 3 or more per CTCAE guidelines must be reported to the Sponsor-Investigator within 24 hours of learning of the event. Any serious adverse events (an adverse event that (1) results in death, (2) is life threatening,
(3) requires inpatient hospitalization or prolongation of existing hospitalization, (4) results in persistent or significant disability/incapacity, (5) results in a congenital anomaly/birth defect, or (6) is an important medical event that jeopardizes the subject or requires medical intervention to prevent one of outcomes listed above) must be reported to the Sponsor-investigator within 24 hours of study staff learning the event.
The Sponsor-investigator will further evaluate the event and its seriousness and suggest further course of actions.

Reportable events must be submitted to regulatory agencies as mentioned in the Regulatory section below.

Individual stopping criteria:

- Development of a lymphoma or any malignancy, independently if it may any relation with the study drug.
- Development of any infection that meets criteria for a Grade 3, Common Terminology Criteria for Adverse Events v4.0 (CTCAE).

- Persistent hyperkalemia defined as values ≥5.5 mEq/L in two separate readings, 48 hours apart.
- Persistent hypomagnesemia that does not respond to oral magnesium supplementation defined as a value <1.3 mEq/L in 2 separate readings in two separate readings, 48 hours apart and while on oral magnesium supplementation [10 meq of slow-release magnesium chloride three times per day].
- Persistent hypophosphatemia defined as levels <2.2 mg/dL in two separate readings, 48 hours apart despite phosphate supplementation (sodium phosphate/potassium phosphate 500 mg up to three times daily).
- Creatinine values of 1.5 mg/dL in 2 separate readings.
- Development of acute kidney injury defined as per Acute Kidney Injury Network (AKIN) diagnostic criteria within 48 hours of starting the drug:
  - Absolute increase in the serum creatinine concentration of ≥0.3 mg/dL from baseline
  - Percentage increase in the serum creatinine concentration of ≥50 percent, or
  - Oliguria of < 0.5 mL/kg per hour for more than six hours.
- Any hypersensitivity reaction attributed to the tacrolimus as per treating physician.
- Blood pressure at any time >160/100 mmHg will trigger a tacrolimus dose reduction by 35%. If in a second reading, it persists >160/100 mmHg, tacrolimus will be discontinued.
- Development of elevated liver enzymes (AST/ALT) 3 times higher than the upper limit of normal and/or the development of a bilirubin ≥1.9 mg/dL without any other potential explanation (starting another pharmacologic therapy, new-onset hepatitis infection or other causes including alcohol intake). If there is no evident etiology is found, the drug will be stopped and liver enzymes followed twice weekly until they normalize.
- Development of a Grade 3 adverse event attributed to the use of tacrolimus defined as severe or medically significant but not immediately life-threatening adverse event including hospitalization or prolongation of hospitalization; disabling; limiting self care activities of daily living. Based on known potential side effects of tacrolimus some of the events that can trigger discontinuation of the drug in the patient include:
  - Seizures
  - Severe infection requiring admission to a hospital
  - Hypersensitivity reaction
  - Any CTCAE grade 3 effect that has any potential association with the use of tacrolimus as described in the current FDA label.
- Development of pure red cell aplasia: while is a potential complication, we also need to consider patients with moderate to severe ulcerative colitis present almost universally with anemia and present with loss of blood through the stool. Because of this, the development of severe anemia

(hemoglobin <7 gr/dL) that does not respond to iron supplementation and with no other potential cause will be considered pure red cell aplasia.
- If the patient presents with classic symptoms of hyperglycemia (thirst, polyuria, weight loss, blurry vision) and has a random blood glucose value of 200 mg/dL and off steroids, the study drug will be discontinued.
- If a random glucose measurement is ≥200 mg/dL, but the patient has no symptoms, he/she will be monitored with glucose levels weekly and the study drug will be discontinued if the level reaches ≥200 mg/dL in two more occasions, tacrolimus/placebo will be discontinued
- In the event of starting any new therapy or required a colectomy for the ulcerative colitis, the patient should be discontinued from the study and will be considered to fail to achieve the study outcome.
- Development of new or worsening neurological symptoms will prompt immediate evaluation. If there is even a minimal clinical suspicious for PML, both drugs (tacrolimus and vedolizumab) will be discontinued.
- The patient will be able to withdraw at any time.

Termination of the study

- If two or more patients develop the same Grade 3 CTCAE v4.0 adverse event attributed to the use of tacrolimus defined as severe or medically significant but not immediately life-threatening adverse event including hospitalization or prolongation of hospitalization; disabling; limiting self-care activities of daily living.
- Any Grade 4 CTCAE v4.0 with potential relationship with the use of tacrolimus.

Assessment and monitoring adverse events

- As detailed in the "Study Procedures" section above, adverse events will be monitored with each visit and weekly trough a phone call to the patient.

- Any side effect for event grade 3 or more per CTCAE guidelines will be reported to the Investigator within 24 hours of learning of the event. Any serious adverse events (an adverse event that (1) results in death, (2) is life threatening, (3) requires inpatient hospitalization or prolongation of existing hospitalization, (4) results in persistent or significant disability/incapacity, (5) results in a congenital anomaly/birth defect, or (6) is an important medical event that jeopardizes the subject or requires medical intervention to prevent one of outcomes listed above) will be reported to the investigator within 24 hours of study staff learning the event. The investigator will further evaluate the event and its seriousness and suggest further course of actions. Side effect will also be communicated to the sponsor.

- If the patient develops an infection (any but Clostridium Difficile) that meets a grade 3 or more per CTCAE, the drug will be discontinued. For infections that meet grade 1 or 2 criteria, tacrolimus/placebo will be held until a full course of antibiotics are given. Then, the drug/placebo will be resumed at the same dose the patient was receiving. If the same infection recurs, tacrolimus/placebo will be discontinued.

- Because of how common Clostridium Difficile can develop in patients with UC and because is usually seen with active disease, if the patient develops worsening symptoms and tests positive, treatment will be started as per standard of care. If the infections warrants admission (as per the patient's primary gastroenterologist), then tacrolimus/placebo will be held until two weeks of therapy are completed.

- An assessment of attribution of adverse events will be performed and each classified as below based on several factors including known adverse event of the drug, temporal relationship to the initiation of the drug and serum drug level and clinical assessment.
  - Definite (clearly related to)
  - Probably (likely related to)
  - Possible (may be related to)
  - Unlikely (doubtfully related to)
  - Unrelated (clearly not related to)

- All the adverse events noted during the study should be followed until resolution or return to baseline levels.

Appendix 1: Mayo UC Score

Stool frequency

0 = Normal number of stools for this patient

1 = 1–2 stools more than normal

2 = 3–4 stools more than normal

3 = 5 or more stools more than normal

Rectal bleeding

0 = No blood seen

1 = Streaks of blood with stool less than half the time

2 = Obvious blood with stool most of the time

3 = Blood alone passed

Mucosal appearance*

0 = Normal or inactive disease

1 = Mild disease (erythema, decreased vascular pattern, mild friability)

2 = Moderate disease (marked erythema, absent vascular pattern, friability, erosions)

3 = Severe disease (spontaneous bleeding, ulceration)

Physician's global assessment

0 = Normal

1 = Mild disease

2 = Moderate disease

3 = Severe disease

(*) Partial UC Mayo score excludes the "mucosal appearance item".

Appendix 2: Definition of steroid dependent or intolerance

- One or more failed attempt(s) to taper corticosteroids to/below a dose equivalent to prednisone 10 mg daily orally on 2 separate occasions.

- History of intolerance of corticosteroids (including, but not limited to Cushing's syndrome, osteopenia/osteoporosis, hyperglycemia, insomnia, and infection).

Appendix 3: CYP3A inducers and inhibitors

CYP3A inducers

Bexarotene

Bosentan

Carbamazepine

Dabrafenib

Dexamethasone

Efavirenz

Enzalutamide

Eslicarbazepine

Etravirine

Fosphenytoin

Lumacaftor

Mitotane

Modafinil

Nafcillin

Phenobarbital

Phenytoin

Primidone

Rifabutin

Rifampin (rifampicin)

Rifapentine

St. John's wort

CYP3A inhibitors

Amiodarone

Aprepitant

Atazanavir

Boceprevir

Ceritinib

Clarithromycin

Cobicistat and cobicistat containing coformulations

Conivaptan

Cyclosporine

Darunavir

Diltiazem

Dronedarone

Erythromycin

Fluconazole

Fosamprenavir

Grapefruit juice (food)

Idelalisib

Imatiib

Indinavir

Isavuconazole

Itraconazole

Ketoconazole

Lopinavir

Mifepristone

Nefazodone

Nelfinavir

Netupitant

Nilotinib

Ombitasvir-paritaprevir-ritonavir

Ombitasvir-paritaprevir-ritonavir plus dasabuvir

Posaconazole

Ritonavir and ritonavir containing coformulations

Saquinavir

Schisandra

Telaprevir

Telithromycin

Verapamil

Voriconazole

Appendix 4: Ulcerative Colitis Disease Activity Index (UCDAI)

Stool frequency

0 = Normal number of stools for this patient

1 = 1–2 stools/day > normal

2 = 3–4 stools/day > normal

3 = >5 stools/day > normal

Rectal bleeding

0 = No blood seen

1 = Streaks of blood

2 = Obvious blood

3 = Mostly blood

Mucosal appearance*

0 = Normal or inactive disease

1 = Mild friability

2 = Moderate friability

3 = Exudation, spontaneous bleeding

Physician's global assessment

0 = Normal

1 = Mild disease

2 = Moderate disease

3 = Severe disease

Maximum Score= 12

Appendix 5: Schedule of Events

Schedule of events	Pre-treatment			Treatment					Follow Up
	Screening	Week 0	Week 2	Week 6	Week 8	Week 12	Week 14	When needed [1]	Week 30
Informed Consent	✔								
Begin tacrolimus		✔							
Begin vedolizumab		✔							
Stop tacrolimus						✔			
Prior medications	✔								
Concomitant medications	✔		✔	✔	✔		✔		✔
Demographics	✔								
Smoking status	✔						✔		
Medical/surgical history	✔								
Vitals signs	✔	✔	✔	✔	✔		✔	✔	✔[2]
Body mass (Kgs)	✔	✔							
Urine pregnancy test (women)	✔	✔	✔	✔			✔		
Adverse events assessment			✔	✔	✔	✔	✔	✔	✔

Mayo partial score	✔	✔	✔	✔	✔		✔		✔[2]
Colonoscopy or sigmoidoscopy	✔			✔				✔	✔[2]
Endoscopic Mayo UC score	✔			✔					✔[2]
UCDAI	✔	✔	✔	✔	✔		✔		✔[2]
CRP	✔	✔		✔			✔		✔[2]
C. diff / stool culture	✔								
Fecal calprotectin		✔		✔			✔		✔[2]
Comprehensive Metabolic panel	✔	✔	✔	✔	✔		✔	✔	✔[2]
Serum magnesium	✔	✔	✔	✔	✔		✔	✔	
Serum phosphorus	✔	✔	✔	✔	✔		✔	✔	
Tacrolimus level		✔	✔	✔	✔		✔	✔	
Complete blood count	✔	✔		✔			✔		✔[2]
Development of Clostridium Difficile infection?			✔	✔	✔		✔	✔	✔
Discontinuation of therapy?			✔	✔	✔		✔	✔	✔
Assessment of Colectomy			✔	✔	✔		✔		✔

(1) “when needed” lab draws will be done to follow-up labs and adjust the drug if needed. They will be performed before the fourth or fifth dose after a change of tacrolimus dose. Therapeutic drug monitoring: patients will be tested for tacrolimus and serum electrolytes (Na, K, Cl, phosphorus, creatinine, blood urea, magnesium)
(2) Only if performed as a standard of care.

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