Study Protocol v.1.0, July 2021

Submitted to Institutional Ethics Committee, Dayanand Medical College and Hospital, Ludhiana

Title of the study: Tofacitinib as an add-on therapy to corticosteroids in acute severe ulcerative colitis: A randomized controlled trial

Intoduction

Ulcerative colitis (UC) is a chronic relapsing and remitting immune-mediated inflammatory condition of the large intestine that is frequently associated with inflammation of the rectum but often extends proximally to involve additional areas of the colon.

Ulcerative colitis is characterized by a relapsing and remitting course and although the majority of patients tend to have a mild to moderate disease course, approximately 15–25% of patients with UC will experience at least one episode of severe flare of their disease and 10–20% will present with acute severe ulcerative colitis (ASUC) at diagnosis. ASUC requires prompt hospitalization and intensive care. The diagnosis of ASUC is based on the Truelove and Witts criteria described in 1955, which combines frequency of bloody stools (⩾6 per day) with at least one marker of systemic toxicity: pulse rate >90 per minute, temperature >37.8°C, hemoglobin <10.5 g/dl and/or an ESR >30 mm/h.

Intravenous steroids are the mainstay of therapy, but 30–40% of patients will fail to respond to steroids and require progression to second-line therapy [infliximab (IFX) or cyclosporine (CsA)]. These second-line therapies are associated with a significant risk for adverse events, and colectomy carries a 5% postoperative mortality risk when done emergently in the hospital setting. Despite improvements in management, ASUC is associated with a 20% risk for colectomy on first admission and this risk rises to 40% after two admissions. Moreover, there remains a 1% mortality associated with severe flares of UC. Therefore, a large therapeutic gap remains and strategies are needed to improve the responsiveness to corticosteroids.

Tofacitinib, a JAK-STAT (Janus kinase-signal transducers and activators of transcription) inhibitor and an anti-inflammatory drug, is a new addition to the treatment modalities for UC. Janus kinases are intracellular tyrosine kinases which mediate the signal transduction of various cytokines involved in the inflammatory reactions in IBD, like IL- 6, IL-7, Il-10, IL-12, IFN α, IFNβ 2, 4, 7, 9 etc. The advantages of JAK-STAT inhibitors over monoclonal antibodies include, the oral administration, predictable pharmacokinetics with reduced plasma half-life, rapid onset of action, quick clearance, lack of

immunogenicity and an intracellular target. Additionally, tofacitinib has a broader spectrum whereas biologics are generally selective for a cytokine or integrin.

It has been postulated that apart from the genetic predisposition to corticosteroid non-responsiveness, a higher expression of pro-inflammatory cytokines (such as interleukin [IL]-6, IL-8, etc.), is associated with corticosteroid resistance in patients with UC. In vitro studies have also shown IL-2 to reduce the nuclear translocation of the glucocorticoid receptor via JAK1 and JAK3 mediated phosphorylation of STAT5, thereby contributing to corticosteroid resistance. Tofacitinib, via STAT inhibition has the potential to block the signalling downstream of the IL-2 receptor, and restore the corticosteroid sensitivity. The efficacy and safety of tofacitinib in patients with moderately severe UC is proven. With a quick onset of action and rapid clearance, no associated immunogenicity and no loss of drug due to hypoalbuminemia, tofacitinib presents itself as an exciting option for use in ASUC. More recently, tofacitinib has garnered attention for use as a rescue therapy in ASUC.

In the case-control study, 40 hospitalized patients treated with second-line tofacitinib after failure to iv steroids and with previous biologic treatment failures were compared with 113 controls matched according to gender and date. In all, 63% of the patients complied with Truelove and Witts criteria and the remaining 37% had laboratory or endoscopic features of severe disease. Four [10%] patients in the tofacitinib group vs 18 [16%, $p = 0.44$] in the control group had colectomy at day 30; six [15%] vs 23 [20%, $p = 0.64$] at day 90; and eight [20%] vs 26 [23%, $p = 0.83$] at day 180, respectively. Nevertheless, a multivariate model adjusting for variables associated with disease severity found that the 90-day risk of colectomy was significantly lower in the tofacitinib group (hazard ratio [HR] 0.28 [0.10–0.81],$p = 0.018$).

We hypothesized that addition of tofacitinib to corticosteroids in hospitalized patients with ASUC can have additive effects on the therapeutic efficacy and improve the treatment response rates. The present double blind randomized controlled trial was therefore performed to determine if addition of tofacitinib to corticosteroids was superior to corticosteroids alone in patients hospitalized with ASUC.

Study Structure

Principal Investigator	• **Dayanand Medical College and Hospital Ludhiana** • Dr. AjitSood

Aims and Objectives

Primary objective

- To compare the efficacy of tofacitinib used as an adjunct to corticosteroids versus corticosteroids alone in inducing treatment response among subjects with acute severe ulcerative colitis.

Secondary objectives

- To compare the rates of rescue medical therapy and colectomy in the two intervention groups at day 90 of randomization.

Endpoints

Primary Endpoint

- The proportion of subjects responding to treatment by day 7.

Secondary endpoints

- The proportion of patients requiring medical (infliximab/cyclosporine) or surgical (colectomy) rescue therapy by day 7.
- The duration of hospital stay
- The proportion of patients requiring initiation of infliximab/cyclosporine or undergoing colectomy after discharge but within 90 days following randomization.

Study Site

This is single center, prospective, double blind, randomized placebo-controlled trial in patients with ASUC. The study will be conducted at Dayanand Medical College and Hospital, Ludhiana.

Definitions

<u>Acute severe ulcerative colitis</u>

Acute severe UC will be defined according to the Truelove Witts Criteria.

The definition is based on 6 or more blood stained stools daily, with 1 or more of the 4 additional criteria: hemoglobin <105g/L, ESR >30 mm/h, fever >37.8℃, and tachycardia >90/min.

Treatment Response

Response to therapy will be defined using Lichtiger index.

The Lichtiger index is a clinical score incorporating the total number of stools, nocturnal frequency of stools, blood in stools, fecal incontinence, abdominal pain, abdominal tenderness, need for antidiarrheal agents and general well-being. A decline in Lichtiger index by >3 points the day 7, and an absolute score <10 for 2 consecutive days without the need for rescue therapy (infliximab/cyclosporine or colectomy) was considered as response.

Additionally, *response to corticosteroids will also be assessed using the following criteria*

- Response: Defined as ≤3 stools/day with the absence of visible blood by day 7
- Non response: Patients with >8 stools/day, or 3–8 stools/day with CRP >45 mg/L by day 7

Inclusion Criteria

1. Adult (aged > 18 years)
2. Subjects hospitalized with ASUC, as defined by Truelove Witts criteria
3. Subjects who are willing and able to comply with treatment plan, laboratory tests, daily bowel movement diary call, and other study procedures.
4. Subjects who are willing to provide a written informed consent.

Exclusion criteria

1. Patients hospitalized with severe UC but did not fulfil the Truelove Witts criteria
2. Prior exposure to intravenous corticosteroids or tofacitinib within 4 weeks before hospitalization
3. Active enteric or extra-intestinal infection (including Clostridioides difficile, tuberculosis, etc.)
4. Crohn’s colitis
5. Toxic megacolon, intestinal perforation, or massive haemorrhage requiring emergency colectomy
6. Pregnancy/lactation
7. Current or prior history of thromboembolic disease
8. Subjects infected with human immunodeficiency virus (HIV) or hepatitis B or C viruses

9. Subjects who have been vaccinated with live or attenuated vaccine within 6 weeks of baseline or scheduled to receive these vaccines during study period or within 6 weeks after last dose of study medication
10. Subjects with malignancies or a history of malignancies, with the exception of adequately treated or excised non-metastatic basal cell or squamous cell cancer of the skin.
11. Subjects with current or recent history of severe, progressive, or uncontrolled renal, hepatic, haematological, gastrointestinal, metabolic, endocrine, pulmonary, cardiac, neurological disease.

Methodology

Informed consent

The protocol, informed consent form(s), recruitment materials and all participant materials will be submitted for IEC review and approval. Approval of both the protocol and the consent form will be obtained before any participant is enrolled. Any amendment to the protocol will require IEC review and approval before the changes are implemented in the study. The process of informed consent will be initiated prior to the individual agreeing to participate in the study and will continue throughout study participation. Extensive discussion of risks and possible benefits of study participation will be provided to participants and their families, if applicable. A consent form describing in detail the study procedures and risks will be given to the participant. Consent forms will be IEC-approved, and the participant will be required to read and review the document or have the document read to him or her. The investigator will explain the research study to the participant and answer any questions that may arise. The participant will sign the informed consent document prior to any study-related assessments or procedures. Participants will be given the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. They may withdraw consent at any time throughout the course of the study.

Good Clinical Practice

This study is to be conducted according to globally accepted standards of good clinical practice (as defined in the ICH E6 Guideline for Good Clinical Practice, 1 May 1996), in agreement with the Declaration of Helsinki and in keeping with local regulations.

Delegation of Investigators Duties

The investigator should ensure that all persons assisting with the trial are adequately qualified, informed about the protocol, any amendments to the protocol, the study treatments, and their trial-related duties and

functions. The investigator should maintain a list of subinvestigators and other appropriately qualified persons to whom he or she has delegated significant trial-related duties.

The person conducting the informed consent discussions must also sign and personally date the consent documents. A copy of the signed consent documents must be given to the participant. The original signed consent documents will be retained by the investigator. The investigator will not undertake any measures specifically required only for the clinical study until valid consent has been obtained. It is suggested, that the investigator inform the participant's primary physician about the participant's participation in the trial, if the participant has a primary physician other than the study investigator.

Confidentiality

Participant names will be kept in strictest confidence. Study data stored on a computer will be stored in accordance with local data protection laws. The participants will be informed that representatives of institutional review board or regulatory authorities may inspect their medical records to verify the information collected and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws. The investigator will maintain a personal participant identification list (participant numbers with the corresponding participant names) to enable records to be identified. Participants will be requested explicitly, whether they provide consent for identifiers and contact details can stored centrally, under strict security, at the Coordinating Center.

Randomization and allocation concealment

The eligible patients will be randomized in a 1:1 ratio based on a computer generated random numbers to receive either tofacitinib or a matching placebo. The randomisation list and numbered packing of the intervention will be prepared by an independent person (site clinical research co-ordinator). Randomization will be held centrally to ensure concealment of allocation. Both the investigators and the patients will be blinded to the intervention.

Breaking the blind/Unblinding

A planned unblinding will be done at day 7, and clinical response assessed. The unblinding will be done by the site clinical research co-ordinator and both the investigators and participants will be made aware of the intervention. The trial will be subsequently continued as open label study till day 90 of randomization.

Emergency unblinding will be done if a serious adverse event (SAE) transpired, to dictate the intervention to mitigate the health risk. At the initiation of the study, the study site will be instructed on

the method for blind breaking. The method will be an electronic process. Blinded codes will only be broken in emergency situations for reasons of subject safety. When the blinded code is broken, the reason must be fully documented on the case report form. Subjects whose code is broken will be discontinued from treatment. Every effort will be made to prevent the information regarding emergency unblinding of a participant's treatment assignment to additional study staff (beyond the participant and the principal/co-investigators).

Procedures

There will be two intervention arms

- Tofacitinib arm: Patients randomized to receive tofacitinib in addition to the standard treatment of acute severe UC
- Placebo arm: Patients randomized to receive tofacitinib in addition to the standard treatment of acute severe UC

The standard of care treatment including intravenous hydrocortisone (100 mg q6h), intravenous fluids, correction of dyselectrolytemia, and enteral feeding will be provided to the patients in both the treatment arms. Additionally, all the patients will receive thromboprophylaxis with enoxaparin (40-60 mg subcutaneously q24h) for the entire duration of hospitalization.

Tofacitinib (10 mg) or a matching placebo will be administered thrice daily for 7 days.

Intravenous hydrocortisone will be stopped in all patients by day 7. After unblinding and response assessment, the patients will be categorized into responders and non responders. For patients who respond to the intervention by day 7, per-oral prednisolone will be started in a tapering dose schedule (40 mg/day, gradually tapered and stopped by week 12). The responders in the tofacitinib arm will continue to receive tofacitinib, at a reduced dose of 10 mg twice daily while standard of care treatment with oral 5-aminosalicylates (3.6-4.8 g/day) ± azathioprine (1.5-2.0 mg/kg) will be continued in responders in the placebo arm. The patients will be followed till 90 days after randomization. In case of increase in disease activity between days 7 and 90, the patients will be considered for either infliximab or colectomy, at the discretion of the treating physician. The non-responders at day 7 will be advised rescue therapy with either infliximab/cyclosporine or colectomy.

Efficacy evaluations

The demographic and disease characteristics will be recorded at the time of enrolment. The investigations, including hemogram, liver and renal function tests, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), fecal calprotectin, and a limited unprepared flexible sigmoidoscopy with biopsy for histopathology and cytomegalovirus immunohistochemistry, will be performed in all patients within 24 hours of hospitalization. The investigations can be repeated at the discretion of the treating physician.

All the patients will undergo daily clinical assessments (including total number of stools, nocturnal frequency of stools, blood in stools, fecal incontinence, abdominal pain, abdominal tenderness, need for antidiarrheal agents and general well-being) till day 7 of the intervention. The follow up clinical disease activity and safety assessments will be done at days 30, 60 and 90.

Safety evaluations

Monitoring for adverse event(s), including opportunistic infections (particularly herpes zoster) and cardiovascular events, will be done daily till day 7, followed by assessments at days 30, 60 and 90. Any adverse event related to the use of tofacitinib resulting in death, or threatening life, requiring prolongation of hospitalization, or resulting in persistent or significant disability/incapacity will be considered as serious adverse event.

Investigations

The following investigations will be carried out at baseline in all the enrolled subjects.

1. Hemogram.
    i. Hemoglobin
    ii. Total leucocyte count
    iii. Platelet count
2. C-Reactive Protein (quantitative)
3. Fecal Calprotectin
4. Liver Function Tests.
    i. AST (Aspartate aminotransferase).

ii. ALT (Alanine aminotransferase).
iii. ALP (Alkaline phosphatase).
iv. Bilirubin.
v. Total protein.
vi. Albumin.

5. Renal Function Tests
    i. Blood Urea.
    ii. Serum Creatinine.
6. Fasting Blood Sugar.
7. Lipid profile
    i. Cholesterol (Total, HDL, LDL, VLDL)
    ii. Triglycerides
8. Sigmoidoscopy and biopsy for cytomegalovirus infection (unprepared)
9. Stool for Clostridioides difficile infection (glutamate dehydrogenase and toxins A and B)

Monitoring for adverse effects (AE)

Safety will be assessed by physical examinations, clinical and laboratory results and the spontaneous reporting of AEs, in all subjects. AEs experienced by participants involved in the study will be reported to the site's IEC in accordance with their procedures. Subjects will be monitored for development of any infection (viral, bacterial, and fungal). A subject who experiences a serious infection (defined as any infection requiring parenteral antibiotics or hospitalization) would be discontinued from the study. A serious infection would be reported as a serious adverse event (SAE). Subjects who experience non-serious infections that require treatment may be continued on the drug but a record would be mentioned in the CRF. All enrolled subjects would be followed closely for identification of a cardiovascular or malignancy event.

Statistical Analysis Plan

This Statistical Analysis Plan (SAP) describes the statistical rationale, methods, rules, and conventions to be used in the study entitled *Tofacitinib as an add-on therapy to corticosteroids in acute severe ulcerative colitis: A randomized controlled trial.* It describes the data to be summarized and analyzed, including specifics of the statistical analyses to be performed.

Statistical analysis

There is no planned interim analysis of this randomized controlled trial. All the analysis will be performed at the end of the study period, i.e. 90 days. At day 7, the principal investigator will authorize unlocking of the datasets. Following breaking of the blind, the study will continue as open label study.

The final analysis set will consist of all randomized subjects who receive at least 1 dose of study treatment. The safety set will also include all randomized subjects who receive at least 1 dose of study treatment. The safety set will be used for all safety analyses. Subjects will be analyzed according to treatment received, regardless of randomization.

Statistical Tests

Baseline data will be reported as number (%), mean (± standard deviation) or median (IQR) as appropriate and categorical variables summarised as frequencies with percentages. The binary end points, including the efficacy end point(s), will be compared between the two arms using the Cochran–Mantel–Haenszel Chi square test. Student's t test will be used for continuous variables with normal distribution and Wilcoxon-Mann-Whitney U test for continuous variables with skewed distribution. Kaplan-Meier survival analysis will be used to evaluate the cumulative probability of need of rescue therapy in the two intervention arms. Log rank test will be used to compare the Kaplan-Meier curves. The default significance level will be 0.05; confidence intervals (CIs) will be 95%, and all tests will be 2-sided, unless otherwise specified in the description of the analyses.

All the statistical calculations will be done using the SPSS v21 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.).

Determination of sample size

The sample size was based on 60% response rates to intravenous corticosteroids in patients with ASUC.[18,19] Assuming 25% higher remission rate in the tofacitinib arm compared to placebo, with 80% power, and an alpha error of 5%, a total of 52 patients were computed to be needed in each arm.

Missing Data

Patients with missing data will be taken as treatment failures.

Disposition

All subjects who provide informed consent will be accounted for in this study. Inclusion criteria not met and exclusion criteria met will be listed. Among the randomized subjects, the number and percent of

subjects who completed/discontinued treatment, reasons off treatment, the number and percent of subjects who completed/discontinued the study, and reasons off study will be summarized.

The following baseline characteristics will be summarized:

- Age, years
- Sex
- Disease Duration, years
- Previous Treatment received (5-ASA, thiopurines, prednisolone, anti-TNF)
- Fecal Calprotectin, µg/g
- C-Reactive Protein, mg/L
- Oral steroids on admission
- Day 0 stool frequency
- Lichtiger score
- Number of criteria of systemic toxicity in the Truelove Witts Criteria
- Hemoglobin, g/dL
- Serum albumin
- Deep ulcers on sigmoidoscopy

The date of first and last study treatment administration will be taken from the Day 1 onsite dosing administration eCRF and the end of study eCRF, respectively. Interruptions, compliance, and dose changes will not be taken into account for duration of exposure. Exposure to study treatment (in days) will be summarized for the safety set.

Safety outcomes

Treatment-emergent adverse events (TEAEs) will be defined as AEs that started or worsened in severity on or after the first dose of study treatment. All AEs, regardless of treatment-emergent status, will be included in an AE listing. Additionally,a listing of other AE details as collected on the CRF will be presented. The severity will be classified as Grade 1: Mild, Grade 2: Moderate, Grade 3: Severe, Grade 4:Life-threatening, Grade 5: Death related to AE, using the Common Terminology Criteria forAdverse Events, v5.0 (CTCAE v5.0). All TEAEs will be summarized. Information collected about death (eg, date of death, primary cause of death) will be presented. TEAEs leading to discontinuation of study treatment will be identified by action taken being recorded as “Drug withdrawal” on the Adverse Events eCRF. All TEAEs leading to discontinuation of study treatment will be summarized.

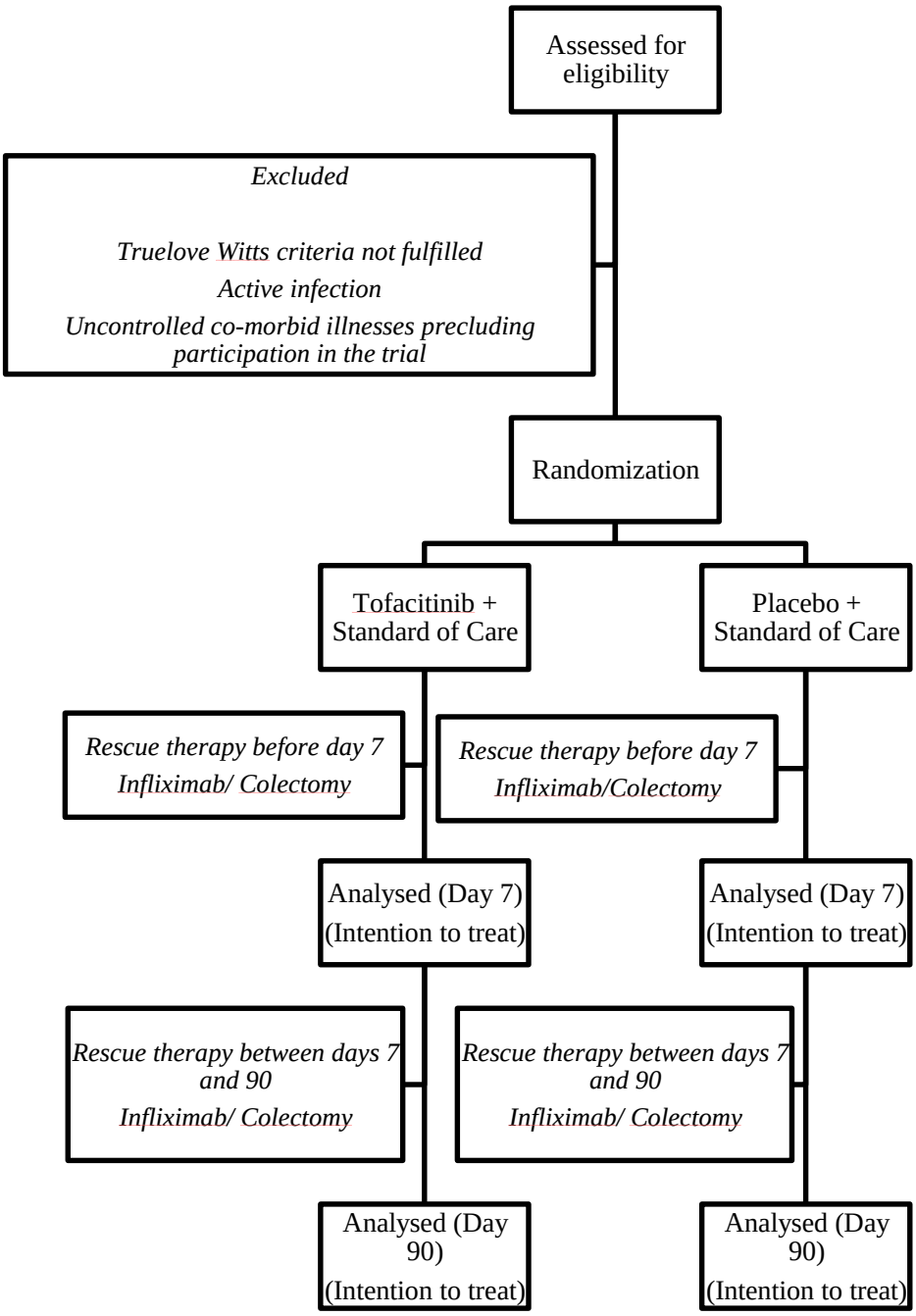
TEAEs of special interest will be summarized as follows

- Major Adverse Cardiovascular Events
  - Acute coronary syndrome
  - Coronary artery disease
  - Bradycardia
  - AV conduction delay
  - Hypertension
- Thromboembolic phenomenon
- Infections
  - Severe infections
  - Herpes zoster
  - Other opportunistic infections, including tuberculosis
- Liver Injury
  - Liver transaminases elevation
  - Bilirubin elevation
- Dyslipidemia
  - Elevation of total cholesterol, triglycerides, low density cholesterol, and very low density cholesterol
  - Lowering of high density cholesterol
- Malignancies
- Elevation of creatinine kinase
- Drug induced hyperglycemia
- Development of cushingoid features

Screening tuberculin skin test and Chest X Ray

- Screening TB results and chest X-rays will be listed. Results of TB questionnaires will also belisted.

Trial Design



Schedule of activities

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 14	Day 30	Day 60	Day 90
Informed Consent	☑											
Clinical History and Physical Examination	☑	☑	☑	☑	☑	☑	☑	☑		☑	☑	☑
Evaluation for Adverse effects and Safety	☑	☑	☑	☑	☑	☑	☑	☑		☑	☑	☑
Laboratory Investigations												
Complete Blood Count	☑							☑				☑
Erythrocyte Sedimentation Rate	☑											
C-Reactive Protein	☑			☑		☑		☑				☑
Faecal Calprotectin	☑											
Blood Sugar	☑	☑	☑	☑	☑	☑	☑	☑				☑
Lipid Profile	☑											☑
Renal Function Tests and Liver Function Tests	☑											☑
HBs Ag and Anti HCV	☑											
Sigmoidoscopy/ Colonoscopy	☑											
Stool for Clostridiodes	☑											

difficile toxin												
Rectal biopsy for CMV (histopathology and IHC)	☑											
Assessment of Disease Activity												
Truelove Witts Criteria	☑											
Mayo Clinic Score	☑											
Partial Mayo Clinic Score	☑	☑	☑	☑	☑	☑	☑	☑		☑	☑	☑
Endoscopic Mayo Score	☑											
Breaking the Blind												
Breaking of Code								☑				