Safety and **T**olerability **O**f Allogeneic **M**esenchymal Stromal Cells in **P**ediatric and Adult Inflammatory Bowel Disea**s**e (STOMP)

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Title of Study:

Safety and Tolerability of Allogeneic Mesenchymal Stromal Cells in Pediatric and Adult Inflammatory Bowel Disease (STOMP trial)

Study Center(s): Children's National Medical Center (CNMC)

Principal Investigator: Laurie S. Conklin, MD

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Objectives:

Primary Objective:

This is a pilot phase 1 study of Mesenchymal Stromal Cells (MSCs) for treatment of children and young adult patients with refractory Crohn's Disease (CD) or ulcerative colitis (UC). The primary aim is to evaluate safety and tolerability of the intervention.

Secondary Objectives:

1. Examine the effects of 4 weekly infusions of MSCs on clinical indices, and endoscopic scores when available.

2. Examine inflammatory and immunologic effects of MSC infusions on peripheral and intestinal mucosal biomarkers including, but not limited to, serum C-reactive protein, fecal calprotectin, anti-HLA antibodies, and viral specific T-cell activity before and after treatment.

Study Design:

This study is a pilot phase 1 study of patients with moderately to severely active Crohn Disease (CD) and ulcerative colitis (UC) (≥ 18 years, Mayo score: ≥6 or CDAI: > 220; <18 years, Pediatric Crohn's Disease Activity Index (PCDAI) :> 30) or Pediatric Ulcerative Colitis Activity Index (PUCAI) : >34). [1] [2] A fixed dose will be studied: 1 x 10^6 cells/kg administered intravenously (IV) weekly for 4 consecutive weeks, with the option of an additional 4 weeks of treatment, at the discretion of the principal investigator. The primary outcomes are regimen-related toxicity, defined in section 5. Toxicity will be scored as a binary variable indicating occurrence or non-occurrence over a monitoring interval from the start of MSC therapy to 45 days after the last infusion. Based on the planned administration schedule of 4 weeks, the total toxicity monitoring period will have duration 28 +45 = 73 days. If additional weekly doses are administered, the safety assessment phase will be 45 days after the last dose given.

Three young adult patients (ages 17≤22 years) will be enrolled, staggered by at least 7 days, and monitored for toxicity over a period of 73 days. Infusions are administered over the first 28 days, followed by a 45 day monitoring period. If we observe no severe (grade 3 or worse) adverse events in the young adult cohort, we will wait a minimum of 45 days after the last MSC administration before beginning to enroll 3 more patients, ages 12≤16 (pediatric cohort). If we observe a severe (grade 3 or worse) adverse event occurring at any time from the start of therapy to 45 days following the last MSC administration in the young adult cohort we will stop the study. If we observe no severe adverse events attributable to MSCs in the young adult cohort but a severe (grade 3 or worse) adverse event occurring at any time from the start of therapy to 45 days following the last MSC administration in the pediatric cohort we will stop the study. A maximum of 6 patients will be enrolled. Assuming an accrual rate of 3-4 patients per year, it is anticipated that it will require at least 18-24 months to accrue all 6 patients.

Eligibility

This protocol allows for the treatment of both male and female pediatric and young adult patients, between the ages of 12 ≤ 22 years, with active moderate to severe CD or UC, who have failed to respond to, or are intolerant of, biologic therapy.

Inclusion criteria

- For the young adult cohort, patients must be ages 17 ≤22 years
- For the pediatric cohort, patients must be ages 12 ≤16 years
- Patients must have moderate-severely active CD or UC (defined in section 2.3), and documented active disease on flexible sigmoidoscopy, colonoscopy or MR enterography within the preceding 2 months.
- Patients who have failed or are intolerant of biologic therapy. Specifically, the patient will have recurrence or persistence of active disease despite current or past treatment with a biologic. At the time of enrollment, study subjects may be currently receiving 5-aminosalicylates, corticosteroids (≤ 20 mg daily or up to 0.5 mg/kg/day if weight <40 kg), methotrexate, 6MP/azathioprine, or a biologic (either as monotherapy or in combination). During the treatment phase, if the treating physician thinks that a medication dose should be lowered to avoid side effects, this should be recorded.
- Patient or parent/guardian capable of providing informed consent.

Exclusion criteria

- Patients < 12 years of age or >22 years of age
- Pregnant or breastfeeding. Serum pregnancy test must be negative at screening for female subjects of childbearing potential. Urine pregnancy test must remain negative at each of 4 infusion visits.
- Patients with toxic mega-colon or intestinal perforation
- Evidence of autoimmune chronic active hepatitis or sclerosing cholangitis.
- Patients with fever > 39° C or clinically significant active infection within 1 week (i.e. chronic infections including Hepatitis B/C or HIV or acute infections, including urinary tract infection and respiratory tract infection)
- Received an agent not approved by the FDA for marketed use in any indication or any small molecule inhibitors (i.e. naltrexone) within 60 days of enrollment.
- Subjects who are taking greater than 20 mg (or if body weight <40 kg, 0.5 mg/kg) of prednisone daily.

- Clinically significant abnormal biochemical and hematological parameters, including:
  - Neutrophil count < 1000 cells/mm^3
  - Hemoglobin < 8 g/dl
  - Platelet count ≤ 130 cells/mm^3
  - Creatinine ≥ 1.2 x the upper limit of normal
  - Alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) ≥ 2x the upper limit of normal
  - Conjugated bilirubin greater than 1.2. mg/dL
- Has active infection with enteric pathogens as evidenced by positive microbiological culture of stool or C.difficile toxin PCR.
- Had bowel surgery other than perianal procedures (fistulotomy, seton placement, abscess drainage) within 3 months of enrollment.
- Has uveitis
- Has known pulmonary disease, excluding mild intermittent asthma

Primary Endpoints

- Safety and tolerability of the intravenous administration of human allogeneic bone marrow-derived MSCs to children and young adults with IBD, measured by the frequency of any SAEs, AEs and/or early treatment discontinuations.

Secondary Endpoints

- Magnitude of decrease in clinical index (for young adults we will use the CDAI and Mayo, and for children the PCDAI and PUCAI) relative to baseline, reporting the average decline and 95% confidence limit.
- Changes in biomarkers of inflammation and immunogenicity (i.e. C-reactive protein, fecal calprotectin, anti-HLA antibodies, viral-specific T cell activity)
- Where available, magnitude of endoscopic improvement, as assessed by change in endoscopic scores (Simple Endoscopic Score Crohn's Disease [SES-CD] for CD or Endoscopic Sub Score within the Mayo Score for UC) relative to baseline, reporting the average decline and 95% confidence intervals [3, 4].

Exploratory Endpoints

Blood, urine, saliva, and stool will be collected for evaluation of biomarkers

Accrual Period: Estimated accrual of 3-4 patients per year for 18-24 months

Study Duration: Approximately 48 months, including follow up

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1 BACKGROUND AND RATIONALE

1.1 Inflammatory Bowel Disease:

The purpose of this protocol is to study the safety and tolerability of MSCs for the treatment of pediatric and young adult patients with moderate to severe Inflammatory Bowel Disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), which is unresponsive to conventional therapy. CD and UC are chronic inflammatory diseases characterized by relapsing inflammation of the gastrointestinal tract. There is no known cure for these lifelong conditions. CD affects the full thickness of the bowel wall, and may affect the different areas of the small and large intestine. Conversely, UC affects only the large intestine.

IBD in children profoundly affects nutrition, growth, education, employment, and social well-being. The prevalence of CD in people < 20 years of age is 43 per 100,000 and for UC is 28 per 100,000 in the US. Overall, the cost associated with IBD care is greater in children than in adults. This likely reflects the high number of incident cases in children, but also likely reflects the increased severity of IBD in children. [5] Though a much lower prevalence is found in other areas of the world, IBD appears to be emerging in areas such as China, South Korea, India, and Iran, correlating with industrialization and westernization. [6] Twenty-five to 30% of all patients with CD and 20% of those with UC are diagnosed before age 20. [7] [8]

Although exact details of the disease pathogenesis are unknown, the generally accepted hypothesis is that IBD occurs as an exaggerated and dysregulated mucosal immune response to environmental factors, including intestinal microbiota, in a genetically susceptible host. The hallmark of IBD is an infiltration of innate immune cells (neutrophils, macrophages, dendritic cells, and natural killer T cells) and adaptive immune cells (B cells and T cells) into the intestinal lamina propria. Innate immune cells provide a rapid response to microbes via sensors (i.e. toll-like receptors) which recognize microbial and nonmicrobial triggers and initiate inflammatory responses. In CD, failure of dendritic cells to induce tolerogenic regulatory CD4+ T cells leads to a cascade of intestinal inflammation. [9] CD is characterized by an imbalance of elevated pro-inflammatory effector T cells (Th17 and Th1 cells secreting cytokines interleukin-17 and 22, interferon γ and TNF α) and decreased inflammatory-suppressing regulatory T cells. [10, 11] Th2 cytokines secreted by natural killer T cells are implicated in UC. [12] [10]

1.2 Current Standard IBD Treatment:

Treatment goals in pediatric IBD include improving quality of life, maximizing growth and nutrition, and preventing complications, such as toxic megacolon, anemia, intra-abdominal abscess, intestinal obstruction, intestinal perforation, growth failure and

pubertal delays. In spite of advances in IBD therapeutics, a significant number of patients continue to have symptoms while on conventional medications. Moderate to severe IBD is treated with systemic corticosteroids, immunomodulators (6-mercaptopurine for CD and UC, methotrexate for CD), and biologics (i.e. infliximab, adalimumab, certolizumab). These medications are variably effective and have potential for severe complications and side effects. From the first introduction of corticosteroids, 20% of patients do not respond, and 35% of the responders develop steroid dependency. [13, 14] 6-mercaptopurine and its parent drug azathioprine possess well-known immunosuppressive and lymphocytotoxic properties. [15] While 50% of patients will achieve a clinical response after 4 months of therapy, only 40-65% of patients maintain remission, and the onset of these drugs is slow. [16, 17] Methotrexate is effective in about a third of patients with CD, although experience with methotrexate in pediatric IBD literature is scant and retrospective. [18] Toxicities, particularly teratogenicity in females, limit its use. Methotrexate may also cause nausea, bone marrow suppression, infections, and liver injury.

Biologic therapies are widely used for treating both UC and CD. Established and emerging therapies for IBD are directed toward the destruction or deterrence of activated effector T cells, or blockade of Th1-driving cytokines. Trials with anti-cytokine therapy, in particular anti-TNF α medications, have been successful for patients with refractory disease. There are 4 anti-TNF monocolonal antibodies currently FDA approved for use in adults with IBD: infliximab, a chimeric IgG1 antibody; adalimumab, a fully humanized IgG1 antibody; certolizumab and golimumab, both PEGylated humanized Fab' fragments. [19] However, there are fewer options for pediatric patients, as only infliximab is FDA approved for pediatric UC, while both adalimumab and infliximab are indicated for treatment of pediatric CD. Despite these advances, one third of patients do not respond to these medications, and an additional third subsequently lose response or become intolerant. [20, 21]

Safety data from the use of TNF inhibitors highlight concerns regarding risk of infections, such as tuberculosis, listeria, pneumocystis, and invasive fungal organisms. It is known that concomitant use of immunomodulators improves clinical efficacy of infliximab in adults. [22, 23] However, serious risks are associated with infliximab and immunomodulators. In addition to infection, include infusion/injection reactions, hepatitis, and an otherwise extremely rare and almost uniformly fatal hepatosplenic T-cell lymphoma in pediatric and young adult CD patients receiving the combination of infliximab and 6MP or azathioprine.[24, 25] Thus, a need for new therapies for refractory pediatric IBD exists, particularly the need for safely modulating the immune system to reduce or reverse the abnormal inflammatory cascade.

1.3 Description of the Study Agent/Intervention

Human Mesenchymal Stromal Cells (MSCs) - "Universal Stem Cells"

MSCs are undifferentiated, non-hematopoietic, pluripotent cells that give rise to mesodermal tissue types, including bone, cartilage, tendon, muscle, and fat. [26] MSCs provide support for the growth and differentiation of hematopoietic progenitor cells in bone marrow microenvironments and, in animal models, promote engraftment of hematopoietic cells. [27] These cells may be isolated from most human tissues, usually bone marrow, and expanded *ex vivo*. [28, 29] MSCs do not express the hematopoietic markers CD45, CD34, and CD14, or the co-stimulatory molecules B7-1, B7-2, CD40, CD40 ligand, CD80 and CD 86 and probably therefore do not activate alloreactive T cells. [30-32]. Minimal classification criteria has been defined as positive for CD146, CD90, and human leukocyte antigen (HLA) class 1 and negative for hematopoietic cell markers. [33] MSCs constitutively express HLA class I molecules, but do not express class II molecules on the cell surface. In the presence of inflammatory cytokines, i.e. IFN γ and TNFα, the surface expression of class I is increased and class II expression is induced. [32] However, mesenchymal tissues, such as bone and cartilage have been successfully transplanted without the need for donor-recipient MHC matching, and the incidence of acute rejection of these tissues is low. Thus, the mesodermal origin of BMSCs, along with lack of co-stimulatory molecules necessary to activate T cells, suggests low immunogenicity of these cells. MSCs isolated from humans and other mammalian species do not elicit a proliferative T cell response from allogeneic lymphocytes. [34, 35]

MSCs have been shown to possess immunomodulatory properties, following the initial observation that bone marrow-derived MSCs suppressed T cell proliferation. [36] As expected, MSCs suppress proliferation of allogeneic T cells in a dose-dependent, non-HLA-restricted manner. [30, 32, 37] MSCs inhibit the division of stimulated T cells by preventing their entry into the S phase of the cell cycle and by mediating irreversible B0/G1 phase arrest. [38] MSCs interact with other immune cells and are capable of altering immune cell responses. MSCs can inhibit the differentiation of monocytes into immature dendritic cells.[39] MSCs also impair the ability of DCs to express co-stimulatory molecules, produce cytokines, and stimulate T cells. [40] MSCs can decrease the pro-inflammatory potential of dendritic cells by inhibiting their production of tumor-necrosis factor (TNF) and IFN-γ.[41] MSCs increase production of IL-10, thereby limiting T cell expansion. [41] Suppression of T cell responses is in part mediated by incompletely defined soluble factors, and MSCs may also suppress T cell reactivity by inducing T regulatory cells. [42]

A critical benefit of MSCs is that they do not seem to induce immunological memory in the recipient even when they are completely HLA mismatched, to the point of avoiding rapid rejection. [43] Given the goal of healing intestinal mucosa, another possible benefit of MSCs is that they have been shown to promote tissue repair and healing after radiation injury. Infused MSCs improve the outcomes of acute renal, neural, and lung injury, perhaps leading to a switch from pro-inflammatory to anti-inflammatory cytokines

at the site of injury. [44] Thus, much research has been focused on the potential use of MSCs for the treatment of chronic wounds, degenerative diseases, autoimmune diseases, and graft-versus-host disease. [45-48] The vast majority of studies of MSCs have been performed in adults.

1.4 Scientific Rationale and Clinical Justification

1.4.1 Rationale for the study of BMSCs in Pediatric IBD

Pediatric IBD is a chronic, life-long condition beginning in childhood, characterized by relapsing inflammation of the gastrointestinal tract. Inflammation is thought to be mediated by T cells, macrophages, and dendritic cells that produced downstream cytokines. Despite the many therapeutic advances in the treatment of IBD, many patients still suffer with refractory disease. Children with IBD are typically exposed to multiple medications over years, and many suffer with associated serious side effects.

According to 2009 US Census data, inflammatory bowel diseases (IBD) (i.e. Crohn's disease and ulcerative colitis) affect about 1.2 million Americans. Of these, 62,000 are children (38,000 CD and 23,000 UC). Thus, 5% of all IBD cases in the USA are of pediatric age (<20 years). This qualifies drugs to treat pediatric UC and pediatric CD as orphan indications. (http://www.accessdata.fda.gov/scripts/opdlisting/oopd/OOPD_Results_2.cfmIndex_Number=340911) Further, while there are options for the treatment of IBD in adults, other than corticosteroids, infliximab (Remicade) and adalimumab (Humira) are FDA-approved for the treatment of moderate to severe pediatric CD. Infliximab has orphan drug designation to treat pediatric UC. Certolizumab pegol, methotrexate, 6MP, and 5-ASA products are NOT FDA approved for use in children with UC or CD. (http://www.accessdata.fda.gov/scripts/cder/drugsatfda/)

The scientific rationale for investigating the use of MSCs in IBD patients is based on known properties of inflammatory and immune modulation and enhancement of tissue repair. This is not a first-in-human study and there is now a sizable body of evidence that this intervention is safe when used in people ≥ 18 years of age. However, the safety of MSCs has not been yet demonstrated in children with IBD. While children cannot be treated as “small adults”, this is not an intervention that is expected to have substantially different impact on children than adults because metabolic differences are not a concern for this intervention. CD and UC are considered similar enough that efficacy in children has been extrapolated from adults in past trials of drugs used to treat IBD.

Therefore, there is an urgent medical need to evaluate novel therapies such as MSCs in pediatric IBD since:
1). Children with refractory IBD have few well-studied treatment options.
2). MSC therapy has already been evaluated in adults

3). There is not only the potential for benefit when using MSC in IBD but also the high likelihood that the allogeneic MSC will not cause the systemic immune suppression seen with other investigational therapies for this disease.

The MSCs that we propose using are different because they are passaged fewer times (up to 4 passages). One of the limitations when expanding MSCs is the time, labor, necessary space and costs associated with the expansion of these cells. For example, to generate 500 million MSCs using standard T-175cm2 flasks requires four-five passages and as many as 200 flasks by the final passage. One way to circumvent these limitations is to use a functionally-closed, automated bioreactor that expands a large number of cells in a short period of time, such as the Quantum Cell Expansion system. Besides being a closed system (which limits the chance of contamination), the bioreactor requires only about 20% of the labor that the normal flask-based expansion requires and manufactures the cells 1-2 weeks sooner. In addition, the Quantum can produce 200-400% more cells during the same time period. In Dr. Hanley's preliminary pre-clinical studies, >500 million MSCs can be expanded after only two passages with the potential to generate over 1 billion MSCs by passage two, and over $2.5x10^{10}$ MSCs by passage three. In these same studies, cells obtained from the Quantum had a similar phenotype to those expanded in flasks. We are one of the few centers with a Quantum system. Dr. Hanley is an established expert in the manufacturing of MSCs for phase 1 trials, as well as the Director of the GMP facility at CNHS. [49, 50]

Our cell therapy product consists of allogeneic mesenchymal stromal cells manufactured from an eligible normal donor. We have a CNMC IRB-approved protocol (Pro00006717) that provides a mechanism for the use of excess bone marrow obtained from healthy donor bone marrow harvests. Up to 100 mL of marrow will be obtained from Lonza (Walkersville, MD) under informed consent and transported to Children's National Health System in a validated process at 2-8°C. The donor will meet the eligibility requirements as described in 21 CFR parts 1271 Subpart C. Testing (Table 1) will be performed using FDA-approved, cleared and/or licensed kits by Lonza within 7 days of collection of the marrow for manufacturing. Manufacturing will commence within 24 hours of marrow collection. Risk factors will be assessed by taking a clinical history, clinical examination, and by use of a standard questionnaire for blood donors.

Table 1:	**Test**
Chagas Disease	
Hepatitis B Surface Antigen	
Hepatitis B Core	
Hepatitis C Core	
HIV 1 and 2	
TAQ Screen MPX (HBV, HCV, HIV-1 group M RNA, HIV-1 Group O)	
NAT HCV	
NAT West Nile Virus	
Serological Test for Syphilis	
CMV	
HTLV-1	

This would not be a first-in-human study and there is now a sizable body of evidence that this intervention is safe when used in people ≥ 18 years of age. However, the safety of MSCs has not been yet demonstrated in

children with IBD. Safety and dosing may not be extrapolated from adult studies, as classic pharmacokinetic monitoring to document equivalent exposure-response is not feasible with cell therapies.

1.4.2 PLAN OF STUDY:

This is a pilot phase 1 treatment protocol for subjects with moderate to severe CD or UC who have failed or are intolerant of one biologic. Subjects must have had a flexible sigmoidoscopy or colonoscopy or MR enterography within 2 months of enrollment, demonstrating active inflammatory bowel disease. Subjects with moderate to severe CD are defined differently in children and adults. In patients <17 years of age, moderate to severe CD will be defined as having a Pediatric Crohn Disease Activity Index (PCDAI) score of > 30 (range 0-100) and subjects with moderate to severe UC are defined as a patients with a Pediatric Ulcerative Colitis Activity Index (PUCAI) score of >34 (range 0-85). In patients ≥ 18 years of age, moderate to severe CD will be defined as a CDAI of ≥ 220 (range 0-600), and moderate to severe UC defined as a Mayo score of ≥ 6 (range 0-12). In our study, at the specification of the FDA IND review, a young adult cohort will be enrolled first and monitored for toxicity. A pediatric cohort will only begin to enroll if there are no serious adverse events observed in the young adult cohort. Subjects will be closely monitored both by the study team to detect and evaluate adverse events (AEs), serious adverse events (SAEs), and toxicity requiring discontinuation of the intervention.

Three young adult patients (ages 17 ≤ 22 years) will be enrolled, staggered by at least 7 days. A fixed dose will be studied: 1 x 10^6 cells/kg administered intravenously (IV) weekly for 4 consecutive weeks. Infusions are administered over the first 28 days, followed by a 45 day monitoring period. Our chosen dose (1 x 10^6 cells/kg weekly for a duration of 4 weeks) is within the dosing parameters of previous trials. In trials of MSCs in pediatric GvHD, doses as high as 8 x 10^6 cells/kg/dose were given up to twice per week for four weeks. [51] The rate of administration (for patients < 35 kg, total infusion over 60 minutes, and for ≥ 35 kg, 4-6 ml/minute via infusion pump) is also consistent with prior pediatric administration in patients with GvHD. [51]

In our study, toxicity will be scored as a binary variable indicating occurrence or non-occurrence over a monitoring interval from the start of MSC therapy to 45 days after the last infusion. Based on the planned administration schedule, the total toxicity monitoring period will have duration 28 +45 = 73 days. If we observe no severe (grade 3 or worse) adverse events in the young adult cohort, we will wait a minimum of 45 days before beginning to enroll 3 more patients, ages 12≤16 years (pediatric cohort). If we observe a severe (grade 3 or worse) adverse event occurring at any time from the start of therapy to 45 days following the last MSC administration in the young adult cohort we will stop the study. If we observe no SAEs attributed to MSC administration in the young adult cohort but an SAE (grade 3 or worse) occurring at any time from the start of therapy to 45 days following the last MSC administration in the pediatric cohort, we will stop the study. If patients with active disease do not have >grade 2 toxicity attributed to

cells and fail to rapidly progress with disease requiring urgent therapy or surgery, and at the discretion of the principal investigator, 4 additional weekly doses may be administered. Subjects are eligible to receive up to 4 additional doses of MSCs at weekly intervals, each of which will consist of the same cell number (1 x 10^6 cells/kg). The safety assessment period will be 45 days from the last dose administered. A maximum of 6 patients will be enrolled. Assuming an accrual rate of 3-4 patients per year, it is anticipated that it may require at least 18- 24 months to accrue all 6 patients. With the additional 24 months of follow up to evaluate longer-term safety and efficacy endpoints, the anticipated trial duration will be about 48 months.

The primary study endpoint is safety and tolerability of the administration of human allogeneic bone marrow-derived MSCs to children and young adults with IBD, measured by the frequency of any SAEs, AEs and/or early treatment discontinuations. Secondary endpoints will include: 1) magnitude of change in clinical indices CDAI, Mayo, PCDAI and PUCAI) relative to baseline, reporting the average decline and 95% confidence limit; 2) changes in biomarkers (i.e. C-reactive protein, fecal calprotectin, anti-HLA antibodies and viral specific T-cell activity.) and 3) where available, magnitude of endoscopic improvement, as assessed by change in endoscopic scores (Simple Endoscopic Score Crohn's Disease [SES-CD] for CD or Endoscopic Sub Score within the Mayo Score for UC) relative to baseline, reporting the average decline and 95% confidence intervals [3, 4].

We will collect serum, saliva, peripheral blood mononuclear cells, stool, and urine for study of known and potential biomarkers of disease response. A potential concern regarding this therapy is the effect upon T cell-mediated immunity. Thus, we will assess peripheral viral-specific T cell activity before and after therapy.

The trial has been registered at clinicaltrials.gov (#NCT02150551). The IND has been reviewed and approved at the Center for Biologics Evaluation and Research at the FDA (IND#15902).

Safety and dosing may not be extrapolated from other populations, and classic pharmacokinetic monitoring is not feasible with cell therapies. A young adult cohort will be enrolled and monitored for toxicity. A pediatric cohort will only begin to enroll if there are no serious adverse events observed in the young adult cohort. Subjects will be closely monitored both by the study team and an independent DRC to detect and evaluate adverse events, serious adverse events, and toxicity requiring discontinuation of the intervention. Participants will be followed closely with weekly follow-up and assessment during the treatment protocol, and a subsequent 45 day follow up period after last MSC infusion. Laboratory and clinical data will be collected per protocol.

1.4.3 Rationale for the Dosage and Treatment Period

Our chosen dose1 x 10^6 cells/kg weekly x 4weeks is within the dosing parameters of previous trials. In trials of MSCs in pediatric GVHD, doses as high as 8 x 10^6 cells/kg/dose were given up to twice per week for four weeks. [51] The rate of

administration (for patients < 35 kg, total infusion over 60 minutes, and for children ≥ 35 kg, 4-6 ml/minute via infusion pump) is also consistent with prior pediatric administration in patients with GVHD. [51]

2 STUDY OBJECTIVES AND DESIGN

2.1 Study Objectives:

2.1.1 Primary Objective:

The primary objective is to examine the safety and tolerability of infusions of fresh bone marrow-derived MSCs in the treatment of children and young adults with moderate to severe IBD.

2.1.2 Secondary objectives:

1. Examine the effects of 4 infusions of MSCs on clinical measures of disease activity in patients, using clinical indices, as well as endoscopic scores (if applicable), histologic scores, and MR enterography when available.

2. Examine inflammatory and immunologic effects of MSC infusions on peripheral and intestinal mucosal biomarkers including, but not limited to serum CRP, fecal calprotectin, anti-HLA antibodies, and viral specific T-cell activity before and after treatment.

2.2 Study Design

This study is a pilot phase 1 treatment protocol for subjects with moderate to severe CD or UC who have failed to respond to or are intolerant of a biologic. Patients must have had a flexible sigmoidoscopy, colonoscopy or MR enterography within 2 months of enrollment, demonstrating active inflammatory disease.

Two types of patients will be enrolled:

- Moderate to severely active CD subjects (defined in Section 2.4)
- Moderate to severely active UC subjects (defined in Section 2.4)

At the beginning of the trial, a history and physical examination will be obtained, and documentation of baseline co-variates[52]:

CD:

- age
- gender

- ethnicity
- history of abdominal surgery
- duration of time with diagnosis
- age at diagnosis
- disease location
- history of failed biologic use
- history of stricturing/penetrating disease
- positive family history of IBD
- history of perianal disease
- severity and phenotype (Montreal classification for adults, or Paris classification for children)

UC:

- age
- gender
- ethnicity
- age at diagnosis
- history of failed biologic use
- duration of time since diagnosis
- positive family history of IBD)

During the course of the patient's treatment with MSCs, standard of care will be administered by the treating physician, at his/her discretion. There are no specific or "antidotal" therapies. Thus, any toxicity that arises during a patient's participation in this treatment will be managed with supportive measures at the discretion of the treating physician.

Three young adult patients (ages 17≤22 years) will be enrolled, staggered by at least 7 days between patients, and each monitored for toxicity over a period of 73 days. A fixed dose will be studied: 1×10^6 cells/kg administered intravenously (IV) weekly for 4 consecutive weeks. Infusions are administered over the first 28 days, followed by a 45 day monitoring period. The primary outcome is regimen-related toxicity, defined in section 5. Toxicity will be scored as a binary variable indicating occurrence or non-occurrence over a monitoring interval from the start of MSC therapy to 45 days after the last infusion. Based on the planned administration schedule, the total toxicity monitoring period will have duration 28 +45 = 73 days.

If we observe no severe (grade 3 or worse) adverse events in the young adult cohort, we will wait a minimum of 45 days before beginning to enroll 3 more patients, ages 12≤16 years (pediatric cohort). If we observe a severe (grade 3 or worse) adverse event occurring at any time from the start of therapy to 45 days following the last MSC administration in the young adult cohort we will stop the study. If we observe no severe adverse events attributable to MSC administration in the young adult cohort but a

severe (grade 3 or worse) adverse event occurring at any time from the start of therapy to 45 days following the last MSC administration in the pediatric cohort we will stop the study. A maximum of 6 patients will be enrolled. Assuming an accrual rate of 3-4 patients per year, it is anticipated that it will require at least 18 – 24 months to accrue all 6 patients. With the additional 24 months of follow up to evaluate efficacy endpoints noted in Section 2, the anticipated trial duration will be about 48 months.

2.2.1 Primary Endpoint

Safety and tolerability of the administration of human allogeneic bone marrow-derived stromal cells to children and young adults with IBD, measured by the frequency of any SAEs, AEs and/or early treatment discontinuations.

2.2.2 Secondary Endpoints

- Magnitude of change in PCDAI, PUCAI, CDAI to baseline, reporting the average decline and 95% confidence limit.
- Changes in laboratory parameters indicating inflammation or immunogenicity(i.e. C-reactive protein, fecal calprotectin, anti-HLA antibodies, and viral specific T-cell activity))
- When available, magnitude of endoscopic improvement, as assessed by change in endoscopic scores (Simple Endoscopic Score Crohn's Disease [SES-CD] for CD or Endoscopic Sub Score within the Mayo Score for UC) relative to baseline, reporting the average decline and 95% confidence intervals.

2.2.3 Exploratory Endpoint

- Blood, urine, saliva, and stool will be collected for evaluation of biomarkers

2.3 Study Population

This treatment protocol allows for the treatment of both male and female young adults and children between the ages of 12 years and 22 years with a diagnosis of moderate to severe CD or UC who have failed or are intolerant of biologic therapy.

Subjects with moderate-to-severe CD are defined as patient with a PCDAI score > 30 with visually active CD at the time of endoscopy/colonoscopy, or signs of inflammation on MR enterography, within 2 months of enrollment.

Subjects with moderate-to-severe active UC are defined as a patient with a PUCAI score >34, and visually active mucosal disease by colonoscopy or flexible sigmoidoscopy within past 2 months.

2.4 Recruitment Plan

Subjects will be recruited from the IBD Program at Children's National Medical Center, and from other sites via provider communication. The trial will be listed at clinicaltrials.gov.

2.5 Informed Consent

The informed consent process will be conducted in a private room to respect subject privacy. Following the briefing of the research study, the coordinator or designee will provide the subject with ample time to read the consent, and will answer any of their questions regarding the document. No study procedures will occur before the subject gives informed consent. To minimize any possible coercion, supervisors or anyone in a dominant position will not be permitted to consent the subject. The treating physician is responsible for ensuring that written informed consent, including written authorization for use and disclosure of PHI and appropriate signatures and dates, is obtained from each patient before any protocol-related procedures, including any pre-treatment procedures, are performed. All patients who are <18 years of age will require a parental or legal guardian signature of informed consent. In addition, all patients between the ages of 12 and 17 years will require a signature of assent.

Prior to the subject's participation in any trial procedures, the investigator or designee will ensure that the potential participant understands the research study and their role in the study. The written informed consent will be signed and dated by the subject and by the person who conducted the informed consent discussion. A signed copy of the consent form will be placed in the subject's trial chart and a photocopy will be given to the subject. Informed consent will be obtained in accordance with the Code of Federal Regulations (CFR) 21 CFR 50.25, 32 CFR 219, 45 CFR 46 and 15-2, ICH Harmonized Tripartite Guidance for Good Clinical Practice, and the Belmont Report. Also included in the consent process, Health Insurance Portability Accountability Act (HIPAA) authorization will be obtained before any study procedure is undertaken.

If subject decides to take part in the research study, their protected health information will not be given out except as allowed by law. Study personnel will work hard to keep this information private. The results of the data from the study may be published. However, subject will not be identified by name.

Subjects may change their mind and withdraw from the study any time they want.

If they decide to stop taking part in the study or if they are removed from the study, they may decide that they no longer allow protected health information that identifies them to be used in this research study. The study staff will respect the subject's decision to

decline access to their medical records and biological samples. Subject decision will be available in their records.

If subject agrees to participate in this research study, the research team (PI, co-investigators, Clinical Research Manager, statistician, Clinical Research Coordinator, Quality Assurance coordinator, and Research Nurse), the research sponsor (when applicable) and the sponsor's representatives, may use coded study data. The coded study data does not include subject name, address, telephone, or social security number. Instead, the researcher assigns a code to the data. The research team or the research sponsor may share the coded study data with others to perform additional research, place it into research databases, share it with researchers in the U.S. or other countries, or use it to improve the design of future studies. They may also publish it in scientific journals, or share it with business partners of the sponsor and to file applications with U.S. or foreign government agencies to get approval for new drugs or health care products.

The research team would store personal health information collected from the study in a database in a password protected computer for future research. The database is maintained by Children's National Medical Center in the division of Blood and Marrow Transplantation.

2.6 Eligibility criteria

2.6.1 Subject Inclusion Criteria

- For the young adult cohort, patients must be ages 17 ≤22 years
- For the pediatric cohort, patients must be ages 12 ≤16 years
- Patients must have moderate-severely active CD or UC (defined in section 2.3), and documented active disease on flexible sigmoidoscopy, colonoscopy or MR enterography within the preceding 2 months.
- Patients who have failed or are intolerant of biologic therapy. Specifically, the patient will have recurrence or persistence of active disease despite current or past treatment with a biologic. At the time of enrollment, study subjects may be currently receiving 5-aminosalicylates, corticosteroids (≤ 20 mg daily or up to 0.5 mg/kg/day if weight <40 kg), methotrexate, 6MP/azathioprine, or a biologic (either as monotherapy or in combination). During the treatment phase, if the treating physician thinks that a medication dose should be lowered to avoid side effects, this should be recorded.
    - Patient or parent/guardian capable of providing informed consent.
-

2.6.2 Subject Exclusion Criteria

- Patient <12 years of age or >22 years of age
- Pregnant or breastfeeding. Serum pregnancy test must be negative at screening for female subjects of childbearing potential. Urine pregnancy test must remain negative at each of 4 infusion visits.

- Patients with toxic mega-colon or intestinal perforation
- Evidence of autoimmune chronic active hepatitis or sclerosing cholangitis.
- Patients with fever > 39° C or clinically significant active infection within 1 week (i.e. chronic infections including Hepatitis B/C or HIV or acute infections, including urinary tract infection and respiratory tract infection)
- Received an agent not approved by the FDA for marketed use in any indication or any small molecule inhibitors (i.e. naltrexone) within 60 days of enrollment.
- Subjects who are taking greater than 20 mg (or if body weight <40 kg, 0.5 mg/kg) of prednisone daily.
- Clinically significant abnormal biochemical and hematological parameters, including:
    - Neutrophil count < 1000 cells/mm^3
    - Hemoglobin < 8 g/dl
    - Platelet count ≤ 130 cells/mm^3
    - Creatinine ≥ 1.2 x the upper limit of normal
    - Alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) ≥ 2x the upper limit of normal
    - Conjugated bilirubin greater than 1.2 mg/dL
- Has active infection with enteric pathogens as evidenced by positive microbiological culture of stool or C.difficile toxin PCR.
- Had bowel surgery other than perianal procedures (fistulotomy, seton placement, abscess drainage) within 3 months of enrollment.
- Has uveitis
- Has known pulmonary disease, excluding mild intermittent asthma

3 STUDY AGENT

3.1 Preparation Administration, and Dosage

On the day of each infusion, the following procedures/assessments will be performed:

- MSCs will be thawed, harvested, counted, the viability will be determined, and appropriate samples will be sent for other release testing (including sterility).Vital signs (heart rate, blood pressure, respiratory rate, and temperature) will be measured within 15 minutes prior to the infusion (time 0) and then every 5 minutes during the infusion. If vital signs are normal after three 5-minute checks, they will be checked every 15 minutes during the remainder of the infusion, and then at 30 and 60 minutes after the infusion for 1 hour.
- Patients will be monitored according to institutional standards for administration of blood products and at a minimum will be monitored according to below:
    - Patients should remain in the clinic for at least one hour
    - Patients should remain on continuous pulse oximetry for at least 30 minutes

    - Vital signs should be monitored at the end of the infusion then at 30 and 60 minutes
- Per institutional guidelines, pre-medication, consisting of oral acetaminophen (10-15 mg/kg/dose, max 650 mg) and diphenhydramine (0.5-1 mg/kg/dose, max 50 mg), will be administered at least 30 minutes prior to the infusion.
- No other medications should be given during the infusion, unless determined medically necessary by the treating physician.
- Supplemental oxygen will be kept by the bedside at all times.
- MSCs suspended in Plasmalyte A (multiple electrolyte injection) will be given as an IV infusion using weight-based dosing. We plan to infuse 1-4x10^6 cells per mL, not to exceed 240 mL. With a mean weight of 40 kg, the infusion volume would be 40-120 mL.
- Cells will be infused on Day 0 followed by repeat infusions weekly over a total of 4 weeks. An additional 4 weekly doses may be infused at discretion of physician. An attempt will be made to administer repeat infusions from the same initial donor, but administration of MSCs from another donor is permitted if same-donor cells are unavailable. Tracking of donor sample identification information will be performed for each sample.
- MSC's will be administered intravenously via a 18 gauge needle at a controlled rate of 4-6 ml/minute via infusion pump for patients weighting 35 kg or more. For patients under 35 kg in weight, the infusion will run in over 60 minutes at a rate no greater than 4-6 ml/minute. A syringe in combination with syringe pump can be used for administration in smaller patients.
- During the administration, the infusion must be stopped for any of the following reasons:
    - If the patient has symptoms or signs of respiratory difficulty, including but not limited to tachypnea, cyanosis, complains of shortness of breath, even if the pulse oximeter reading is normal. If the symptoms resolve within 30 minutes after standard supportive oxygen therapy, the infusion may be resumed at half the initial rate and the infusion must be completed within 2 hours of starting the infusion. If the symptoms recur, then the infusion will be stopped and will not be resumed.
    - If SaO2 decreases to ≤ 90% over a continuous period of > 3 minutes, whether or not the patient has symptoms of respiratory distress. If the SaO2 is ≤ 90% in the absence of symptoms or signs, the accuracy of the pulse oximeter reading should be confirmed by using another machine, relocating the sensor, or testing the device on another patient.
    - Infusion may be stopped at the discretion of the treating physician if there is another AE that the treating physician believes is related to the infusion, or if there is an issue with the infusion, or if the patient withdraws consent.

3.2 Assessment of Subject Compliance

All infusions are given under the direct supervision of the clinical study team, so study agent compliance is insured.

3.3 Concomitant Medications and Procedures

All prescription medications, over-the-counter medications, non-prescription medications (including herbal medications), and concomitant surgical or interventional procedures will be recorded for up to 45 days after last MSC infusion. At the time of enrollment, study subjects may be currently receiving mesalamine, corticosteroids (≤ 20 mg daily, if body weight < 40 kg, up to 0.5 mg/kg/day), immunomodulators, or biologics. If the treating physician thinks that a medication dose should be lowered to avoid side effects, this should be recorded.

3.4 Prohibited Medications and Procedures

Concomitant non-steroid anti-inflammatory (NSAID) medications will not be permitted during study participation. Other medications used to treat IBD are prohibited other than those outlined in the eligibility criteria. Subjects required rescue IBD therapy before the day assessment will be considered treatment failures. Medications used to treat medical conditions other than IBD are permitted after review and approval of the Principal Investigator. Systemic immunosuppressant for conditions other than IBD is not permitted.

3.5 Study Schedule

Please see below table for detailed schedule of study procedures and assessments (table 1).

3.6 Table 1: Protocol of Events

Visits/Procedures	Screening visit	Treatment and Safety Monitoring phase								Follow-up Phase		
Visit Day	**Day -30 to -1**	**Day 0 (Dose#1)**	**Day 7 (Dose #2)**	**Day 14 (Dose #3)**	**Day 21 (Dose #4)**	**Day 28**	**Day 45**	**Day 56**	**[a]Day 73**	**Day 180**	**Day 360**	**Day 720**
Visit Window	**Day -30 to -1**	**± 1 day[b]**	**± 3 days**	**± 3 days**	**± 3 days**	**± 3 days**	**± 3 days**	**± 3 days**	**±3days**	**±28 days**	**±28 days**	**±28 days**
Physical Examination	X					X	X	X	X	X	X	X
Medical & Surgical History	X											
Informed Consent	X											
Active disease on sigmoidoscopy, colonoscopy, or MR enterography	X											
Vital Signs (temp, HR, RR, BP)	X	X	X	X	X	X	X	X	X			
SaO2 via pulse oximetry	X	X	X	X	X	X	X	X	X			
AEs/SAEs		X	X	X	X	X	X	X	X			
Concomitant Medications	X	X	X	X	X	X	X	X	X			
Eligibility Criteria		X										
Collection of serum[c], saliva, urine, stool for research	X	X	X	X	X	X	X	X	X			
Urine pregnancy test (before each infusion)		X	X	X	X							
MSC infusion		X	X	X	X							
Disease activity assessed[a]	X	X	X	X	X	X	X	X	X	X	X	X
Routine Labs												
CBC	X	X	X	X	X	X	X	X	X			
Electrolytes, liver enzymes, urinalysis	X	X	X	X	X	X	X	X	X			
Quantitative CRP, fecal calprotectin	X	X	X	X	X	X	X	X	X			

[a]Disease activity assessed using: PCDAI, PUCAI, CDAI, Mayo, SES-CD

[b]Only applies to routine labs and pregnancy test

[c]We will draw up to 40 ml of blood at 9 time points for research studies (at baseline before the first infusion, before each subsequent infusion, and at the 45 day safety follow up).

3.7 Baseline assessment/screening

During the assessment visit, which will occur during the one month period prior to MSC administration, a discussion of the informed consent and signing of the consent will be completed prior to the initiation of study procedures. Medical and surgical history, physical examination and labs will also be performed. To satisfy enrollment criteria, a colonoscopy or flexible sigmoidoscopy, or MR enterography, must have been performed within the preceding 2 months for clinical purposes to document active inflammation. If possible, endoscopic assessment using the SES-CD for CD and the Mayo Score (see appendix 4) will be performed. An upper endoscopy with biopsies may also be performed if the physician deems this necessary for clinical assessment. These procedures may be done for clinical purposes at an outside facility, in which case endoscopic scores may not be available.

3.8 Treatment Phase (Day 0, 7, 14, 21)

On Days 0, 7, 14, and 21, subjects will arrive at the hospital for infusion of study drug, eligibility criteria, laboratory assessments, assessment of AEs/SAEs, collection of serum, saliva, urine and stool, and assessment of clinical indices.

Safety Monitoring Phase (Day 28, 45, 56, 73) +/- 3 days

During this visit, the patient will return to Children's National, or be seen by a local physician for a follow-up visit, physical exam, laboratory assessments, AEs/SAEs assessment, assessment of clinical indices, bloodwork, saliva, urine and stool collected for fecal calprotectin. Assessment of symptoms and screening for adverse events may be done by telephone if necessary.

Follow-up Phase (Day 180, 360, 720) +/- 28 days

Subjects will return to Children's National, or be seen by a local physician, for a physical examination and assessment of clinical indices. If physical examination is obtained by a local physician, reports will be emailed or faxed to the study investigator. Assessment of symptoms and screening for adverse events may be done by telephone if necessary.

3.9 Study Procedures

Any procedures performed during this study, such as endoscopy, colonoscopy, or MR enterography, will be done for clinical purposes.

3.9.1 Phlebotomy

Research blood will be drawn during screening visit, treatment phase, and safety monitoring phase but will not exceed 2 cc/kg or a maximum of 40 ml per blood draw.[53] Blood tests run (CBC, CMP, CRP) are considered standard of care. However, additional tissue specimens (urine, saliva, blood, and stool) may be collected and banked, if the subject provides consent.
Any remaining specimens that will be stored for future research will be stored safely and securely in a research specimen storage laboratory at the CETI Laboratory at CNMC.

4 POTENTIAL RISKS AND BENEFITS

4.1 Allogeneic BMSC Infusion

To date, serious complications or side effects from MSC infusions have not been described. In the trial by Forbes, et al., of MSCs in the treatment of adults with luminal CD, there was one SAE reported: a subject diagnosed with sigmoid adenocarcinoma at the day 42 endoscopy. The patient was subsequently found to have had low-grade dysplasia on sigmoid mucosal biopsy specimen from the entry endoscopy and there had been a previous recommendation for colectomy because of mucosal atypia within a known proximal rectal stricture. [54] Reported infusion side effects are mild and include headache, hives, fever, congestion, and dysguesia (altered taste). Transfusion reactions and allergic reactions have not been reported in published Phase 1 and II trials.

4.2 Phlebotomy

Blood draws may cause pain and bruising. Sometimes drawing blood causes people to feel lightheaded, and very occasionally causes fainting.

4.3 Possibility of Direct Benefit

Subjects may or may not receive any direct benefit from participating in this study. Subjects may potentially have a decrease in active IBD symptoms, possible induction of remission, and possible improved quality of life. Study medications and study-related routine clinical monitoring tests will be provided free of charge. Results of any specialized tests done as part of the study will be made available to interested study subjects, their parents, and their healthcare providers.

5 DRUG TOXICITY AND/OR ADVERSE REACTIONS (AES)

Adverse events will be collected as per SOP BMT_SOP003_AE Routine and serious adverse events will be collected and reported as per SOP BMT_SOP_004_SAE Report.

5.1 ADVERSE EVENT

An AE, as defined by the ICH guideline for GCP, is:

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug."

An AE is considered to be any adverse change or exacerbation from a baseline condition which occurs following the initial administration of an investigational product whether or not the event is considered to be related to the investigational product. Examples of this include but are not limited to the following:

- Adverse changes including new signs and symptoms, intercurrent illness modifying the clinical course, or the worsening of a baseline condition including the increased frequency of an event or an increased intensity of a condition
- Concomitant disease with onset or increased severity after the start of study product administration
- A new pattern in a pre-existing condition, occurring after the receipt of investigational product that may signal a clinically meaningful change
- Clinically significant changes in laboratory values

5.2 SERIOUS ADVERSE EVENT

As defined by the Code of Federal Regulations (CFR), a serious adverse event (SAE) is any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- Life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Congenital anomaly/birth defect.

An adverse event or suspected adverse reaction is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at

immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

5.3 UNEXPECTED ADVERSE EVENT

As defined by 21 CFR 312.32 (a), an unexpected adverse drug experience is:

> “An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.”

5.4 OTHER ADVERSE EVENT

Other adverse events will be identified by the PI during the evaluation of safety data. Significant adverse events of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the subject from the study, will be classified as other adverse events. For each, a narrative may be written and included in the clinical study report.

5.5 RELATIONSHIP TO TREATMENT

The PI or Sub-I must assign a relationship of each AE to the receipt of the investigational product. The investigator will use clinical judgment in conjunction with the assessment of a plausible biologic mechanism, a temporal relationship between the onset of the event in relation to receipt of the investigational product, and identification of possible alternate etiologies including underlying disease, concurrent illness or concomitant medications. The occurrence of adverse events will be monitored at each

visit. The following guidelines should be used by investigators to assess the relationship of an AE to study product administration:

Not related: No relationship to study treatment. Applies to those events for which evidence exists that there is an alternate etiology. For example, events that are clearly related to another medical condition (i.e. diabetes), are related to an outside circumstance (i.e. trauma), or that are not temporally related to administration of the treatment.

Unlikely: Likely unrelated to the study treatment. Likely to be related to factors other than study treatment, but cannot be ruled out with certainty. The need for medical or surgery related to progressive IBD, or known symptoms directly related to IBD such as diarrhea, perianal disease, intra-abdominal or perianal abscess, intra-abdominal fistula, rectal bleeding, and weight loss are unlikely to be related to the study treatment. This also includes patients with known history of extraintestinal manifestations related to IBD (such as arthralgias, arthritis, uveitis, erythema nodosum, pyoderma gangrenosum, or mouth ulcers) who experience such symptoms during the treatment period.

Possible: An association between the event and the administration of study treatment cannot be ruled out. There is a reasonable temporal association, but there may also be an alternative etiology such as the subject's clinical status or underlying factors including other therapy. This includes known potential risks of IBD and treatments that the patient is receiving, such as infection and lymphoma

Probable: There is a high degree of certainty that a relationship to the investigational product exists. There is a reasonable temporal association, and the event cannot be explained by known characteristics of the subject's clinical state or factors including other therapy.

Definite: An association exists between the receipt of investigational product and the event. An association to other factors has been ruled out.

5.6 INFUSION-RELATED ALLERGIC REACTIONS

The investigators should ensure that appropriate best clinical practices and treatment are initiated should an event indicating an allergic or hypersensitive reaction occur. Each event will be recorded as an AE or SAE, depending on which criteria are met by the event. The start and stop/restart times must be recorded in the CRF.

Should an infusion-related allergic reaction occur the infusion will be stopped immediately and the subject closely monitored until stable. An infusion-related allergic reaction may appear as flushing, sudden rash, or shortness of breath or difficulty breathing.

Definitions and grading will follow the current NCI-CTCAE criteria. Current definition and suggested treatment plan for infusion-related allergic reaction:

Grade 1: Mild transient reaction

1. Stop infusion and evaluate for severity. If reaction remains a grade 1 then restart at reduced rate.
2. Reduce infusion rate by 50%,
3. Treat subject per good clinical practice (suggest antihistamines, corticosteroids, etc. as medically indicated) and monitor for worsening condition.
4. If the reaction persists or worsens the infusion will be discontinued.

Grade 2: Readily responds to clinical treatment (e.g., antihistamines, corticosteroids); should consider prophylactic treatment for ≤24 hours.

1. Stop infusion for up to 3 hours (product should not be infused after 4 hours).
2. Administer clinical treatment for allergic reaction as medically indicated.
3. Resume infusion at 50% of previous rate once reaction has decreased to Grade 1 in severity. Monitor closely for any worsening.
4. If the reaction reoccurs, stop infusion. Study treatment is to be discontinued.

Grade 3: Prolonged or severe reaction (e.g., not rapidly responsive to treatment or a recurrence of reaction after an initial improvement of a Grade 1 or Grade 2 reaction.

1. Discontinue infusion immediately. Study treatment is to be discontinued
2. Administer clinical treatment for allergic reaction as medically indicated.
3. Report as a SAE

Grade 4: Characterized as life-threatening AE; urgent intervention indicated to maintain hemostasis.

1. Discontinue infusion immediately. Study treatment will be discontinued.
1. Administer clinical treatment for allergic reaction as medically indicated.
2. Report as a SAE

5.7 Severity Assessment

All AEs will be assessed for severity by the investigator. Inherent in this assessment is the medical and clinical consideration of all information surrounding the event including any medical intervention required. Each event will be assigned one of the following categories: mild, moderate, severe, or life-threatening. The criteria below may be used for any symptom not included in the grading scale. Any grade 4 (life-threatening) AE must be reported as an SAE.

Mild	Grade 1	Does not interfere with routine activities Minimal level of discomfort
Moderate	Grade 2	Interferes with routine activities Moderate level of discomfort

Severe	Grade 3	Unable to perform routine activities Significant level of discomfort
Potentially life-threatening	Grade 4	Hospitalization or ER visit for potentially life-threatening event

FDA guidelines for toxicity will be followed; however, if a subject is evaluated in an emergency room for nonlife threatening illness or symptoms (i.e., visits emergency department on weekend for mild problems because the physician's office is closed), the information from that visit will be reviewed and severity of the adverse event will be assessed according to the subject's clinical signs and symptoms.

The term "severe" is often used to describe intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious", which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

6 OFF TREATMENT AND OFF STUDY CRITERIA

Off Treatment Criteria for an Individual Subject

All patients, regardless of reason for withdrawal, will be asked to continue in the follow-up phase of the study. An individual subject may be withdrawn from the study for any of the following criteria:

- An individual subject's decision (the Investigator will attempt to determine the reason for the subject's decision).
- Non-compliance with the study medication and procedures to the extent that it is potentially harmful to the subject or to the integrity of the study data.
- Worsening of IBD symptoms as defined by a PCDAI greater than 50 or PUCAI greater than 65.
- Subjects requiring rescue treatment for IBD
- Any clinical AE, laboratory abnormality, or other medical condition or situation such that continued participation in the study would not be in the best interest of the subject (including changing medication doses due to concern for side effects).
- A change in the subject's baseline condition after enrollment, but prior to start of the treatment period so that the subject no longer meets the inclusion/exclusion criteria.
- The investigator deems a subject to be no longer fit to participate in the study.

Off Study Criteria for an Individual Subject

- Completion of study specified procedures.
- Refusal of further study follow-up by patient or legal guardian
- Lost to follow up
- Death
- Physician's discretion
- Patient withdrew from study
- Other

7 STATISTICAL CONSIDERATIONS

7.1 Statistical Analysis Plan

7.1.1. Sample size and follow up. A maximum of 6 patients will be treated in this pilot study. It is anticipated that it will require 18-24 months to accrue 6 patients. With the additional 24 months of follow up to evaluate efficacy endpoints noted in Section 2, the anticipated trial duration will be 48 months.

7.1.4. Safety Monitoring and Stopping Rules. For the purpose of safety monitoring, "toxicity" will be defined as a probable or definite regimen related severe (grade 3 or worse) adverse event occurring at any time from the start of therapy to 45 days following the last MSC administration. If we observe 1 severe (grade 3 or worse) adverse event occurring at any time from the start of therapy to 45 days following the last MSC administration in the young adult cohort we will stop the study. If we observe no attributable severe adverse events in the young adult cohort, but do observe 1 severe (grade 3 or worse) adverse events occurring at any time from the start of therapy to 45 days following the last MSC administration in the pediatric cohort, we will stop the study.

7.1.3. Future Trials. If the trial is not stopped early, then the toxicity and efficacy data will be used to plan a future trial of MSC cell therapy in pediatric patients with either CD or UC, including (i) blinded placebo-controlled phase II trials optimizing dose or administration schedule, or (ii) randomized comparison to standard therapies.

8 RECORDS TO BE KEPT

The CETI Research Office will maintain a password protected, restricted access database documenting the dates and doses of therapy as well as clinical chemistries and hematologic parameters. The clinical status and occurrence of any adverse events and subsequent interventions are to be kept on all patients.

- Imaging reports
- Surgical summaries
- Autopsy summaries, where appropriate

- Informed consent documents

All required clinical evaluation records will be the responsibility of Principal Investigator who will also be responsible for analysis of the clinical outcome and toxicity.
The laboratory evaluation of immunological efficacy will be the responsibility of Principal Investigator.

8.1 Data Handling and Record Keeping

8.1.1 Data Management Responsibilities

The Investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner. Source documentation (the point of initial recording of information) should support the data collected in the electronic data system and must be signed and dated by the person recording and/or reviewing the data. All data should be reviewed by the Investigator and signed as required with electronic signature.

8.1.2 Data Capture Methods

Data Collection: The OpenClinica database, a 21CFR11.10 compliant database, will be used for data capture. Access to the system will be limited to the study members only. The OpenClinica database will be used to capture data as per the protocol required assessments (see Table 4.2.2).

All required clinical evaluation records will be the responsibility of principal Investigator who will also be responsible for analysis of the clinical outcome and toxicity.
The laboratory evaluation of immunological efficacy will be the responsibility of Principal Investigator.

8.1.3 Types of Data

Source documents include, but are not limited to, the subject's medical records, laboratory reports, ECG tracings, biopsy reports, clinic notes, pharmacy records, and any other similar reports or records of procedures performed during the subject's participation in the study.

8.1.4 Source Documents and Access to Source Data/Documents

Source documents include all recordings of observations or notations of clinical activities, and all reports and records necessary for the evaluation and reconstruction of the clinical trial. Data will be collected directly from subjects during study visits and telephone calls, or will be abstracted from the subjects' medical records. The subject's medical record must record his/her participation in the clinical trial and study treatment

(with doses and frequency) or other medical interventions or treatments administered, as well as any AEs experienced during the trial.

8.1.5 Record Retention

The investigator is responsible for retaining all essential documents listed in the ICH GCP Guideline. All essential documentation for all study subjects will be maintained by the investigators in a secure storage facility for a minimum of 3 years. The FDA requires study records to be retained for up to 2 years after marketing approval or disapproval (21 CFR 312.62), or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational agent for a specific indication. These records will also be maintained in compliance with IRB, state and federal medical retention requirements, whichever is the longest. All stored records will be kept confidential to the extent required by federal, state, and local law. Destruction of research records will not proceed without permission of the IRB.

The FDA requires that the Investigator must retain study files for a period of at least 5 years after study completion or 2 years following the date that a marketing application is approved for the indication in which the study agent is being investigated (whichever is later).

9 REPORTING REQUIREMENTS

- Discuss all patients with CNMC Research Nurse
- Enter/Register all patients after calling Principal Investigator. The following forms should be completed:

  - ☐ Eligibility checklist
  - ☐ On study form
  - ☐ Drug Toxicity and/or Adverse Reactions
  - ☐ Adverse events will be collected as per SOPs (BMT_SOP003).
  - ☐ Serious adverse events will be collected and reported as per SOP (BMT _SOP004).

9.1 Adverse Event Reporting

Reporting of patient serious adverse events (SAEs) will be consistent with standard BMT SOP (BMT _SOP004_SAEReport). Unexpected, grades 4-5AEs must be reported within 24 hours of knowledge of the event. Unexpected, grade 3 AEs must be reported within three business days of knowledge of the event. All other unanticipated problems will be reported to the IRB within seven (7) business days of knowledge of the problem. The Data Review Committee (DRC) Board will receive summary reports of all adverse experiences on at least an annual basis.

9.2 Reporting to the IRB

Following AEs should be considered as unanticipated problems that must be reported to the IRB within seven business days of the knowledge of the AE(s):

Single occurrence of a serious, unexpected, and uncommon event that is strongly associated with investigational products exposure

- A single or small number of a serious, unexpected event that is not commonly associated with investigational products exposure but is uncommon in the study population
- Multiple occurrences of an AE that, based on aggregate analysis, is determined to be an unanticipated problem. There should be a determination that the series of AEs represents a signal that the AEs were not just isolated occurrences and involve risk to human subjects
- An AE that is described in the investigator's brochure, protocol, or informed consent documents, but occurs at a specificity or severity that is inconsistent with prior observations
- A serious AE that is described in the investigator's brochure, protocol, or informed consent documents, but for which there is a clinically significant increase in the expected rate of occurrence
- Any other AE or safety finding, including those based on animal or epidemiologic data that would cause the sponsor to modify the investigator's brochure, protocol, or informed consent documents, or would prompt other action by the IRB to ensure the protection of human subjects

9.3 Pregnancy Reporting

Each pregnancy must be reported immediately (within 72 hours of identification) by reportable event submission system to the CNMC IRB. Subjects who become pregnant after Day 0 will be followed to term, and the following information will be gathered for outcome, date of delivery, health status of the mother and child including the child's gender, height and weight. Complications and or abnormalities should be reported including any premature terminations. A pregnancy is reported as an AE or SAE only when there is suspicion that the treatment may have interfered with the effectiveness of contraception or there was a serious complication in the pregnancy including a spontaneous abortion or an elective termination for medical rationale.

9.4 IND Annual Report to the FDA

The PI will be responsible for the preparation of a detailed annual synopsis of clinical activity, including adverse events, for submission to the sponsor. Each annual report

will summarize IND activity for 1 year beginning approximately 3 months before the IND FDA anniversary date. The sponsor's representative will notify the PI of the due date with sufficient time for the PI to assemble the required information.

9.5 Final Report

A report will be submitted to the FDA in accordance with ICH E3 Guideline "Structure and Content of Clinical Study Reports". A final report will also be submitted to the IRB at the time of study closure.

9.6 Reporting to the FDA

Serious adverse events (SAEs) that are unlisted/ unexpected, and at least possibly associated to the drug for this study should be reported promptly to the Food and Drug Administration (FDA). Fatal or life threatening SAEs that meet the criteria for reporting to the FDA must be reported to the FDA within 7 calendar days after awareness of the event by submitting FDA form 3500/MedWatch form. All other SAEs that meet the criteria for reporting to the FDA must be reported to the FDA within 15 calendar days after awareness of the event. A clear description of the suspected reaction should be provided along with an assessment as to whether the event is drug or disease related.

10 CLINICAL TRIAL OVERSIGHT AND MONITORING

This protocol will be conducted in accordance with the CNMC BMT Monitoring Plan. This protocol will be monitored in accordance with federal regulations and FDA guidance documents.

The conduct of this clinical trial will be evaluated in accordance with the CNMC BMT Quality Assurance Policy and Procedure Plan (SOP: BMT_SOP010_QA).

10.1 Safety monitoring

Safety monitoring will be conducted throughout the study; therefore safety concerns will be identified by continuous review of the data by the PI, clinic staff, and Data Review Committee (DRC).

10.2 Data Review Committee (DRC)

The Data Review Committee (DRC) is primarily being established to monitor serious adverse events which may occur during the trial, complete an annual review of the safety data, and to make any recommendations as appropriate. The DRC is required to review all unanticipated problems involving risk to subjects or others, SAEs, and all subject deaths associated with the protocol and provide an unbiased written report of

the event. The DRC should indicate concurrence or non-concurrence with the details of the report provided by the investigator. Reports for events determined by either the investigator or DRC to be related or unrelated to participation and reports of events resulting in death should be promptly forwarded to the IRBs.

11 APPENDIX 1:

11.1 PCDAI

History (Recall, 1 week)

Abdominal Pain

0=None	5= Mild: Brief, does not interfere with activities	10= Moderate/Severe: Daily, longer lasting, affects activities, nocturnal

Patie It Functioning, General Well-Being

mitation of activities, well	5=Occasional difficulty in maintaining age-appropriate activities, below par	10= Frequent limitation of activity, very poor

As (per day)

liquid stools, no blood	5= Up to 2 semi-formed with small blood, or 2-5 liquid	10=Gross bleeding, ≥ 6 liquid, or nocturnal diarrhea

Laboratory

HCT

<10 years (male and female)

0= > 34% 2.5 = 29-33% 5 = <29%

11-14 years (Male)

0 = > 35% 2.5 = 30-34% 5 = < 30%

11-19 years (female)

0 = ≥ 34% 2.5 = 29-33% 5 = < 29%

15-19 years (Male)

0 = ≥ 37% 2.5 = 32-36% 5 = < 32%

ESR

0 = < 20 mm/hr 2.5 = 20-50 mm/hr 5 = > 50 mm/hr

Albumin

0 = ≥ 3.5 g/dL 5 = 3.1-3.4 g/dL 10 = ≤ 3.0 g/dL

Examination

Weight

0 = Weight gain or voluntary weight stable/loss	5 = Involuntary weight stable, weight loss 1%-9%	10 = Weight loss ≥ 10%

Height at Diagnosis

0 = < 1 channel decrease	5 = ≥ 1, < 2 channel decrease	10 = > 2 channel decrease

Height at Follow-Up

0 = Height velocity ≥ -1 SD	5 = Height velocity < -1 SD, > 2 SD	10 = height velocity ≤ 2 SD

Abdomen

0 = No tenderness, no mass	5 = Tenderness or mass without tenderness	10 = tenderness, involuntary guarding, definite mass

Perirectal Disease

0 = None, asymptomatic tags No tenderness	5 = 1-2 indolent fistula, scant drainage,	10 = Active fistula, drainage, tenderness, or abscess

Extra-intestinal Manifestations

Fever ≥ 38.5 for more than 3 days in the past week, definite arthritis, uveitis, E. nodosum, P.gangrenosum

0 = None 5= 1 10= ≥ 2

12 APPENDIX 2:

12.1 PUCAI:

Item	Points
1. Abdominal Pain	
No pain	0
Pain can be ignored	5
Pain cannot be ignored	10
2. Rectal Bleeding	
None	0
Small amount only, in <50% of stools	10
Small amount with most stools	20
Large amount (>50% of stool content)	30
3. Stool consistency of most stools	
Formed	0
Partially formed	5
Completely unformed	10
4. Number of stools per 24 hours	
0 to 2	0
3 to 5	5
6 to 8	10
>8	15

5. Nocturnal stools (any episode causing wakening)	
No	0
Yes	10
6. Activity Level	
No limitation of activity	0
Occasional limitation of activity	5
Severe restricted activity	10

13 APPENDIX 3:
13.1 CDAI

Clinical or laboratory variable	Weighting factor
Number of liquid or soft stools each day for seven days	x 2
Abdominal pain (graded from 0-3 on severity) each day for seven days	x 5
General well being, subjectively assessed from 0 (well) to 4 (terrible) each day for seven days	x 7
Presence of complications	x 20
Taking Lomotil or opiates for diarrhea	x 30
Presence of an abdominal mass (0 as none, 2 as questionable, 5 as definite)	x 10
Hematocrit of <0.47 in men and <0.42 in women	x 6
Percentage deviation from standard weight	x 10

One point each is added for each set of complications:

- arthralgias or frank arthritis
- inflammation of the iris or uveitis
- presence of erythema nodosum, pyoderma gangrenosum, or apthous ulcers
- anal fissures, fistulae, or abscesses
- other fistulae

14 APPENDIX 4:

14.1 MAYO SCORE

Endoscopic findings	Normal, inactive disease	0
	Mild disease, erythema, decreased vascular pattern, mild friability	1
	Moderate disease, marked erythema, absent vascular pattern, erosions, friability	2
	Severe disease, spontaneous bleeding	3
Stool frequency	Normal for the patient	0
	1-2 more than normal	1
	3-4 more than normal	2
	5 or more stools than normal	3
Rectal bleeding	No blood seen	0
	Streaks of blood with stool less than half the time	1
	Obvious blood with stool most of the time	2
	Blood alone passed	3
Physician's Global Assessment	Normal	0
	Mild disease	1
	Moderate disease	2
	Severe disease	3

15 APPENDIX 5:

15.1 SES-CD SCORE

Score per Segment				
Variable	0	1	2	3
Size of ulcers	None	Apthous ulcers (0.1-0.5 cm)	Large ulcers (0.5-2.0 cm)	Very large ulcers (> 2 cm)
Ulcerated surface	None	<10%	10%-30%	> 30%
Affected surface	Unaffected segment	< 50%	50%-75%	>75%
Presence of narrowing	None	Single, can be passed	Multiple, can be passed	Cannot be passed

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